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Review

The hunt for sustainable biocontrol of oomycete plant pathogens, a case study of *Phytophthora infestans*

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ABSTRACT

Late blight caused by the oomycete *Phytophthora infestans* is considered to be one of the most severe diseases of potato and tomato worldwide. Whilst current synthetic fungicides are efficient at controlling this disease, they are an environmental and economic burden. In line with EU directives to reduce the use of synthetic pesticides and increase the use of sustainable alternative disease control strategies that can form part of integrated pest management systems, practical biological control solutions are urgently needed. Despite the fact that there has been a large body of scientific research into microorganisms with potential for the biological control of late blight disease, relatively few commercial biocontrol agents, licensed to control late blight, exist. Furthermore, the practical uptake of those in Europe is lower than might be expected, suggesting that such solutions are not yet feasible, or effective. Here we review the scientific literature, focusing on the most recent developments in the hunt for efficient and sustainable biological control of late blight disease. We discuss the progress in our mechanistic understanding of mycoparasite–prey interactions, in the context of late blight and the challenges and limitations to the use of such knowledge in practical disease control within a European context.

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IPM
Integrated pest management
IDM
Integrated disease management
In-planta
Studies conducted in the host plant,
under controlled conditions,
for instance in greenhouse
In-agro
Field studies
DSS
Decision support system
VOC
Volatile organic compound

1. Introduction

1.1. *A rational for the hunt: the need for biocontrol*

In the last decades, chemical pesticides have been widely used to diminish yield losses caused by plant pathogens and pests (Hillocks, 2012). However, their continuous and abusive application has been associated with harmful side effects which have led to environmental and human health concerns. As a result, the number of registered synthetic chemical pesticides has decreased within the EU through restrictions imposed by directives 2009/128 and 2019/782 to reduce pesticide application risks on human health and environment (Baker *et al.*, 2020; Junaid *et al.*, 2013; Popp *et al.*, 2013). These directives promote the use of Integrated Pest Management (IPM) and alternative approaches to synthetic pesticides, such as biological control and low-risk compounds with the aim of achieving a sustainable and ecologically sound use of plant protection measures. Combined with a heightened public awareness of sustainability issues, research into alternative control strategies, particularly biological control, is a rapidly growing area (Barratt *et al.*, 2018).

Biological control of plant diseases can be defined as the application of beneficial micro, (or macro) living organisms to control aerial or soilborne plant pathogens. We consider biological control in the strictest sense of the definition, i.e., always involving a living organism that targets the pest directly or indirectly (Heimpel and Mills, 2017; Stenberg *et al.*, 2021). The term biocontrol agent (BCA) refers to the organism (typically a bacterium, fungus, oomycete, nematode, insect, virus or occasionally plant) that is used to control the disease-causing agent (Stenberg *et al.*, 2021). Mechanisms of biocontrol are broadly divided into four categories (Köhl *et al.*, 2019). 1) Competition for resources, including nutrients, space, and/or water and often for rare nutrients such as iron. Biocontrol agents that use this mode of action are termed competitive saprophytes. Here, disease suppression by mixed communities of microbes or other organisms in the soil in combination with physiochemical soil properties that discourage the growth of disease-causing microbes may also occur (e.g., through the action of suppressive soils) 2) Mycoparasitism, whereby the biocontrol agent is termed a facultative hyperparasite and where direct infection of the plant pathogen (prey species) occurs. 3) Antibiosis, also a feature of facultative hyperparasites, where antimicrobial compounds or toxins and lytic enzymes are produced to destroy the prey (plant pathogen). 4) Induced host resistance, whereby plant hormone mimics or precursors are produced and/or innate immunity is triggered by a facultative plant symbiont to confer resistance to incoming pathogens. Biocontrol ability, and mode of action, is generally strain-specific depending on the host, plant, pathogen, and environmental factors and most BCAs employ more than one mode of action to control pathogens (Köhl *et al.*, 2019). The methods through which biocontrol is used can be classified into four main categories depending on whether native BCA species are utilized, with or without targeted human intervention (conservation biological control and natural biological control, respectively) or if BCAs are added into the agroecosystem for permanent or temporary establishment (classical biological control and augmentative biological control, respectively) (Stenberg *et al.*, 2021).

1.2. *Know your prey: oomycete threats to our crops and the environment*

Oomycetes, or water moulds, are fungus-like eukaryotic microorganisms that genetically belong to the Stramenopila (which includes brown algae) but resemble fungi in both their filamentous growth and absorptive nutrition (Baldauf *et al.*, 2000). The oomycete lineage contains both pathogenic and non-pathogenic species. The pathogenic species have a wide range of hosts and affect plants, insects, crustaceans, fish, vertebrate animals, and various microorganisms (Judelson and Ah-Fong, 2019). Oomycetes are able to swiftly develop resistance to synthetic fungicides, overcome the resistance genes that have been bred into crop plants (Vleeshouwers *et al.*, 2011) and are known to deploy a vast array of effectors to facilitate destruction of their hosts (Bozkurt *et al.*, 2012; Hamed and Gisi, 2013; Schornack *et al.*, 2009).

Phytopathogenic oomycetes, such as *Phytophthora infestans*, are the causal agents of some of the most devastating plant diseases known to man (Birch *et al.*, 2012). *P. infestans*, the causal agent of potato and tomato late blight now has an almost global reach (Birch *et al.*, 2012) and is arguably the most destructive of the oomycete plant diseases both in terms of economic damage

(Haverkort *et al.*, 2016) and of fundamental food loss (Fisher *et al.*, 2012). *P. infestans* is able to trigger stomatal opening, through an as yet unknown mechanism (Yang *et al.*, 2021). This allows asexual propagation to occur via the emergence of aerial sporangiophores containing sporangia. A single potato late bight lesion can contain more than 300 000 *P. infestans* sporangia that can be rapidly dispersed via the wind, or after cleavage into motile zoospores, through air or soil -borne water droplets (Fry, 2008). The biflagellated wall-less zoopores are able to swim towards host plant cues, and differentiate to form walled cysts that germinate upon contact with the host, producing a thick-walled penetration structure, the appressorium (Grenville-Briggs *et al.*, 2008). The pre-infection stages of the asexual lifecycle occur outside of the host plant, and thus *P. infestans* is particularly vulnerable to fungicides or to attack by other microbes, such as potential biocontrol agents during these stages of development. The infection potential of an oomycete spore on plant tissue is probably much lower than 100% (Kong and Hong, 2016), meaning that not all of the 300 000 sporangia produced in a lesion will continue to propagate the disease. Thus, the progress of an epidemic, and to some extent the effectiveness of control treatments, will be influenced by factors that raise or lower this infection potential (Judelson and Ah-Fong, 2019; Willocquet *et al.*, 2017). This can include aspects of the cultivation system (Brylińska *et al.*, 2016), temperature (Lurwanu *et al.*, 2021; Wu *et al.*, 2020), UV index (Wu *et al.*, 2019), carbon dioxide levels (Plessl *et al.*, 2007) and population structure, including sexual reproductive capacity (Klarfeld *et al.*, 2009). There are indications that over the past 20 years, the aggressiveness of *P. infestans* has increased (Cooke *et al.*, 2011; Lehsten *et al.*, 2017), whilst at the same time global populations of the pathogen are in flux, with sexual recombination and diverse rapidly evolving genotypes contributing to unexpectedly severe epidemic outbreaks worldwide (Fry *et al.*, 2015). Thus *P. infetans* can be considered to be a reemerging pathogen (Fry *et al.*, 2015) and under a warming climate, we will require new and/or more adapted control measures in the different growing regions of the world. However, the common feature of those control measures must be in line with the UN sustainability goals and the EU directives, to reduce the burden of synthetic inputs in agricultural systems, whilst maintaining biodiversity and effective disease control. Biological control has the potential to be an important component of such an integrated disease management program. Thus, here we discuss progress and challenges to the development and deployment of BCAs against late blight disease of potato and tomato.

