Biological control of plant diseases – what has been achieved and what is the direction?

Article

Accepted Version

Running head: COLLINGE et al.

Review

Biological control of plant diseases – what has been achieved and what is the direction?

David B. Collinge[1], Dan Funck Jensen[2], Mojgan Rabiey[3], Sabrina Sarrocco[4], Michael W. Shaw[5], Richard Shaw[6]

[1]*University of Copenhagen - Plant & Environmental Sciences, Thorvaldsensvej 40 , Frederiksberg C 1871, Denmark*

[2]*SLU - Forest Mycology and Plant Pathology, Uppsala, Sweden*

[3]*University of Birmingham – Biosciences, Birmingham, UK*

[4]*University of Pisa - Dipartimento di Coltivazione e Difesa delle Specie Legnose, sezione patologia vegetale, Via del Borghetto 80, Pisa I56124, Italy*

[5]*University of Reading, School of Agriculture, Policy and Development, Whiteknights , Reading, RG6 6UR, UK*

[6]*CABI, Wallingford, UK*

Correspondence

David B. Collinge; email: dbc@plen.ku.dk

The global sustainability agenda is increasing the demand for reduction in inputs into agricultural production whilst maintaining profitable yield of quality products. Plant diseases are a major constraint for both yield and product quality, but often tools for their control are ineffective or lacking. Biological control using antagonistic microorganisms has long been a subject of research which has resulted in a wide range of products that are now available and marketed in specific territories around the world. These preparations are often niche products with narrow uses. The research effort is intense both to develop new biological control agents (BCAs) and to obtain knowledge of the mechanisms underlying biological disease control. The prospects for biological control are promising. As a minimum, BCAs supplement other sustainable disease management practices such as disease resistance and presents opportunities for controlling diseases for which other approaches are ineffective or unavailable. We can realistically expect an increasing usage of BCAs to control crop diseases in ways, which will benefit the environment. This review paper arose from a webinar held by BSPP as part of the International Year of Plant Heath (IYPH2020). Many of the 300 participants posed or discussed interesting questions. This review is based on that input and the panel members at the webinar are all included as co-authors in this review.

Keywords

plant diseases, plant pathology, virus

1 Introduction

The green agenda, specifically the need to focus on sustainable use of the resources available on our planet, is receiving increasing attention. The discipline of Plant Pathology can contribute to this agenda by improving agricultural efficiency, both in terms of increased yield and reduced environmental impact, specifically by reducing the estimated 20%–30% losses caused by pests and diseases (Oerke, 2006, Savary et al., 2019) and the side-effects of disease and pest control actions. Both can be achieved by reducing inputs per unit of production (e.g., watering, spraying pesticides and applying inorganic fertilizers) and reducing food and fodder spoilage after harvest. Disease resistance is also an important means of disease management but effective resistance is often not available, whether introduced by conventional means (plant breeding) or biotechnologically by genetic engineering including NGT – new genomic technologies (Collinge & Sarrocco, 2021).

Biological control (BC) is receiving increasing attention as an alternative means of disease control, both pre- and postharvest, especially where disease resistance or chemical control are not available. This review was motivated by a webinar held 21 September 2020 as part of the British Society for Plant Pathology's (BSPP) contribution to plant health week and the UN-initiative "International Year of Plant Health 2020" (IYPH2020). The authors were in the panel and were inspired by enthusiastic participants from around the globe – see BSPP News #93 (2021). The recording is available via https://www.bspp.org.uk/conferences/webinar-biocontrol/. Many interesting issues were brought up by the participants, who represented undergraduate and graduate students, researchers, practitioners and industry as well as others challenged by or fascinated with plant diseases. We discuss many of the points raised in discussion.

2 What is biological control?

For plant diseases, biological control is most usually defined as direct or indirect inhibition of a disease, or the pathogen causing the disease, by another organism (antagonist) or group of organisms (Cook & Baker, 1983). The beneficial organism is termed the biological control agent (BCA) (Jensen et al., 2016) (Tronsmo et al., 2020). A broader definition also includes specialized metabolites, isolated, for example, from interactions or plant extracts that can be useful for controlling diseases. These include substances with signalling, antibiotic or attractant activities (e.g., pheromones), and are often termed biopesticides (Roberts & Taylor, 2016). However, we recommend that the misleading term biopesticides is avoided and the new term bioprotectants is used as proposed by Stenberg et al. (2021). Thus, the term bioprotection should replace this wider use for biological control mentioned above and then include both where non-living extracts and natural products are the agents used and the narrow definition of biological control so the term biological control be reserved for situations where a living BCA is applied (Stenberg et al., 2021).

Classical BCAs are defined as natural enemies that self-propagate and establish in the introduced environment to suppress pest populations. Augmentative BCAs are not expected to establish and are defined as mass produced natural enemies that are periodically introduced into a specific environment to suppress pest—and pathogen—populations. Augmentative BCAs can be further subdivided into seasonal inoculative agents, which can reproduce and persist throughout the growing season, inundative agents, which cannot reproduce and must be frequently reapplied throughout the growing season (Stenberg et al., 2021).

Biological control is seen to offer several opportunities for improved disease control methods, especially where conventional approaches are limited or compromised. Alongside the use of disease resistant cultivars, BC is seen to have an important role in integrated pest management (IPM) strategies aiming at reducing the use of chemical pesticides. A BCA is an organism or collection of organisms rather than a chemical per se. It is likely to be more specific in effect than most commercialized agrochemicals and less likely to leave potentially harmful residues in the environment. A living organism may be able to penetrate the diseased plant or affect the target pathogen in a way that a chemical cannot. In addition, in some situations, the risk of the evolution of pathogens resistant to a chemical pesticide is greatly reduced by applying a BCA. Biological control is also publicly perceived as natural and therefore less environmentally harmful than chemical control; in many cases this is true, because no completely novel molecule is being introduced to the environment. Because of these favourable perceptions, many forms of biological control are accepted for use also in organic cultivation. It is also claimed that—again only in some cases—a BCA may be cheaper than a pesticide.