2. Preparing and executing the hunt: towards biocontrol of *Phytophthora infestans*

In the hunt for new or improved biocontrol agents, most studies firstly employ *in vitro* assays of growth inhibition or death of the prey species, typically in confrontation assays (e.g., Fig. 1 lower panel). This approach allows mechanistic understanding of direct interactions between BCAs and their prey to be elucidated under laboratory settings. In the case of *P. infestans,* this allows researchers to test potential BCAs for direct control of the vulnerable pre-infection stages of the lifecycle. The "*in vitro* first" approach is attractive for several reasons, including the low cost, high throughput nature of such assays, and the ease of experimental set up and data analysis for individual experimental factors under controlled conditions. However, such approaches run the risk of years of work failing to identify BCAs that perform effectively in an agricultural setting. In fact, it may be argued that research starting from field (*in agro*) or greenhouse (*in planta*) (e.g., Fig. 1 middle and top panel) studies has a higher chance of implementation and may be more informative for practitioners than *in vitro* analysis; since, differences in efficacy of disease control in controlled environments versus in the field, coupled with the high costs of field trials mean that some BCAs never move beyond *in vitro* or *in-planta* studies to field applications. Furthermore, promising agents that may work in the field but not *in vitro* can be mistakenly eliminated from screens. *In vitro*, and in particular associated molecular and 'omics studies may stimulate new research avenues including important questions regarding microbe–microbe interactions and generate important fundamental knowledge including mechanistic understanding of BCAs and their interactions with their prey. However, if we want to bring about meaningful change in pest management practices, in terms of a reduction in the reliance on synthetic pesticides and an increase in the use of BCAs, we need more applied studies. The knowledge obtained from these applied studies is essential to the development of durable formulations of BCAs for sustainable disease control.

In the last 20 years, there have been more than 95 peer-reviewed scientific publications in which the potential of a microorganism for the biocontrol of *P. infestans* has been investigated (Table S1). Here, we review that literature with particular emphasis on research from the last 7 years, in the hunt for sustainable biocontrol of late blight disease of potato and tomato. Our hunt takes us from mechanistic studies or "target practice" as revealed by *in vitro* studies, through to *in planta* and *in agro* (full agricultural field) studies to bring down our prey *P. infestans,* as summarised in Fig. 1. Finally, we discuss "the struggle to make the kill" – the challenges we face in the use of biocontrol against oomycete diseases and prospects for the future, focusing on the integration of biocontrol into IPM strategies (summarised in Fig. 2) and the current situation in Europe.

2.1. *Target practice: biocontrol mechanisms revealed by* in vitro *studies*

Despite the fact that *in vitro* biological control studies often fail to translate to field applications, valuable mechanistic knowledge can be gained from such experiments. One of the most studied and most well-known fungal genera harbouring BCAs is *Trichoderma* (De Silva *et al.*, 2019; Rai *et al.*, 2019). The biocontrol mechanisms used by *Trichoderma* spp. against *P. infestans* encompass classical mycoparasitism and antibiosis behaviour, by coiling around prey hyphae, and secreting lysing enzymes, secondary metabolites and/or other toxins that directly inhibit *P. infestans* growth and sporulation *in vitro* (Kariuki *et al.*, 2020; Yao *et al.*, 2016).

Within the oomycetes, *Pythium oligandrum* is known for its antagonistic properties and its mycoparasite behaviour of a

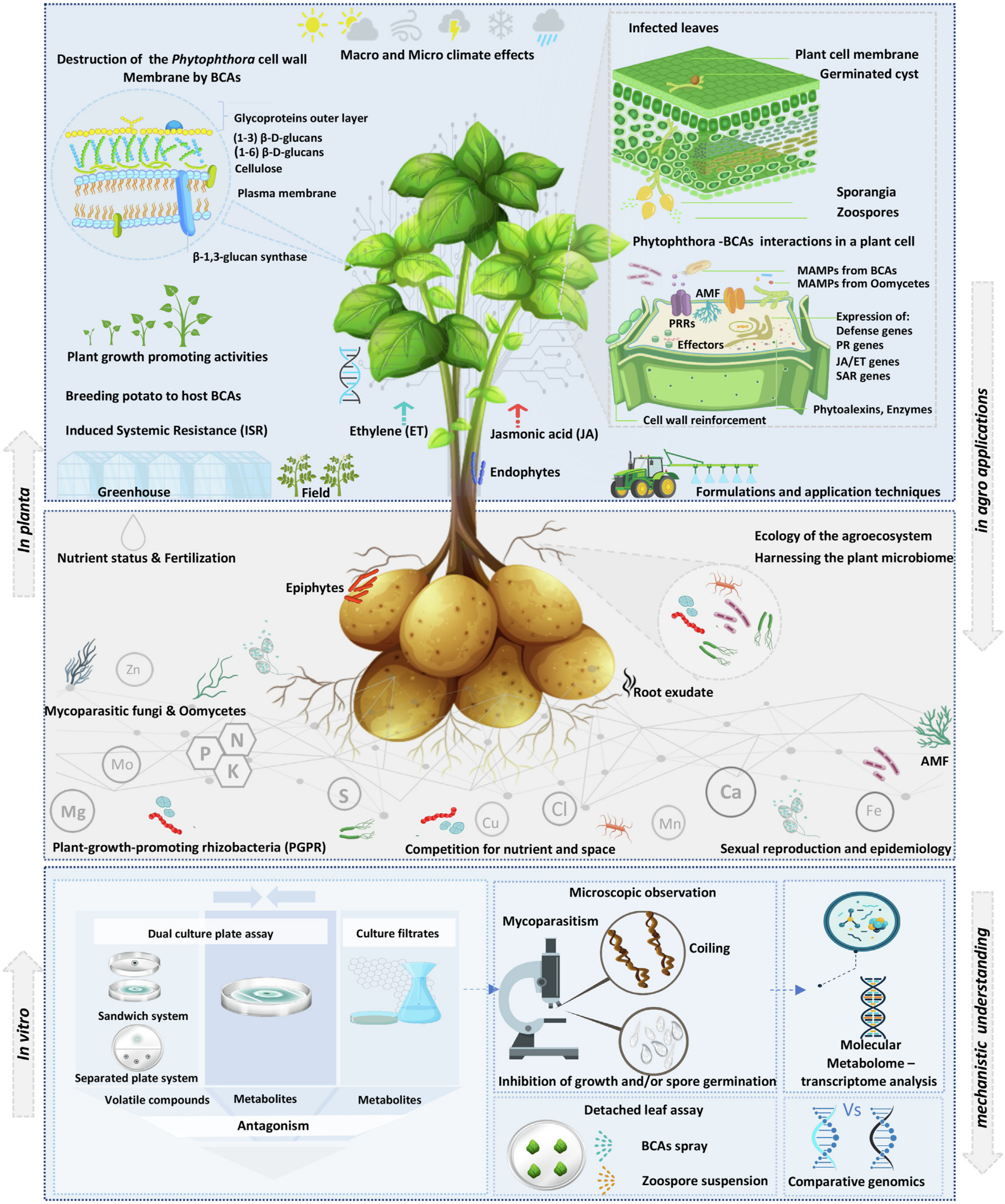

Fig. 1 – Towards both a mechanistic understanding and practical use of biocontrol agents against potato late blight disease. *In vitro* assays may lead to the identification of metabolites, volatiles or direct mycoparasitic effects of BCAs, which, combined with molecular tools such as metabolomics, transcriptomics or comparative genomics provide a mechanistic understanding of mycoparasite-prey interactions between BCAs and *P. infestans* (bottom panel). *In planta* and *in agro* experiments allow us to understand these interactions within the agroecosystem, and in the context of plant responses to both the pathogen and the potential BCAs (top panels). Within the soil BCAs have to compete for space and nutrients with the indigenous plant microbiome and with each other, and may be affected by the nutritional status of the plant (middle panel).

diverse set of fungal and oomycete prey species (Gerbore *et al.*, 2014). It displays mycoparasitic behaviour and secretes cell wall degrading enzymes and putative effectors during colonisation of *P. infestans in vitro* (Horner *et al.*, 2012; Liang *et al.*, 2020). Interestingly, a recent comparative genomics study revealed *P. oligandrum* may have evolved its mycoparasitic capabilities by tandem gene duplication and horizontal gene transfer of specific carbohydrate-active enzymes (CAZy) from fungal and bacterial species, giving it the potential to utilize fungal and oomycete species for nutrition (Liang *et al.*, 2020).

Many genera of bacteria display biocontrol characteristics and the major genera that have been investigated in relation to control of *P. infestans* are *Bacillus, Pseudomonas* and *Streptomyces* (Table S1). Several Bacillus species show direct antagonism toward *P. infestans* providing effective growth reductions *in vitro* (Cray *et al.*, 2016; Caulier *et al.*, 2018; Wang *et al.*, 2020a, 2020b). Bacterial volatile organic compounds (VOCs) play important ecological roles in both soil microbial and host plant interactions (De Vrieze *et al.*, 2015) and have also been shown to be important for inhibition of *P. infestans* growth (Anand *et al.*, 2020; Bailly and Weisskopf, 2017; Guyer *et al.*, 2015; Joller *et al.*, 2020; Lazazzara *et al.*, 2017). Among these, hydrogen cyanide, long-chain aldehydes, alkenes and short-chain ketones as well as sulphur-containing compounds and some longer-chain ketones all have an inhibitory effect on *P. infestans* growth, spore development and germination *in vitro* (Bailly and Weisskopf, 2017). The majority of these VOCs have been identified from *Pseudomonas* species that are native inhabitants of the potato rhizosphere, and do not negatively affect potato growth (Bailly and Weisskopf, 2017).

Numerous other bio-active metabolites are produced by bacterial BCAs, and those from species of *Bacillus* and *Pseudomonas* have been particularly well studied. For example, cyclic lipopeptides produced by several *Pseudomonas fluorescens* strains have specific activity against the vulnerable wall-less zoospores of *P. infestans* (De Vrieze *et al.*, 2020; Zachow *et al.*, 2015) and siderophore production has also been associated with anti-oomycete activity, in the *Pseudomonas* genus (De Vrieze *et al.*, 2020). Iron acquisition genes including those linked to the production of Pyoverdines have been linked to both the ability of *Pseudomonas* strains to survive in the soil and colonise plant roots, as well as to direct antagonism of *P. infestans* (De Vrieze *et al.*, 2020) suggesting iron competition could be one of the mechanisms used by some *Pseudomonas* species to inhibit *P. infestans* development. Although members of both the *Pseudomonas* and *Bacillus* genera are known to be prolific producers of numerous bioactive compounds including those with anti-oomycete activities, the *in vitro* activities of such compounds are not easily transferable to *in planta* or *in agro* assays (Caulier *et al.*, 2018), thus new methods for selection of BCAs need to be developed. One possibility for the screening of potential bacterial BCAs could be to screen for the production of biosurfactants and siderophores, since in their large-scale analysis of over 2800 bacterial isolates (Caulier *et al.*, 2018), identified that the strains that were effective *in planta* at controlling late blight were prolific producers of both, and indeed the production of such compounds might be the significant factor contributing to the success of these strains as BCAs. As microbial competition for nutrients and ecological niches, on, or within plants, contributes to the antagonistic activity of competent bacterial strains (Vorholt, 2012), isolates naturally associated with potato plants, such as some of these *Bacillus* and *Pseudomonas* species, have the highest chance to be artificially reintroduced to a crop for control purposes, through classical biocontrol strategies.

Molecular and genomic studies, in combination with *in vitro* assays can also reveal important information on the biology, modes of action, and genetics of BCAs. For example, comparative genomics studies have revealed that specific loci in the genomes of some *Pseudomonas* species control aggressiveness of these species towards *P. infestans*. Furthermore, this aggressiveness can increase through increasing exposure to the prey *in vitro* (De Vrieze *et al.*, 2020), meaning that in the future it may be possible to genetically engineer hyper-aggressive strains for use in the field. Additionally, such studies can determine the life history of BCAs, for example, mycoparasitic *Pythium* species likely acquired their facultative hyperparasitic abilities by horizontal gene transfer and tandem gene duplication, meaning the ancestral state of these species was likely to be as phytopathogens (Liang *et al.*, 2020). In contrast, *Trichoderma* species are likely to have an ancestral state as facultative hyperparasites, with some members of the genus later developing abilities to parasitise plants (Kubicek *et al.*, 2011).

Understanding the biology of both the BCA and the prey is important for the development of effective biological control strategies. For example, the *P. infestans* cell wall consists predominantly of β-D-glucans and cellulose, and the correct formation of the cell wall during encystment of wall-less zoospores and subsequent differentiation into appressoria, is required for establishment of disease (Grenville-Briggs *et al.*, 2008). The cell wall is already the target of several anti-oomycete fungicides, such as the CAA fungicides including Mandipropamid (Blum *et al.*, 2010). Breaching the cell wall is also necessary for BCAs that act as facultative hyperparasites, and thus many bacterial (Caulier *et al.*, 2018), fungal (Karlsson *et al.*, 2015; Kubicek *et al.*, 2019), and oomycete (Grenville-Briggs *et al.*, 2013; Liang *et al.*, 2020) BCAs secrete extensive cocktails of cell wall degrading enzymes, (CWDEs) which are likely to contribute to their success as mycoparasites. Indeed it has recently been proposed that such CWDEs from such hyperparasites are major pathogenicity determinants (effectors) in these species, since they display the genetic hallmarks of rapidly evolving effectors (Linag *et al.*, 2020; Karlsson *et al.*,

Within the leave, *P. infestans* produces effectors to manipulate the host and evade immunity, whilst the plant responds with defence related genes that are often hormonally regulated. Within this battle ground, BCAs have to succeed to break open the cell wall of the prey and to obtain the nutrients they require (top panel). Finally, this knowledge must be harnessed and BCAs manipulated to withstand both macro and micro climate effects, to produce correctly formulated products for practical plant protection within greenhouse or open field systems (top panel) in order to successfully control late blight disease.