3 History and origin of BCAs

From 1932 on, Weindling published several papers (Weindling, 1932, 1934, 1941) demonstrating that a *Trichoderma* isolate was able to reduce damage to citrus seedlings caused by *Rhizoctonia solani* and describing some of the possible mechanisms of action. *Trichoderma* spp. are today probably the most widely used organisms in BCAs for plant disease control worldwide (see below and Table 1) (Lorito et al., 2010). In another example, inoculation of freshly cut pine tree stumps with the commercially available *Phlebiopsis gigantea* has been used

as a biocontrol against *Heterobasidion annosum* in pine plantations in parts of Europe since the 1960s (Pratt, 1999), following research by Rishbeth (1963). These and other seminal projects—for example, influential work on take-all of wheat from 1970s and 1980s (Cook, 2007), and, from the 1970s, biological control of crown gall in stone fruit trees caused by *Agrobacterium tumefaciens* with the BCA *Agrobacterium radiobacter* K84 (syn. *Rhizobium rhizogenes*) (Kerr, 2016), paved the way to a large body of research aimed to demonstrate that beneficial microorganisms could be used to control plant pathogens. During the 1980s, biological control was seen not only as a strategy but also as a philosophy to reduce crop loss due to plant diseases. In 1981 Papavizas highlighted that BC could find its roots in earlier farming practices including rotation of crops, burial of infected crop residues and fertilization with organic manures, all allowing time and opportunity for biological destruction of pathogens (Papavizas, 1981). However, in 1974 Baker and Cook had already introduced the term "pathogen-suppressive soils" to describe examples of natural, apparently biological, control of soilborne plant pathogens where a precise mechanism of control was still uncertain (Baker & Cook, 1974). These suppressive soils were initially recognized because of the absence of a disease despite an environment apparently favourable for its occurrence and the presence of a susceptible host and virulent pathogens. Suppressiveness to specific pathogens was explained as the result of a natural "microbiological makeup" of the soil, or of management practices encouraging antagonists, which can control disease (Papavizas, 1981). For key contributions over the last 30–40 years to understanding the biology of disease or pathogen suppressive soils, we should mention pioneering researchers like Claude Alabouvette, Dijon, France and from Washington State, USA, David Weller and Linda Thomashaw together with R. J. Cook cited above. A further step to transforming interesting research results into tools available for farmers was the appearance on

the market of additional crop protection products based on microorganisms. BCA products based on *Agrobacterium radiobacter* and *Plebiopsis gigantea* were mentioned above. Already in 1972, Jacques and Suoma Ricard founded the firm Binab^{R} in Sweden producing the *Trichoderma*-based BCA product Binab-T™ and were subsequently among the first to commercialize *Trichoderma*-based BCAs. Now in 2021 the firm has several products on the market based on *Trichoderma* spp. Several other BCA products from the mid-1980s and 1990s can be mentioned like Mycostop™, a Finnish product based on a strain of *Streptomyces griseoviridis*, Polygandrum™—a *Pythium oligandrum*-based product that was also sold in Europe (Veselý, 1989) and in the USA, GlioGard™ based on *Gliocladium virens* (syn. *Trichoderma virens*) (Lumsden et al., 1996). A more well-known example from the USA came later in the 1990s where G. E. Harman and two others cofounded TGT Inc., later BioWorks Inc., to commercialize an isolate of *Trichoderma* (T22) originating from the fusion of protoplasts of two different *Trichoderma* isolates (Harman, 2000). Since then, a number of other BCA products have been developed and commercialized worldwide (Table 1). These include both bacteria (especially *Pseudomonas* and *Bacillus* strains in addition to the *Agrobacterium radiobacter* strains) and fungi (especially *Trichoderma* spp. but, for example, *Clonostachys rosea* is also used worldwide).

BCAs identified so far include bacteria, fungi, oomycetes and viruses (Table 1). Successful BCAs have been isolated from soil, especially disease suppressive soils as was the case for the parent strains of the BCA T22 mentioned above or isolated in association with plants, for example, phyllosphere or rhizosphere—or from within plants, the endosphere. Many of the organisms identified occur naturally in several of these niches. In essence, there is a

151 continuum from soil to rhizosphere (root surface) to endosphere (inside the plant) and
152 phyllosphere (above-ground plant surface) (Jørgensen et al., 2020).

153

154 4 How to find a new BCA

155 Two fundamentally different approaches are commonly used in attempts to identify novel BCAs
156 (Figure 1). These are, first approach, the indirect screening of microbial libraries for antagonistic
157 properties in planta or in silico and, second procedure, isolating organisms from the habitat
158 where the product would be used and then screening directly for activity in planta (Collinge et
159 al., 2019; Köhl et al., 2011; Knudsen et al., 1997; Teperi et al., 1998). The in vitro approach has
160 been used as a high-throughput approach to screen existing collections of strains for activities
161 against one or more pathogens. We do not know of documented examples of successful products
162 for plant protection from this approach. The direct screening approach is less suited to high
163 throughput but facilitates the identification of organisms where the mode of action involves plant
164 responses, for example, induced resistance or the ability of an organism to colonize and compete
165 in plant niches (e.g., rhizosphere, phyllosphere, endosphere or in wounded tissue). The advantage
166 of the in vitro approach is that many strains can be tested for the production of antimicrobial
167 metabolites and, for example, mycoparasitic (also termed hyperparasitic) activity. However, both
168 positive and negative results may be misleading as one cannot be sure that the mechanisms
169 would be active in the plant, nor, conversely, that useful mechanisms are not activated in vitro.
170 The latter has led in many cases to discarding promising BCAs based on in vitro screening
171 (Knudsen et al, 1997; Teperi et al 1998). There have been many disappointments but a few
172 promising BCAs (Whipps & Lumsden, 2001). The in planta first approach, in its extreme form,

involves testing potential BCAs under field conditions that has been a successful approach for some selecting isolates that now are commercialized (e.g., the product Cedomon; Table 1). In practice, it is, however, in most cases necessary to develop tests on plants in growth chamber or greenhouses (Knudsen et al., 1997), or even in a few examples on leaves (Latz et al., 2020) or wheat heads (Rojas et al., 2020a) (Figure 2). Although these are a compromise, they can simulate conditions, which are more comparable to the field. Also, these in planta tests can often be carried out throughout the year and thereby do not depend on a brief growth season. Thus, they can give a reasonable level of throughput to select promising candidates for extensive tests in production systems.

Recently, the availability of next-generation sequencing tools has allowed research on biocontrol agents to take a directly functional approach. In *Clonostachys rosea* and species of *Trichoderma*, for example, genomics and metabolomics are currently allowing the discovery and investigation of a vast repertoire of specialized metabolic pathways (Karlsson et al., 2015). Study of the roles these metabolites play in the environmental and biotic relations of these organisms may represent a new route to development of BCAs (Mukherjee et al., 2013; Vicente et al., 2020). However, genomic or metabolomics screens are necessarily restricted to looking for signatures derived from study of organisms known to have biocontrol activity. Such screens should therefore, if used, be following after an in planta selection of potential organisms and not as a stand-alone approach.