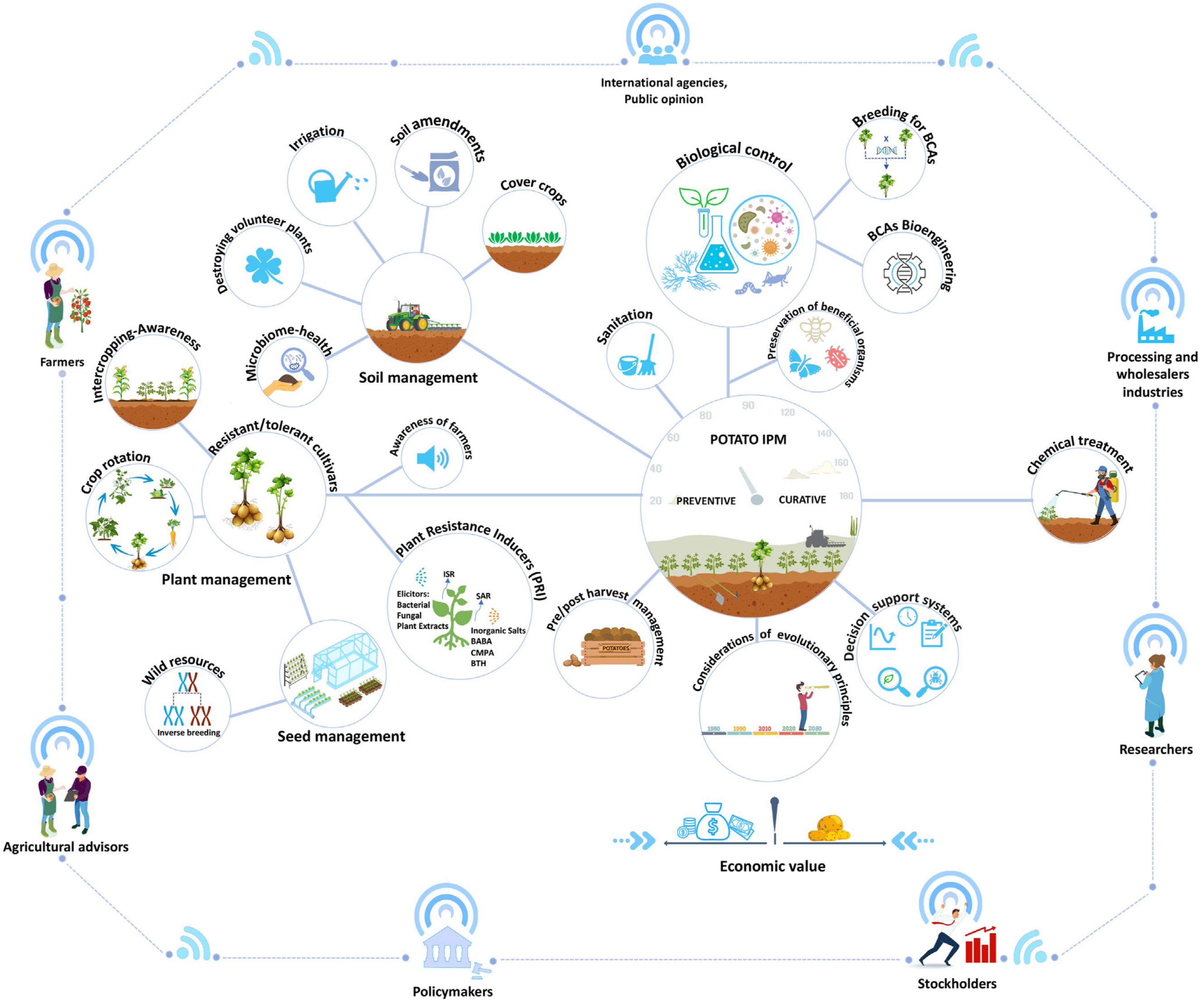

Fig. 2 – Integrated Pest Management (IPM) in potato. A schematic of the potential components of an IPM system for the protection of potato from pests such as late blight disease. For successful implementation of IPM in potato, greater emphasis should be placed on preventing pest problems (traveling left from the central bubble) than curative measures (traveling right from the central bubble). Bubble size reflects the importance of the action in the system. Biological control should be a central component of IPM that can be applied both preventatively and curatively, and should include methods to preserve beneficial microbes and breeding of potato to host beneficial microbes including BCAs. Soil management, should include microbiome health, as well as traditionally included measures such as soil amendments, irrigation and destruction of volunteer plants. Plant management should include crop rotations, resistant cultivars and the use of low-risk compounds that act as plant resistance inducers. IPM needs to take into account evolutionary principles and the use of curative synthetic pesticides should be a last resort, and they should be applied only when needed, with the help of decision support systems. IPM involves many actors, including farmers, advisors, policymakers researchers, and the processing and wholesale industries.

2015). The secretion of the most potent mixtures of CWDEs could therefore be used to select Eukaryotic microbes that will be efficient BCAs in the future.

2.2. *The thrill of the chase:* in planta *studies of late blight disease control by microbial BCAs*

A substantial number of studies have tested BCAs against late blight *in planta*, either in controlled environment detached leaf assays, or in whole plant greenhouse bioassays (Table S1), although the efficacy of control varies. Since commercial tomato cultivation in Northern Europe is almost exclusively carried out in greenhouses, it is relatively easy to mimic this production system to accurately analyse BCA efficacy in a research setting. Valuable knowledge that might also translate into potato cultivation systems can also be gained in this manner. In addition to direct disease control measures, *in planta* assays have the advantage of evaluating biostimulation, for example Kariuki *et al.* (2020) observed that both *T. harzianum* and *T. asperellum* stimulated growth of

Table 1 – Table of approved products against Phytophthora *infestans* in potatoes or tomatoes. Product name, licence approval, regislation, active components and Application is displayed.

Product Name	License approved	Licence expire	Approval under Reg. (EC) No 1107/2009	Component(s)	Application method
TAEGRO®	2017-06-01	2032-06-01	Yes	*Bacillus subtilis* var. *Bacillus amyloliquefaciens* strain FZB24	Sprayed product.
Polygandron WP®	2009-05-01	2022-04-30	Yes	*Pythium oligandrum*	Sprayed product.
Sonata®	2014-09-01	2024-08-31	Yes	*Bacillus pumilus* QST 2808	Sprayed product.

above ground tomato plants and increased the biomass by over 30% and 19%, respectively compared to control plants as well as providing protection against late blight, reducing disease symptoms to 40% of that seen in control plants. Stephan *et al.* (2005) used the commercial product Trichodex® based on *Trichoderma harzianum*, in greenhouse and detached leaf assays to assess the potential for this product to control potato late blight, but despite a previously reported *in vitro* growth reduction of *P. infestans* by 40% when co-cultured with this species (Fatima *et al.*, 2015), there was no significant effect of the commercial preparation on late blight disease development.

Endophytes, or other antagonistic indigenous soil microbes from healthy plants in a habitat where disease is a problem are more likely to be competitive BCAs, than introduced species, since such endophytes are already adapted to the appropriate environment (Collinge *et al.*, 2019). The large-scale screening study conducted by (Caulier *et al.*, 2018) took this approach. They first isolated 2800 strains of *Bacillus*-like and *Pseudomonas*-like bacteria from potato agroecosystems and then tested a subset of them for *in planta* activity against *P. infestans*. Of the 11 strains tested *in planta*, four strains (*Bacillus amyloliquefaciens* 17A-B3, *Bacillus subtilis* 30B-B6, *Pseudomonas brenneri* 43R-P1 and *Pseudomonas protegens* 44R-P8), decreased disease symptoms in the greenhouse study, and one of these, *B. subtilis* 30B-B6, significantly decreased late blight disease severity *in agro*, in a small-scale field trial (Caulier *et al.*, 2018), validating the endophyte-based approach for the identification of new BCAs.

2.3. *Bringing down the prey: translation of these data to an in agro setting*

Since potato is the third most important food crop globally (Fisher *et al.*, 2012), potato late blight arguably has a greater potential impact on food and economic security than tomato late blight, and thus to actually transform late blight disease control in a sustainable manner, biocontrol strategies that are durable in open field agriculture (*in agro*) are desperately needed.

Many *Trichoderma* species have been tested for potato late blight disease control *in agro* (Al-Mughrabi, 2008; de Souza *et al.*, 2015; El-Naggar *et al.*, 2016; Wharton *et al.*, 2012; Yao *et al.*, 2016). For example, Al-Mughrabi (2008) showed a significant effect on late blight incidence when tubers were pretreated with Trichoderma in field trials. The commercial product Planter-Box® containing *Trichoderma harzianum,* is effective against late blight seed pierce incidence on potato tubers, and microscopic investigations confirm *T. harzanium* coiling around *P. infestans* hyphae and thus exhibiting classical mycoparasitic behaviour *in agro* (Wharton *et al.*, 2012). Yao *et al.* (2016) showed that Trichoderma can significantly reduce late blight disease severity in potato field trials and, most recently, Lal *et al.* (2021) tested Neem, a bioextract, in combination with *Trichoderma viride* and found a significant reduction of potato late blight *in agro*. However these studies did not determine if secondary metabolites also played a role in the efficiency of these treatments.

In agro studies with other fungal BCA have also been carried out, for example, Shanthiyaa *et al.* (2013) demonstrated that application of a spore suspension of *C. globosum Cg-6* as a tuber, soil and foliar treatment inhibited late blight infections by 72% *in agro*. The application of *C. globosum* even resulted in an increased tuber yield. They further identified a metabolite “Chaetomin” belonging to epidithiodioxopiperazine potentially responsible for the antagonistic activity; a great example of antibiosis working *in agro*. This study also highlights the importance of secondary metabolites produced by BCAs in effective disease control.

Within the oomycetes, the BCA *Pythium oligandrum* has been formulated into the commercial products Polyversum® and Polygandron®. These products have been tested *in agro* against potato late blight through applications as both tuber dressings and as foliar sprays. A multi-year field trial in Poland demonstrated a significant yield increase and foliar protection against late blight from these applications (Kurzawińska and Stanisław, 2007), and a Swedish study demonstrated effective control of potato late blight for the first 20–30 days of the growing season after applications of Polygandron® early in the season (Wiik *et al.*, 2020).

The effects of bacterial BCAs under field conditions have been reported in several publications (El-Naggar *et al.*, 2016; Huang *et al.*, 2007; Wang *et al.*, 2020a; Yan *et al.*, 2021). Moreover, their application as a seed treatment for late blight control under storage conditions has been investigated (Cray *et al.*, 2016; Wharton *et al.*, 2012). However, the results are variable. For instance, Yan *et al.* (2021) showed that *in agro* application of a low concentration of the fungicide Fluopimomide and a high concentration of the antagonistic bacteria *Bacillus velezensis* SDTB038 can be effective in controlling potato late blight. *Bacillus subtilis* applied both to the soil and as a foliar treatment has significantly reduced late blight occurrence rate *in agro* compared to control plants (Kumbar *et al.*, 2019). Open field foliar applications of crude bacterial suspensions of *Pseudomonas protegens* strain 44R-P8 and *B. subtilis* strain

30B-B6 were shown to decrease late blight severity by approximately 20% (Caulier *et al.*, 2018). However, most BCAs do not perform to the same level as synthetic chemical fungicide treatments (Caulier *et al.*, 2018; Kumbar *et al.*, 2019). It may therefore be more promising currently, to combine BCAs with low-risk fungicide treatments, such as resistance inducers or biopesticides in an integrated disease management approach. For example, it may be effective to apply BCAs early on, i.e., before the onset of disease, whilst the infection pressure within the field is low, and before the crop canopy has closed. After canopy closure, when living BCAs might find it difficult to access all areas of the plant and when the infection pressure is higher, (bio)fungicide treatments may well be more effective. This combinatorial approach proved successful when the bacterium *Rhodopseudomonas palustris*, GJ-22 was combined with the two synthetic fungicides cymoxanil and mancozeb (Zhang *et al.*, 2020). The challenge for the future is to provide effective disease control not only when combining BCAs with synthetic fungicides, but rather with biopesticides, such as those refined from secondary metabolites produced by BCAs, resistance inducers or other low-risk compounds that are more sustainable.

3. The struggle to make the kill: challenges and limitations for the development of biocontrol solutions to late blight

P. infestans displays the nature of a super pathogen, adapting and evolving, to constantly stay on top of the host-pathogen evolutionary arms race, in terms of overcoming both host resistance and synthetic fungicides through adaptive evolution of effectors and fungicide target genes (Dong and Ma, 2021). Nevertheless, sustainable control measures are still needed and for the future, this should include biological control as a major component of disease management.

3.1. *Licence to kill: registration of BCAs*

Recently the International Organisation for Biological Control (IOBC) reviewed the barriers to uptake of biological control by practitioners and advisors, and concluded that the reasons why biological control is not widely used are the difficult and risky regulatory processes involved, as well as bureaucratic barriers to access to biocontrol agents (Barratt *et al.*, 2018). The same barriers serve as limiting factors for developing and registering BCAs for late blight disease control.

The EU Commission Regulation No 546/2011 of 10 June 2011, implementing directive (EC) No 1107/2009, specifically regulates the use of plant protection products, including biological control agents, in agriculture. Requirements for registration to ensure safe usage are important and needed. However, these regulations also create an unwanted effect, namely that it is a long and potentially expensive process to get a product approved in the EU (Hauschild, 2017). The process is more extensive in the EU than in other parts of the world, (taking on average 65,7 months in the EU compared to 15,7 months in the USA) (Balog *et al.*, 2017; Frederiks and Wesseler, 2019; Kiewnick, 2007). In general the US approval process is much faster since microbial BCAs are registered under the regulatory framework of Biopesticides, and the US is accustomed to such. To formally have a microbial BCA approved in the EU, it needs to pass two steps within the legislation. The first step is the evaluation of the active substances. This step consists of three phases, the rapporteur member state phase, the risk assessment phase and the risk management phase (Frederiks and Wesseler, 2019). Generally, during the first step applicants provide a dossier of documents regarding the active substance to a member state. After evaluation of the dossier, the European Food Safety Authority starts assessing the risks in all aspects of food safety and the risk management is then carried out by the European Commission. The second step is to approve and register the plant protection product at a member state level. BCAs used in field crops are usually registered and approved to be used within specific zones of Europe, however country-specific or cross-zonal approval occurs too. EU Approved active substances and BCAs can be found here: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en.