Useful organisms are not found only by targeted searches. For example, a *C. rosea* strain (IK726), originally found in the rhizosphere of a barley root, is effective against many diseases of diverse organs in a wide range of hosts ranging from brassicas to strawberry, oak and cereals (Jensen et al., 2007). Similarly, *Serendipita indica* (syn. *Piriformospora indica*), a plant growth-

promoting organism, was found in the root of a desert shrub, but has positive effects for protection against both abiotic stress and attack by certain pathogens in many plant species in very different environments (Cheng et al., 2020; Rabiey et al., 2015; Shrivastava & Varma, 2014). It is commercially available both for biological control and as a biofertilizer (Table 1). In both cases, several mechanisms of action may be operating. Another example is the isolate *Trichoderma gamsii* T6085, isolated from an uncultivated soil in Crimea but effective, when applied on spikes at anthesis, in reducing the incidence of Fusarium head blight on wheat. Like several examples quoted here, it also possesses several quite diverse modes of action, from mycoparasitism to induction of plant defence responses (Matarese et al., 2012; Sarrocco et al., 2013, 2020). Different pathogen lifestyles may necessitate different strategies for identifying and isolating appropriate BCAs. For example, biotrophic parasites of a fungal (or bacterial) pathogen would benefit from the development of methods for isolating and subsequently cultivating them on bait organisms. This is especially true for viruses as BCAs which can only live as parasites, for example, bacteriophage (Carstens et al., 2018, 2019; Sabri et al., 2021) on bacteria and mycoviruses and other biotrophic hyperparasites on fungi (Milgroom & Cortesi, 2004; Xie et al., 2011; Yu et al., 2013; Zhang et al., 2020) and Table 1. It can also be a challenge to isolate specialized organisms which may be slow growing or require a host to grow at all—but equally it may be difficult to exploit a slow-growing BCA.

5 Improved efficacy – a key to implementation?

One of the challenges of biological control is reliable efficacy. Biological control is often considered to be less reliable and efficient than chemical control or host resistance, probably

because exposure to the external environment is largely an uncontrollable variable. A counterargument is that some types of biological control (unlike some mechanisms of host resistance) may have an effect against multiple diseases, especially where induced resistance or resistance priming is an underlying mechanism. In addition, it has been shown that, for example, *C. rosea* can be a mycoparasite of diverse fungal plant pathogens such as *Fusarium graminearum* and *Botrytis cinerea* (Jensen et al., 2021). This seems to rely on the response of both general-purpose and specific gene expressions in *C. rosea* depending on which fungal species it parasitizes, indicating that the BCA can work through different modes in biocontrol interactions (Nygren et al., 2018).

Most of the successful BCAs are effective competitors in the harsh biotic environment of soil and in the plant holobiome (the combination of the plant and its associated microbiome), as they have evolved mechanisms for tolerating toxins from other organisms and are adapted to stressed conditions in those environments, including growth on roots, stems, leaves and flowers and in wounded tissue. Endophytes—defined as microorganisms colonizing the interior of plants (the endosphere) without causing disease (Jørgensen et al., 2020) (Figure 3)—are adapted to the ecological niche of the endosphere and are also partly protected from the external environment (and colonize the same niche as pathogens). It is therefore suggested that endophytes have the potential to be more consistent as BCAs than purely epiphytic organisms, especially those in the phyllosphere. However, this hypothesis is speculative, based on knowledge that many plant pathogens compete poorly, with an advantage only inside the plant. The hypothesis remains to be demonstrated experimentally for potential endophytic potential BCAs (Latz et al., 2018). One example is the use of endophytic fungi associated with the invasive weed Japanese knotweed (*Fallopia japonica*). Some endophytes can increase the effectiveness of the rust *Puccinia*

241 *polygoni-amphibii* var. *tovariae* as a potential control agent against of *F. japonica* (Kurose et al.,
242 2012). Another example concerns grass endophytes of the genus *Neotyphodium* and *Epichloë*
243 that can produce alkaloid mycotoxins (e.g., ergovaline) affecting ruminants (especially cattle and
244 sheep). However, some *Neotyphodium* and *Epichloë* endophyte isolates can provide a very high
245 level of protection of the host plant against insect pests (e.g., Argentinian weevil) or fungal
246 pathogens of grasses including *Rhizoctonia* spp., *Bipolaris sorokiniana*, and *Curvularia lunata*
247 (Panka et al., 2013b), *Sclerotinia homoeocarpa* (Clarke et al., 2006) and *Fusarium oxysporum*
248 (Reddy & Faeth, 2010). This appears to be mediated through priming of defences (Pańka et al.,
249 2013a).

250 Many endophytes only enter the apoplast, but may still have a control function there,
251 either directly inhibiting the pathogens or indirectly by inducing or priming defence responses in
252 the plant (Veloso et al., 2016). However, these organisms might also be adapted to function
253 outside the plant, as it is known for *Trichoderma* spp. and *Clonostachys* spp. As good root
254 colonizers, these fungi are also adapted to the harsh environment in the rhizosphere. That an
255 organism was originally isolated from the rhizosphere or endosphere thus does not mean that it
256 only colonizes as an endophyte or epiphyte or vice versa. Most endophytes will, however, be
257 specialized to some extent to survive inside a plant and would be predicted to compete poorly
258 with microbes outside the plant endosphere. That notwithstanding, there is a continuum in
259 lifestyle, and the same organism may behave as an endophyte, epiphyte or pathogen under
260 different environmental conditions (Jørgensen et al., 2020). This must of course be considered
261 already in the selection of potential BCAs to prevent accidental selection of plant pathogens.

262 Consortia, that is, mixtures of microorganisms, are receiving increasing attention as a
263 way of addressing multiple problems. Thus, the insect pathogen *Metarhizium brunneum* was

combined with the fungal BCA *Clonostachys rosea* and effects observed on both the pest and pathogen, though the efficacy was reduced compared to treatment with either separately (Keyser et al., 2016). It is tempting to assume that a mixture of BCAs will be more effective than a single agent. However, modelling suggests that—depending on exactly how the organisms compete and act—this may often be untrue (Xu & Jeger, 2013). Different associations can have opposite or antagonistic effects, thus the ability of *S. indica* to control *R. solani* or *F. oxysporum* infections depended on associated bacteria (del Barrio-Duque et al., 2019). It has also been difficult, except in a few cases, to demonstrate significant additional or synergistic biocontrol efficacy by combining different BCAs in consortia (Xu et al., 2011a, 2011b). A challenge is to ensure that the different agents can operate together under variable environmental conditions and do not have incompatible modes of action. For example, two BCAs acting mostly by bulk nutrient competition would be expected to counter each other's activity. Thus, the idea of forming complex consortia—"synthetic biomes" or "synthetic communities", abbreviated SynComs (Großkopf & Soyer, 2014)—consisting of several different microorganisms with biocontrol effects which could be used as mixtures does not seem to be the most promising route. It can be predicted that there will be selection within consortia to favour the best adapted to a particular environment and that the dominant consortia members will change following treatment in response to local environment. Nevertheless, a special case, where several products comprising bacteriophage consortia have been released for combating bacterial disease seems feasible (Table 1).

BCAs are an attractive component in management of postharvest disease, by application at harvest or shortly before. An example is Alfasafe and similar products for controlling aflatoxin contamination using nontoxigenic *Aspergillus flavus* strains to compete with the toxigenic forms

(Amaike & Keller, 2011; Bandyopadhyay et al., 2019). Consumer sensitivity over the use of artificially synthesized chemical application is greater for applications made postharvest than during crop growth; the environment is usually less variable or much less variable than in the field, and doses applied can be much more uniform, assisting the use of BCAs acting by resource competition or breakdown of mycotoxins produced by other microbial species. However, biological control using applications of BCAs postharvest is currently not allowed in the EU. Indeed, several products mentioned in Nunes (2012) for European use are no longer approved in the EU, namely, Candifruit™ (*Candida sake*, Sipcam-Inagra, Spain), Pantovital (*Pantoea agglomerans*, Biodurcal, Spain and Boni-Protect® (*Aureobasidium pullulans*, Bio-protect, Germany. Furthermore, Candifruit™ is considered inefficient (Carmona-Hernandez et al., 2019). In contrast, postharvest BCAs have been used for many years in the USA, for example, to protect soft fruit from postharvest decay before they reach the consumers. Postharvest BCA treatment of soft fruit for controlling *Penicillium* and *Aspergillus* species and other spoilage pathogens like *Botrytis cinerea* and *Rhizopus* spp. therefore seems to be an important way forward in the EU in view of its successful commercial use in the USA *Pseudomonas syringae* ESC-10 is commercialized by Bio-save 10LP in USA and marketed for several products for postharvest disease control. Examples include citrus fruit, pome fruits, cherries and potatoes to control various fungal pathogens postharvest (product information; Stockwell & Stack, 2007).

Product spoilage can in some cases also be avoided by BCA treatments before harvest, depending on the epidemiology of the pathogen–host association. Postharvest problems with mycotoxin production may be also addressed long before harvest to reduce the populations of producing organisms or the rate at which they produce toxins, and to increase the rate and extent that mycotoxins are degraded (Abdallah et al., 2018). For example, mycotoxin production by

ear-inoculated *Fusarium graminearum* and *F. culmorum* in wheat was greatly reduced in outdoor (but pot-grown) wheat inoculated with *Serendipita indica* at sowing (Rabiey & Shaw, 2016). This must be an indirect effect on host resistance, because the *S. indica* remained restricted to the roots. The doses of BCA culture used here were very large (equivalent to 60 g/m^2 or 600 kg/ha), but the effect is intriguing. There are interesting examples concerning beneficial fungi able to degrade mycotoxins: the ability of *Clonostrachys rosea* whose ability to detoxify the mycotoxin zearalenone (ZEA) through the enzyme zearalenone lactonohydrolase has been demonstrated (Kosawang et al., 2014) and there are promising results from the field where *C. rosea* has reduced the DON content in harvested wheat grain (authors' unpublished data). Similarly, the ability of some *Trichoderma* isolates to degrade mycotoxins has recently been studied. In the case of *T. aggressivum*, its zearalenone lactonohydrolase was expressed in *Escherichia coli* BL21 (DE3) and successfully purified (Chen et al., 2021).

Postharvest pathogens on soft fruit such as mycotoxin producing species of *Aspergillus* and *Penicillium* are not likely to be controlled efficiently preharvest even though it is often suggested that application of beneficial organisms preharvest can reduce mycotoxin accumulation postharvest (Sarrocco & Vannacci, 2018). There are exceptions. This is the case for beneficial yeasts such as *Aureobasidium pullulans* whose preharvest application on grape resulted in a reduction of ochratoxin A contamination by around 95% (Dimakopoulou et al., 2008). Another interesting example is *Kluyveromyces thermotolerans*, able to control the growth of *Aspergillus carbonarius* and *A. niger* in the field by up to 100% and to reduce mycotoxin accumulation by up to almost 80% (Ponsone et al., 2011).

6 Mechanisms – modes of action

There are four main modes of action underlying biological control of plant diseases (e.g. Jensen et al., (2017)): (a) exploitation competition for resources (oxygen, carbon, nitrogen, and other essential resources); (b) interference competition for space via antibiosis where the BCA inhibits the pathogen through effects of toxic secondary (specialized) metabolites; (c) hyperparasitism, where the antagonist acts as a predator and exploits the pathogen as a prey; (d) induced resistance—the indirect interaction of a BCA via induction of plant defence mechanisms against invading pathogens. A fifth mechanism that can contribute to disease control is plant growth stimulation by better nutrient absorption and/or by affecting plant hormone pathways, as demonstrated by, for example, various rhizosphere bacteria and fungi. A strongly growing plant may be better able to withstand a pathogen and a rapid establishment of seedlings in the field can lead to avoidance of damping-off diseases. However, some researchers would not consider this as biological control, as discussed earlier in this review (Stenberg et al., 2021).