Dominating factors that slow the EU registration process are investigations into the environmental fate, toxicity and resilience of the BCA, questions that reappear throughout literature and are seldom studied (Ehlers, 2011; Hajek and Eilenberg, 2018; Köhl *et al.*, 2019). Based on the assumption that an increase in population levels of microbial species can have adverse effects on other organisms and ecosystems, data on the environmental fate and persistence of microbial BCAs are required (Deising *et al.*, 2017). However, long-term experiments show that applications of non-pathogenic microorganisms into the environment have not generated situations where the released organisms became overwhelming and the dominating species within the habitat (Alabouvette and Cordier, 2011; Köhl *et al.*, 2019; Sundh and Goettel, 2013).

3.2. *The armoury: formulation of microbial BCAs*

A major challenge in using living microorganisms as BCAs is that they can be difficult to formulate in a way so they can both be sprayed efficiently and still be storable over longer time, whilst remaining viable. Formulation is often beyond the scope of most research projects, even those with applied aspects, and thus to move from research to practice, practitioners may be reliant on larger agrochemical companies seeing the value and need in a BCA and taking on both the formulation and registration process for these organisms. Very few of the BCA presented in this review have been commercially formulated for late blight control (Table 1), considering the amount of research conducted (see S.1). To produce a successful formulation it is necessary to take into consideration how the microbial BCA can be affected by temperature, humidity, soil type, pH and UV light. Some notable species that have been commercialised such as *Clonostachys rosea,* and members of the *Bacillus*, *Pseudomas* and *Trichoderma* genera have been well studied in this regard (Costa *et al.*, 2016; Maruyama *et al.*, 2020; Panpatte *et al.*, 2016; Wang *et al.*, 2018; Zin and Badaluddin, 2020). Such data is essential to allow formulation of microbial BCAs that need to establish themselves and proliferate within agroecosystems, where they are expected to be effective within a single growing season, and then may be disturbed by tillage and crop rotation in a standard intensive European farming system. This has led practitioners to use BCAs in a curative manner, utilising existing machinery used for application of synthetic pesticides. However, given the lack of success in translating *in vitro* studies to *in agro* applications, it might be time to rethink this approach. Future efforts should be focused on better understanding the modes of action and environmental interactions of current BCAs, and reformulating them for use at or before planting, to help stabilise host plants before pathogens enter the agroecosystem. Furthermore, for those species where active secondary metabolites have been identified from BCAs, isolating and stabilising those active metabolites in a formulation suitable for spray applications would allow practitioners to use only the active substance from the BCA in curative applications where needed.

3.3. *The weapons factory: utilising microbial secondary metabolites for biological control*

A range of secondary metabolites with activity against plant pathogens such as *P. infestans* have been identified from many microbial BCAs. Therefore, we suggest that microbial BCAs could be utilized as cell factories to produce compounds with anti-pathogen properties such as secondary metabolites, and indeed screening for the ability to produce biosurfactants, siderophores or other secondary metabolites may be a more successful approach to identifying competent BCAs rather than *in vitro* confrontation assays. Many secondary metabolites from BCAs have been demonstrated to have good efficacy in the suppression of late blight disease. For example, the cyclic dipeptide 2,5-diketopiperazine cyclo (L-Pro-L-Tyr) from *Lysobacter capsici* AZ78 can directly inhibit development of *P. infestans* sporangia (Puopolo *et al.*, 2014). Bikaverin and fusaric acid from *Fusarium oxysporum* EF119 are effective at controlling tomato late blight in greenhouse settings (Son *et al.*, 2008) and *Trichoderma* species are well known to produce a wide variety of metabolites and other compounds such as peptaibols (i.e., trichokonins, alamethicin), small non-ribosomal peptides (NRP) (i.e., gliotoxin, siderophores), polyketides (PK) (i.e., aspinolides, trichodermaketones), terpenes (i.e., trichothecenes), and pyrones (i.e., 6-pentyl-2H-pyran-2-one (6-PP) (Hermosa *et al.*, 2014; Vinale *et al.*, 2014).

Commercial formulations such as Serenade®, a broad spectrum biofungicide based on *Bacillus subtilis*, are thought to derive much of their success from the secreted metabolites and lipopeptides produced by the bacterium and included in the commercial formulation (Stephan *et al.*, 2005). This sets a precedent for the development of low risk biopesticides that are based solely on secreted metabolites and that thus will not be as sensitive to environmental changes as formulations containing living BCAs.

Trichoderma species have been harnessed for novel plant biotechnology approaches. One notable example is the use of *T. atroviride* as a “cell factory” to produce selenium nanoparticles (SeNPs) as an eco-friendly plant protection product. These fungal-derived SeNPs have an inhibitory effect on *P. infestans* growth and spore production *in vitro* (Joshi *et al.*, 2019) and *in planta* when used as a seed coating in tomato, where they prime the plant defence responses and stimulate induced systemic resistance (ISR) (Joshi *et al.*, 2021). The SeNP treated tomato plants showed a significant deposition of callose and lignin as well as elevated H_2O_2 consistent with an ISR response, and an upregulation of general defence enzymes such as lipoxygenases, and cell wall degrading enzymes, with 72% of the primed plants showing complete disease protection against late blight (Joshi *et al.*, 2021). Such bioengineering approaches are highly promising for the future of plant protection.

3.4. *Hunting in the wilderness: agroecological effects on BCA efficacy*

Even if natural secondary metabolites are not necessarily suitable for direct use as commercial fungicides, the identification of such mechanisms are key for the development of control strategies, in both Integrated Pest Management systems and organic agricultural production systems. However deeper knowledge about the adaptation to environmental conditions, and an understanding of microbiome interactions is crucial to find a better approach for BCA selection.

The variability in efficacy of BCAs *in planta* or *in agro* is generally attributed to climatic variations (temperature, humidity, radiation) encountered in field conditions, a lack of ecological competence (survival, colonization ability) of the biocontrol agent, intrinsic traits of the antagonistic microbe (variable production of required metabolites or enzymes) and/or an unstable quality of the formulated product (Bardin *et al.*, 2015; Mark *et al.*, 2006; Ruocco *et al.*, 2011). Another factor contributing to varying efficacy of BCAs is interactions with the native microbial community in the soil, or leaf, microbiome associated with the host plant. Studies have shown that for instance the bacterial community in potatoes are recruited from the soil (Buchholz *et al.*, 2019; De Vrieze *et al.*, 2015). Abiotic factors such as environmental conditions (Rasche *et al.*, 2006) or soil types (Inceoğlu *et al.*, 2012) are known to influence the structural and functional diversity of the bacterial microbiota of potato plants. Similar trends have been seen for fungi. Hou *et al.* (2020) reported that the change of the microbiome in potato plants was most significant at seedling stage, and that potato root exudates contributed to the growth of the rhizobiome. Zimudzi *et al.* (2018) reported that the rhizospheric fungal microbiome of potatoes were different between the two seasons and in the different plant growth stages within a given season, indicating the significance of the rhizosphere in shaping microbial communities.