A single BCA may exhibit a combination of these modes of action. The individual modes of action have different but not exclusive population dynamic consequences. It can be quite difficult to prove that a particular mechanism is operating in planta even though it can be operating in vitro (Latz et al., 2018). More than one of these mechanisms can contribute to a concerted action in a particular case and the importance of a specific mechanism used can vary from case to case, even using the same organism, for example, species of *Trichoderma* and *Clonostachys* may act as hyperparasites, metabolite producers, competitors and/or modulators of plant defence responses (Benítez et al., 2004; Harman, 2006; Jensen et al., 2021; Mukherjee et al., 2013). Exploitation competition can be independent of the pathogen population size, simply reflecting efficient local resource capture. Competition through more efficient resource use does

not rely on direct interaction as the BCA has taken over resources and space so the pathogen cannot benefit from the resource. Being the first to colonize new resources is another important way of exploitation competition that can deprive a pathogen of resources needed, especially in the critical early stages of colonization. In addition, the ability of beneficial organisms to colonize a substrate that is not preferred by the targeted pathogens could improve competitiveness of the biocontrol agent against the biocontrol agent in the targeted pathogen community (Lasinio et al., 2021). Alternatively, interference competition through antibiosis, depending on how close the organisms need to be to interact, may allow the BCA to monopolize the habitat (Sarrocco et al., 2019). Hyperparasitism requires that the BCA occurs and is metabolically active spatially close to the target pathogen (normally in the niche where the pathogen would infect, or which is occupied by fruiting bodies or resting structures of pathogens that are parasitized by a BCA).

The question was raised in the webinar whether pathogens could evolve to be resistant to BCAs, as frequently occurs with repeated use of pesticides with specific modes of action. Over more than four decades of using biological control, resistance in the target bacterial and fungal pathogens has yet to be demonstrated to be a problem. The direct use of metabolites and extracts—leading to high pathogen exposure (and not included in the definition of biocontrol discussed earlier)—is much more risky, and seems similar to the use of chemical pesticides for resistance development. In the case of bacteriophages, it is known that bacteria can adapt rapidly to bacteriophages and are expected to overcome single strains. Products are therefore based on cocktails of bacteriophage to reduce this problem (see below).

Although resistance has not been considered a serious problem for most other practical uses of biocontrol we will next discuss the issue and its relation to mode of action. It is not easy

to see how a pathogen could evolve resistance to exploitation competition in nature. However, as for chemical pesticides, resistance towards BCA metabolites in pathogen populations is a theoretical possibility if a substantial proportion of a pathogen population is regularly exposed to a metabolite, leading to high selection pressure, and resistant phenotypes could in principle arise. Some BCAs may mainly rely on antibiosis due to production of one or a few specific toxic metabolites and resistant phenotypes could be possible, perhaps with a consequent risk of field resistance. An example of one stage in this process has been observed in take-all decline of wheat in suppressive soils induced by monoculture. Isolates of the pathogen involved (*Gaeumannomyces tritici*) showed variation in sensitivity to two metabolites produced by strains of *Pseudomonas fluorescens* that were claimed to be important for disease suppressiveness (Mazzola et al., 1995). Such variation in different traits is to be expected but, based on the studies by Mazzola et al. (1995), there is no clear evidence that the population as a whole has become less sensitive to the two metabolites tested (phenazine-1-carboxylic acid or 2,4-diacetylphloroglucinol) despite heavy exposure to these metabolites. Furthermore, no evidence of resistance to 2,4-diacetylphloroglucinol was found in of *G. tritici* populations from Washington State, USA (Kwak et al., 2009).

In general, pathogenic organisms can be expected to vary in traits allowing them to thrive in variable but competitive environments (Dubey et al., 2014; Karlsson et al., 2015). Because resistance to a metabolite can be conferred by changes in the target site, detoxification, excretion (efflux) or general metabolic adjustments, intensive use of a BCA acting via antibiosis and based on one or a few specific toxic compounds could lead to the evolution of resistant pathogens. The selection pressure is increased if pathogen populations experience heavy (long term and/or highly effective single dose) exposure to the metabolite. For this reason, vulnerability to

resistance should be considered on a case-by-case basis when creating strategies for biocontrol use. There is a strong argument for the development of many different BCAs for a given problem, to avoid exposure of large proportions of the pathogen population to the same selection pressure.

A special case where a strategy for avoiding resistance in pathogen populations has been addressed is the biocontrol of crown gall caused by the bacteria *Agrobacterium tumefaciens* by the BCA *Agrobacterium radiobacter* (syn. *Rhizobium rhizogenes*) strain K84 that produces the toxin agrocin responsible for the antibiosis (reviewed by Penyalver et al., 2000). Here the BCA harbours a plasmid that encodes resistance to its own agrocin toxin and at the same time encodes mobility of this plasmid with resistance to other *Agrobacterium* strains. In this case, the concern was that the plasmid might be transferred to the plant-pathogenic *Agrobacterium* bacterial strains making them resistant to agrocin. As this was demonstrated to happen both in field and in laboratory experiments and information accumulated that it also might be happening under commercial use, a gene modification of the BCA was created in which the plasmid mobility trait was deleted—strain K1026. This strain K1026 has been used commercially in Australia and in the USA although biocontrol with commercial use of the wild-type strain K84 still provides effective biocontrol in many crops worldwide, after almost 50 years of commercial use (Kerr & Bullard, 2020). Strict legislation for regulating BCAs has until now prevented the use of *R. rhizogenes* for biocontrol in the EU, but both the mutant K1026 and the wild-type K84 are approved now in many other countries (Kerr & Bullard, 2020).

A specific (biotrophic) hyperparasite requires a pre-existing host population to parasitize as well as a living host for activity and growth, so they will be effective in the short term only if applied inundatively. An exception could be if a biotrophic hyperparasite could function

effectively and survive in the longer term in an environmental reservoir. Unfortunately, such biotrophic hyperparasites will not usually compete well in the absence of a host (Bennett et al., 2003). Nonetheless, there are some examples of commercialized biotrophic hyperparasites used for biocontrol such as *Ampelomyces quisqualis* (Figure 4), used against powdery mildew (Karlsson et al., 2018) and *Coniothyrium minitans*, a parasite of several sclerotia-forming plant pathogens (Whipps et al., 2008). A special example of a potential BCA is the hyperbiotrophic fungus *Pseudozyma flocculosa* (a yeast) that parasitizes powdery mildew and in this way obtains access to resources from the leaf infected by the mildew fungus. *P. flocculosa* is dependent on living host–pathogen combination and thus needs to find a new host mildew as a mildew colony dies (Laur et al., 2017). Interestingly, *P. flocculosa* also produces an antifungal glycolipid, flocculosin suspected to have a role in the interaction. However, A CRISPR-Cas9 mutant impaired in the biosynthesis of flocculosin was apparently unaffected in its ability to antagonize powdery mildew (Santhanam et al., 2021). This is an effective lifecycle as powdery mildews are polycyclic, the organism attacks multiple species of powdery mildew, and new infections are found throughout the growing season in many crops, continually offering new living hosts for the BCA.