Individual BCAs have different survival capacities in the rhizosphere or in host plants and this is an area where we still lack a lot of knowledge. *Pseudomonas protegens* has been reported to survive down to 2 m below the soil surface (Troxler *et al.*, 2012) and, whilst some studies have shown that *Trichoderma* species can survive for up to two years after inoculations in soils (Longa *et al.*, 2009), other studies show that these BCAs are not able to persist long-term (Feng *et al.*, 2011). Hence, it matters greatly into which environment and existing interactive microbial community the BCA will be amended, and thereby to what extent it will persist in the environment and provide effective disease control.

3.5. *Timing the shot: when and how to apply BCAs*

Targeting *P. infestans* at the right moment, of both the epidemic within the field and also of the lifecycle, is challenging. Directly combatting late blight when already established in the host by augmented release of a BCA is possible, but, as discussed above, can have varying results. To increase the efficacy of many of the microbial BCAs discussed in this review, we may have to move away from using BCAs curatively as we do synthetic fungicides and start using them as fertilisers or soil improvers, ensuring they can establish themselves in the field at or before planting. For example, Stephan *et al.* (2005) reported that a combination of preventive and curative application of the BCA had a better effect than just a curative application alone. It is likely that the BCA needs to establish itself in the agroecosystem first, and then a population threshold may need to be met before it will be effective at disease suppression or controlling a pathogen. The population dynamics and survival of BCAs in agroecosystems is a huge gap in the biocontrol research field. Future research therefore needs to investigate this aspect in agroecosystems. There are several disease predictions models of late blight in potatoes, many of which are widely used by agricultural advisers and farmers, to predict the best timing for synthetic fungicide applications based on local weather conditions and potato phenology (Cooke *et al.*, 2011), although they may also need to be adapted to local agroecosystems and climates e.g., (Lehsten *et al.*, 2017). The challenge for the future is to incorporate the biology and ecology of potential BCAs into such models so that they can be used more effectively in the field and crucially at the right time.

3.6. *Multifunctional weapons: combining multiple modes of action and creating synergistic effects*

Most of the BCAs discussed in this review display a combination of different modes of action, although many of the mechanistic studies focus on a detailed understanding of one of these, probably due to the notorious difficulties of elucidating modes of action in microbe–microbe interactions. Indeed, a combination of several modes of action is likely to ensure better phytopathogenic disease control (Köhl *et al.*, 2019). Resistance to BCAs or their metabolites has been reported in pathogens such as *Botrytis cinerea* (Ajouz *et al.*, 2011), which makes BCAs with several modes of action advantageous in terms of limiting the risk of resistance emergence among phytopathogens such as *P. infestans,* which is well known to adapt resistance to synthetic fungicides and to overcome resistance bred into commercial potato and tomato cultivars (Bardin *et al.*, 2015). Furthermore, targeting more than one stage of the lifecycle may also enhance the efficacy of a BCA.

Given the adaptability of *P. infestans* towards fungicides with only one mode of action, it is worth considering combining more than one BCA to combat late blight, or at least using a BCA with several different modes of action. Synergistic effects of combining two or more BCAs against late blight in potatoes and tomatoes have been reported (De Vrieze *et al.*, 2018; El-Naggar *et al.*, 2016; Kumar *et al.*, 2015; Lourenço Júnior *et al.*, 2006; Maksimov and Khairullin, 2016; Wharton *et al.*, 2012). Combinations of metabolites produced by BCAs can also be effective at disease control, particularly if these metabolites function through synergistic modes of action. For example, Fengycin B from *Bacillus pumilus* is directly toxic to *P. infestans,* whereas the surfactin metabolites produced by the same organism induce defence responses in potato, and the combination of these two metabolites is more effective at treating late blight in potato than either metabolite alone (Wang *et al.*, 2020a). Tripartite combinations of resistance inducers such as chitinosan, with BCAs and low doses of fungicides, should be considered in an integrated pest management (IPM) program, and have recently shown promising results in protecting potato from *P. infestans* (Shukla *et al.*, 2021). Combinations of fungicides, such as metalaxyl, and BCAs, such as *Trichoderma asperellum*, have shown effective disease control and allowed the intervals between fungicide applications to be prolonged in potato (Jackson *et al.*, 2020). However, whilst this might be a workable approach currently, moving away from combinations that include synthetic fungicides should be seen as a long term goal, that will allow IPM to become more environmentally sustainable.

In some studies, the combination of two BCAs was not effective in controlling late blight disease, even though each individual BCA was effective separately (Zegeye *et al.*, 2011). It is important to note that even though strategies based on combinations of two BCAs may be currently unrealistic in practice, given the high registration costs for BCAs, we need to better understand any potential added value of combining different BCAs or their metabolites to control diseases such as late blight. Such research may lead to more effective biological control strategies that may become more affordable in the future.

3.7. *The magic bullet? IPM solutions in practice*

Integrated pest management (IPM) is now recognised as the most sustainable pest management practice, in most cases, and is therefore now mandatory in all EU member states, being regulated through directives 2009/128 and 2019/782. It is a complex, knowledge-intensive management practice, that needs to be optimised for every crop and location. Potato lags behind many other crops in terms of reductions in the use of synthetic pesticides, (which are currently very effective against late blight) (Eriksson *et al.*, 2016) and in the uptake of IPM. For example, whilst potato typically occupies around 0.9% of the cultivated land in Sweden, at least 20% of the synthetic fungicide usage in Swedish agriculture is directed to protecting potatoes (Eriksson *et al.*, 2016). Thus, we urgently need more sustainable IPM practices for the management of late blight disease in potato.

Decades of potato and tomato resistance breeding have led to the commercial use of dozens of Resistance to *Phytophthora infestans* (Rpi) genes (Vleeshouwers *et al.*, 2011). However, little to no attention has been given to potential plant genetic components of biological control. Since most BCAs need to form close associations with plants in order to be effective at controlling phytopathogens, this is a hugely overlooked area with great potential to enhance the effectiveness of biological control. Plant growth promotion by *Trichoderma* species, is highly dependent on plant genetics (Schmidt *et al.*, 2020) and thus future breeding efforts in crops such as potato would benefit from approaches that include genetic compatibility with biological control agents. This should be considered a key component in the IPM of potatoes, as shown in Fig. 2.