Whether the use of specialized hyperparasitic BCAs would be risky in an inundative strategy should be considered case by case. It is possible to set up an effective strategy for their use provided knowledge of the target pathogens and their disease cycles, the environmental conditions the biology and ecology of the BCA allow the prediction of the right timing and placement of the BCA in the niches where it is to act. *Ampelomyces quisqualis*, for example, is effective against powdery mildew on cucumber (Sundheim, 1982) but less effective in controlling powdery mildew on grapevine caused by *Uncinula necator* as it mainly parasitizes

447 the fruiting bodies (chasmothecia) late in the season (Falk et al., 1995a, 1995b). Parasitism of the
448 conidial stage throughout the growing season is highly dependent on humidity, which is not a
449 requirement for conidial production by the pathogen. Therefore, the BCA is less efficient in
450 periods with low rainfall/humidity. However, as Falk et al. (1995a, 1995b) point out, parasitism
451 of chasmothecia might have an important role in integrated disease control by reducing primary
452 inoculum for the following year.

453 Although not a crop example, the rust hyperparasite *Sphaerellopsis filum* appears to have
454 specific genotypes which are adapted to attack only some genotypes of individual species of
455 grass-infecting rusts (Kajamuhan et al., 2015) a phenomenon that also might be relevant with
456 other biotrophic hyperparasites. Viruses can also be considered as obligate hyperparasites with
457 more or less specific host ranges (see below).

458 However, most BCAs that work via hyperparasitism are necrotrophic parasitic fungi that
459 compete well and survive without a living host pathogen. Examples are species of *Trichoderma*
460 and *Clonostachys* that can work as mycoparasites as part of their lifestyle but also grow and
461 multiply via other ways of life, as addressed in more detail in Karlsson et al. (2018).
462 Necrotrophic hyperparasites are considered more aggressive as BCAs than the more specialized
463 hyperparasites and are more competitive, for example, in the rhizosphere and in root
464 colonization.

465 Induced resistance is a well-studied phenomenon in the laboratory and there are good
466 laboratory examples of this as a mode of action. However, induced resistance will be ineffective
467 against existing high population densities of pathogen. Interaction with target pathogens via
468 induced resistance does not require close proximity of the target and the BCA. For example, root

application of *Serendipita indica* can stimulate both plant growth and induced resistance in the shoot (Ntana et al., 2022; Rabiey et al., 2015). Volatile specialized metabolites can act as signals between plant parts and at least in principle between neighbouring plants (Farag et al., 2013). Moreover, application of BCAs can induce resistance in the progeny of treated plants (Medeiros et al., 2017), a phenomenon termed "transgenerational systemic acquired resistance" (Luna et al., 2012). Several phytohormones have been shown to be involved in the induced resistance induced by *S. indica* (Hilbert et al., 2012; Jacobs et al., 2011; Khatabi et al., 2012). Hormones have complex and sometimes antagonistic effects, which can influence both abiotic and biotic stress modifying cellular physiology to respond and adapt to the stress. For pathogens, the activated defence responses provide induced resistance (PAMP-induced immunity; Ray et al., 2018).

Understanding the evolutionary response to the use of host resistance inducers raises the question of why plants do not trigger these defences constitutively. The obvious answer is that induced resistance needs energy or involves intrinsic damage such as cell death, and that is a fitness cost. This means that the induced defences are regulated (for example by transcriptional modulators such as NPR1) and not deployed unless needed. In this case, therefore, the use of a BCA to induce resistance in the absence of a substantial subsequent pathogen attack should lead to loss of yield. This would be a serious set-back in developing BCAs as part of an integrated disease management toolbox. Negative effects of application in the absence of pathogens are, however, hard to demonstrate. Experiments involving transgenic plants where constitutive expression of *R* genes (Oldroyd & Staskawicz, 1998) and regulators such as *Npr*1 (Backer et al., 2019; Silva et al., 2018) were used can result in enhanced induced resistance with demonstrable fitness costs (Collinge et al., 2010). One of the great challenges for the genetically modified organism (GMO) approach in recent decades has been the identification of appropriate

promotors for driving the expression of such genes. The use of tissue-specific promotors can mitigate the negative effects of inappropriate expression (Tripathi et al., 2016).

The effect of *S. indica* (and some other agents) has been suggested to be first and foremost growth promotion (Gill et al., 2016) allied to effects such as drought tolerance. In that case, nonetheless, the question remains of what prevents evolution of constitutive expression of the growth promoting traits. Apparently, defences can be, if not activated, primed, with effects on growth and yield which are too slight to measure (Conrath et al., 2006). There are two hypotheses which could explain why the defences remain facultative: (a) the costs are expressed in specific circumstances, not usually encountered in experimental or field-crop settings; (b) less probably, perhaps it is the case that in natural settings, with a diverse and microbe rich soil, priming always happens, so there is no selective advantage or disadvantage in facultative control —it is just a normal stage in development. If (a) is correct, there is the important practical conclusion that we should be looking very hard for side-effects of these priming organisms before they are too widely deployed on crops.

7 Environmental manipulation and suppressive soils

Environmental manipulation is often used as an approach to achieve biological control against insect pests, such as the promotion of biodiverse crop margins to encourage predators to provide biological pest management under the title of Conservation Biological Control. This is used rather less against pathogens. Reduction in attack by pathogens can be achieved in principle by manipulating the habitat to encourage one or more BCA in the soil, or perhaps by using adjacent vegetation to encourage the right individual microorganisms or microbiomes. The use of

elemental sulphur to lower the local pH and discourage *Streptomyces scabies*, causing scab on potato is perhaps an example (Vlitos & Hooker, 1951). Another example is watering potato plants during tuber formation to stimulate colonization of the new lenticels with antagonistic bacteria (Cook & Papendick, 1972; Ryan & Kinkel, 1997). Similarly, damage from eyespot of wheat in the later season caused by *Oculimacula* spp. was—counterintuitively—reduced by ceasing straw burning (Jalaluddin & Jenkyn, 1996). Compost and especially "compost tea" may provide a source of BCAs or alter the nutritional environment to favour BCAs which are responsible for the activity of the compost tea (St Martin, 2015). Biochar is hypothesized to provide increased surface area suitable for microbial growth and may interact desirably with compost teas (Edenborn et al., 2018). These approaches are a ripe subject for study, though reliability has been a major problem. Metagenomic and community metabolism methods may improve this (Edenborn et al., 2018). Part of the effect of good cultural practices—though perhaps unconsciously—is likely to be the encouragement of microbial communities that either prime or induce plant defences, or act as direct BCAs.

Microbiota can increase natural soil suppressiveness against soilborne pathogens particularly when intensive cropping systems (with high inputs of synthetic chemicals, low soil organic matter accumulation, little humification and frequent soil tillage) are the primary reasons for soil depletion (Cook, 1992). Soil microbiota associated with biocontrol can be a key factor in the beneficial influence of agronomic practices on plant health (French et al., 2021). Next-generation sequencing often offers a deeper characterization of the soil microbial community during microbiome manipulation. This may allow more mechanistic understanding of what is happening and the effect on crops in terms of soil suppressiveness and so help to limit inconsistencies, drawbacks and failures related to soil microbiota disturbance (De Corato, 2020).

More generally, the 'omics sciences——through a combination of metagenomics, meta-transcriptomics, meta-proteomics and metabolomics approaches——should help to understand the whole microbial activities and the potential of the plant-associated microbiota to suppress plant disease (De Corato, 2020; Schlatter et al., 2017).

8 Host genotype and plant breeding

Another exciting research area related to biocontrol is the interaction between plant host genotype and microbiome. Just as disease resistance is inherited, it is predictable that the microbiome of a plant, which is relevant to biological control activity, is affected by genotype. We can predict that deepening knowledge of how agronomically important traits relate to plant function will increasingly contribute to our ability to predict the effects of genotype variation on responses to BCAs. For example, the effect of *S. indica* on wheat response to drought stress is strong but variable, with quantitative trait loci with large effects apparent (Amer, 2020). It seems very likely that genotype would also affect the control of *Fusarium* spp. on the crown and ears shown in previous work (Rabiey et al., 2015; Rabiey & Shaw, 2016). While this would complicate management, diverse varietal susceptibility to multiple diseases is routinely part of farm decision making.

Another factor that plant breeders should consider is the genotype of the host and native microbiome. Some *Trichoderma* isolates, by endophytically colonizing host roots and shoots, establish a molecular dialogue resulting in desirable effects on plants (Macías-Rodríguez et al., 2018; Ramírez-Valdespino et al., 2019). This phenomenon was first described in 1952, when Mostafa and Gayed (1952) reported that *Trichoderma* improves fresh and dry weight in cotton

559 plants. More than 20 years later, exudates from lettuce were reported to have a beneficial effect
560 on germination of *Trichoderma viride* conidia, indicating that fungus and plants obtain mutual
561 benefits (Catskă et al., 1975). What was not known, and indeed drove researchers to more basic
562 studies, was that the beneficial effects of *Trichoderma* application depend on the plant genotype.
563 This concept has been proven in the interaction between *T. harzianum* T22 and maize (Harman,
564 2006) and between *Trichoderma* and tomato (Tuccci et al., 2011), where the beneficial effects of
565 *Trichoderma* are shown to be influenced by the plant genotype. However, the influence of the
566 microbiome must be studied on a case-by-case basis; a recent study on wheat looked at the
567 endomycobiome (i.e., fungal endophytic microbiome) of wheat but could find no relation to
568 resistance to *Zymoseptoria tritici* (Latz et al., 2021). In contrast, Mahoney et al. (2017) observed
569 that wheat cultivars may consistently alter the rhizospheric bacterial operational taxonomic units
570 (OTUs) thus providing beneficial services to the host. Plant genotype, including hosts already
571 affected by a disease, seems to play a crucial role in the recruitment of rhizosphere bacterial
572 microbiota, at least in controlled environment, an approach suggesting the need for further
573 investigation in soilborne plant disease suppression (Dilla-Ermita et al., 2021; Yin et al., 2021).

574

575 9 Risk assessment

576 Just because something is “natural” does not mean that it is “safe”. For approval, biological
577 control agents have to be assessed for potential harmful activities to farmers and consumers, and
578 for negative effects on the environment and other crops. Several categories of risk need to be
579 considered before a BCA (or any other novel product) can be considered reasonably safe for
580 possible commercialization or recommendation (Ehlers, 2011; Sundh & Eilenberg, 2021).

Screening and isolation of new organisms concentrate on looking for promising organisms before worrying what they are. However, already in an early stage of serious screening programmes it is necessary to identify the organisms that are being selected as potential BCAs. This is to avoid selection of plant pathogens, human and/or farm animal pathogens or mycotoxin-producing strains. Aspects to consider when starting a screening programme are discussed by Köhl et al. (2011).

A few examples of potential BCAs, when finally identified, have turned out to be potential human pathogens. For example, a bacterial strain which had good activity against *Didymella bryoniae*, was isolated from watermelon roots. It turned out to be the human pathogen *Pseudomonas aeruginosa* (Nga et al., 2010). The *Burkholderia cepacia* complex, defined by Eberl and Vandamme (2016) as "good and bad guys", includes several BCAs of plant diseases and actively exploited in bioremediation. However, because the *B*. *cepacia* complex also contains strains described as plant pathogens or opportunistic pathogens of humans affected by cystic fibrosis, the U.S. Environmental Protection Agency reassessed the risk of several isolates already registered by for biological control (Parke et al., 2001). Another risk is, as mentioned above, the production of harmful metabolites or even mycotoxins by a successful BCA. The greatly reduced costs and improved efficiencies in genomic sequencing over the last decade provide excellent opportunities to avoid this type of unpleasant surprise. The ascomycete *Chaetomium globosum* can control the serious apple pathogen *Venturia inaequalis* of the phyllosphere but its production of toxins led to it being abandoned as a commercially viable BCA already in the 1980s (Boudreau & Andrews, 1987). It is to be expected that plant pathogens will be isolated and enter into the first stage of screening for potential BCAs, because the sources of promising microorganisms will often be plant biomes including endophytes (Latz et al., 2021;

Manzotti et al., 2020; Rojas et al., 2020b). However, a universal exclusion of possible BCA candidates based on their species-level taxonomy risks missing useful organisms. For example, fungi within the species *F. oxysporum* can be grouped into either nonpathogenic or pathogenic individuals. Those belonging to the pathogenic group can again be subdivided into *formae speciales* depending on the specific host plant they can infect and cause wilt disease in. Indeed, nonpathogenic *F. oxysporum* strains are promising BCA derived from disease suppressive soils (Alabouvette, 1986). These strains are for example good at controlling wilt in tomato caused by *F. oxysporum* (Alabouvette et al., 2009) or *Verticillium albo-atrum* in pepper (Constantin et al., 2019; Veloso et al., 2016). The basis of host range among pathogenic strains in *F. oxysporum* has been shown to reside on supernumerary chromosomes (Ma et al., 2010). Similarly, the acquisition of *Tox*A from *Parastagonospora nodorum* by *Pyrenophora tritici-repentis* (Friesen et al., 2006) has led to serious new disease problems. Would it be possible to ensure that a successful BCA could not gain a chromosome or chromosome segment and become a pathogen in its own right or a pathogen of other crops? This scenario seems, fortunately, to be rather unlikely as, for example, nonpathogenic *F. oxysporum* coexist naturally with the pathogenic strains and with other species of *Fusarium* in many soils, apparently without leading to new pathogenic strains. Furthermore, such transfer of pathogenicity has not been observed in augmentative biocontrol experiments with nonpathogenic *F. oxysporum*, although clearly the process cannot be totally ruled out.