In Fig. 2 we present an overview of an IPM strategy for potato, in which biological control is a central component. Progress is being made towards more integrated disease control approaches, for example, as discussed above, combinations of BCAs, resistance inducers, and if absolutely necessary, reduced doses of fungicides show promising results for the future (Shukla *et al.*, 2021). Such treatments are likely to be most effective in cultivars that display some level of resistance, as is the case with the use of the resistance inducer phosphite (Liljeroth *et al.*, 2016). However, as with resistance breeding and the use of synthetic fungicides, BCAs are likely to have a pest load beyond which the pathogen develops resistance and thus, IPM strategies should be designed with evolutionary principles in mind, to ensure sustainability (Karlsson Green *et al.*, 2020). Using genetic information from wild resources for inverse breeding – keeping in mind various evolutionary factors (Egan *et al.*, 2018; Thormann *et al.*, 2014), maintaining intermediate levels of both tolerance and defence in plants (Fornoni *et al.*, 2004), increasing spatial (Yang *et al.*, 2019) and temporal (Mariotte *et al.*, 2018) genetic diversity, manipulating the off-season survival of the pathogen in the agroecosystem (Vetukuri *et al.*, 2020) and optimising plant health and the resilience of BCAs within the plant microbiome are some tactics that, when combined, can help reduce the risk of oomycetes developing resistance to IPM strategies such as biological control. For instance, very little is known about the longevity and survival of BCAs in the field, and more importantly about the time for a BCA to establish in the field. Nemec (1997) evaluated the longevity, survival and compatibility of *Bacillus subtilis*, *Trichoderma harzianum*, *Streptomyces griseoviridis*, and experimental single isolates of *Serratia plymuthica*, a *Pseudomonas flouorescens* parent, and its lacZY mutant, with the mycorrhizal fungus *Glomus intraradices* in a commercial planting mix. The study showed that the number of *Trichoderma* isolates increased slightly within 2 weeks after application and were stabilized through to the end of the test, around 8 weeks. In this study, *Bacillus* and *Trichoderma* species were the microorganisms with higher survival rates in a mix for potential use as BCAs in tomato. There are not many IPM strategies that include an evolutionary approach. It is, therefore, crucial to develop novel IPM strategies, that also fit the *P. infestans* pathosystem.

3.8. *Conclusions*

Large-scale *in vitro* screening approaches have had some notable successes in the identification of BCAs that reduce late blight disease severity *in agro* (Caulier *et al.*, 2018), however these successes are often down to the fact that large numbers of isolates have been screened to find a single successful BCA, or that the microbes tested were identified as potato or tomato endophytes initially. Whilst *in vitro* approaches are very useful for the identification of the modes of action of BCAs, new studies should focus on testing BCAs *in agro* both in terms of disease control as well as in terms of environmental survival in the agroecosytem. A better understanding of the interactions between BCAs and the soil microbiome will provide valuable ecological risk data and allow better formulations of existing BCAs. Furthermore microbiome studies, which are just in their infancy in potato, have the potential to allow us to identify potentially new BCAs that are adapted to the potato rhizosphere or live endophytically in close association with potato, and are crucially already adapted to the correct agroecological environment. Screening thousands of new microbes in the same manner as Caulier *et al.* (2018) whilst admirable is not always practical. Rather, we propose that such microbes should be screened for their production of secondary metabolites and CWDEs as well as their evolutionary potential in the agroecosystem. This is likely to aid in the identification of new BCAs with traits already adapted to the same environment as the pathogen. Further utilising these or existing well characterised BCAs as cell factories to bioengineer effective formulations of secondary metabolites as biopesticides is also a promising new direction in the hunt for sustainable control of late blight disease. Finally, practitioners should be encouraged not to simply replace their synthetic pesticide sprays with BCAs, but to utilise BCAs preventatively as soil amendments before or at planting, to better allow the establishment of healthy rhizosphere soil, in a similar manner to the use of pro and pre-biotics to support human health.

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Declaration of competing interest

This manuscript has not been published previously, and is not under consideration for publication elsewhere and we have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbr.2021.11.003.

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Dania Tabet is originally from Lebanon where she completed her bachelor's degree in Agricultural engineering specialization in plant protection at The Lebanese University, faculty of agriculture. She completed her her master's degree in Integrated Pest Management of Mediterranean fruit tree crops (IPM) from the Mediterranean Agronomic institute of Bari (CHIAM-Bari-Italy). Her master thesis focused on soil borne citrus and olive pathogens in nurseries. Currently she is pursuing her doctoral studies at Tuscia University in Italy under the supervision of Anna-Maria Vettraino. Dania hopes to identify new Eco-friendly control strategies against *Phytophthora* species and is investigating various species as biocontrol agents.

Murilo Sandroni has a background in Agricultural Engineering which he studied in his home country, at the University of São Paulo, Brazil. Murilo undertook a professional science master degree in Agricultural production at the University of Illinois Champaign–Urbana, USA followed by a double masters through the Erasmus Mundus program from the Polytechnic University of Valencia, Spain and the Georg-August-University of Göttingen, Germany, specialising in Plant Health in Sustainable Cropping Systems. Murilo is a PhD student at the Department of Plant Protection Biology, SLU, under the supervision of Erik Alexandersson and Laura Grenville-Briggs. Murilo is studying alternative methods of resistance to *Phytophthora infestans* in potato, by investigating the effects of Plant Resistance Inducers (PRIs) as low-risk alternatives to synthetic pesticides.

Clara Benavent-Celma has a background in Forestry Engineering, which she studied at the Polytechnic University of Valencia, Spain. She is studying for her PhD at the University of Aberdeen under the supervision of Steve Woodward and Pieter van West. Clara is interested in the study of alien invasive pest and pathogens, their detection, the damage they cause, how they are spread globally and the mitigation methods that can be used to reduce their impacts on natural ecosystems and is thus studying the biocontrol properties of *Pythium oligandrum* for the control of Phytophthora species in nurseries and the plants for planting pathway.

Jenifer Seematti completed a Bachelor of Technology in Genetic Engineering at SRM University, India. She completed her BSc thesis on kidney toxicology in mouse models at Harvard Medical School, USA. Following this, she completed an MSc in Plant Biotechnology at Wageningen University and Research, Netherlands. She is currently a PhD student at in Laura Grenville-Briggs' lab at the Department of Plant Protection Biology, at SLU, Alnarp. Her PhD project focus on functional characterization and population genetics of *Phytophthora infestans* effectors. She hopes to identify effectors to use in resistance breeding and biocontrol against potato late blight.

Christian Benjamin Andersen graduated from the University of Copenhagen with a master's degree in Agronomy before joining SLU as a PhD fellow. Christian is pursuing his PhD studies in Laura Grenville-Briggs' lab at the Department of Plant Protection Biology, SLU. He researches oomycetes for biological control of various plant pathogens and the effector biology of the mycoparasitic oomycete *Pythium oligandrum*. He also aims to improve the use of *P. oligandrum* as a biocontrol agent. Through his investigations into the ecological risk of using conventional pesticides and biological control agents in commercial agriculture he hopes to benefit both producers and society alike.

Laura Grenville-Briggs was educated at the Universities of Bath and Birmingham (PhD, 2003). After a post-doctoral period at the University of Aberdeen, UK, and a Marie-Curie Intra-European Fellowship at the Royal Institute of Technology, Stockholm, Laura joined the Swedish University of Agricultural Sciences (SLU) as an independent researcher in 2014 and was appointed Professor of Integrated Plant Protection in 2018. Her research group focuses on the molecular and genetic determinants of disease in oomycete and fungal phytopathogens and their interactions with microbial biocontrol agents. The goal is to develop more durable, environmentally sustainable integrated pest management strategies for plant diseases.