For some BCAs, perhaps particularly for species operating by induced resistance, there is also a risk that weedy hosts might be made more competitive by interaction with the BCA, particularly if it has a wide host range. For example, *S. indica* improves the growth of many wheat cultivars, as mentioned above—but also has, as do other *Serendipita* spp., beneficial

effects on some competing weeds (Edenborn et al., 2018; Rabiey et al., 2017; Ray et al., 2018). More research is needed to clarify whether this can really be a problem in crop production.

Whereas a BCA needs to be sufficiently aggressive to be active against its target without uneconomic volumes or numbers of applications, we should also be able to recover from unexpected ecological or medical effects. This leads to the argument that an agent should not persist too long in the environment. Commercially, the advantage of this is that the product has to be sold every year, allowing recovery of the research investment over a long period. Perhaps an average of one growing season should be enough? Is it ethical to develop BCAs which can persist and become permanent components of the local microbiome or would this be a godsend for agriculture—if they do not spread to natural habitats and change ecosystems? For perennial plants, would it be sufficient to ensure that they do not spread from the inoculated host? This requirement, of course, is in conflict with the desire to be able to encourage BCAs in the environment by habitat manipulation.

There is a political movement to speed up the process of approval for BCAs, on the probably spurious grounds that they are intrinsically safer than artificially synthesized molecules. For instance, in the EU, where the process is considered to be as painstaking as for new pesticides or GMOs (Sundh & Eilenberg, 2021), the argument has been made that strains closely related to existing approved products should not need the same level of documentation before being licensed for release. Of course, there would still be risk and some kind of "yellow card" system, like that used to report possible side-effects of medical interventions, would be desirable.

However, in some countries, most prominently in Brazil, people from farms are starting to use home-grown biomass of beneficial isolates (such as *Trichoderma* spp.) in order to have the

quantity required to treat their fields. Without being supported by adequate facilities and without a basic knowledge of the organisms the growers are managing, the risk of contamination of the target strains is likely. The products applied to crop fields could therefore be completely different from the original strains, with the consequences that (a) any kind of beneficial effect is reduced or eliminated; and (b) the supposed BCA could be dangerous for the producers and the consumers of the final product. Lastly, but of no less importance, almost 90% of a BCA product is usually represented by coformulants that guarantee the survival and quality of the active BCA, and therefore assure good disease control (Lana et al., 2019). The correct mix of coformulants cannot be expected to be reproducible in home-made BCA products. Strict regulation is needed in these countries in order to reduce the risks connected with this trend and to ensure that the products sold actually work and are not just harmless—or worse—mixtures. Quality control is vital to achieve effective biological control and home-made products, including compost teas, cannot be controlled for consistency and safety.

10 Legislation and registration

Factors, that are considered in the approval processes around the world, include production of toxic metabolites, pathogenicity to humans or crops, allergenicity and ability to survive and spread. Some countries have very little regulation whereas other regions (EU, USA) impose strong constraints on the documentation for safety and—in the EU but not the USA—efficacy, before permitting commercialization. The challenges regarding registration of biological control agents were the focus of a white paper from the EMPHASIS project (EMPHASIS, 2016) which called for more harmonization, as did a workshop in the same year convened by the IOBC and

671 summarized in Ward (2016). Another important recommendation was that benefits as well as
672 risks need to be taken into account when considering biocontrol agent release permissions. The
673 current system of Pest Risk Analysis only focuses on the latter.

674 As mentioned above, the first GMO product was the strain K1026 modified from
675 *Agrobacterium radiobacter* K84 originally marketed as NOGALL® originally in Australia (Kerr
676 & Bullard, 2020). It is interesting to consider other categories of BCA and how their use and
677 regulation has evolved alongside agents for disease management. BCA intended to reduce weed
678 populations can be considered to be "classical"—that is, agents which are expected to offer long-
679 term reduction in target populations, without repeated widespread release—or as
680 mycoherbicides, requiring regular and widespread release. The regulatory requirements differ.

681 In the case of classical weed biocontrol agents, the focus in the early part of the 20th
682 Century was on safety to crops and little else. Then protecting native species became politically
683 important and a thorough risk assessment is demanded prior to the release of any BCA active
684 against weeds. This includes centrifugal (testing close relatives first) host specificity testing
685 based on plant phylogeny and typically includes 50–80 species of nontarget test plant being
686 exposed to the potential agent, be they fungal or arthropod. However, with the advent of
687 molecular tools to better determine phylogeny much shorter test plant lists are proposed (Briese,
688 2006). This level of investigation normally satisfies the licensing authority of recipient countries,
689 most of which have legislation banning the introduction of non-native organisms. In the UK,
690 permission to release arthropods is done through the Wildlife and Countryside Act 1981 often
691 with input from the Advisory Committee on Releases to the Environment, a public consultation,
692 and ministerial approval. The fact that weed biocontrol agents are "likely to be injurious to plants
693 in the UK" puts them under Plant Health Regulation (Shaw et al., 2016; Box 1).

694 If one is considering developing a mycoherbicide, then the registration process, at least in
695 Europe, is the analogue of registering BCAs for plant disease control and the chemical pesticide
696 registration process and this is often cited as a reason for the slow development and poor pipeline
697 of alternative products for pest and weed management (Bale, 2011; Zaki et al., 2020).

698 In the case of classical arthropod biocontrol, the restrictions are technically the same as
699 agents targeting weeds. As the predators and parasitoids are not plant pests, there are no plant
700 health quarantine restrictions placed on the research, but responsible researchers would take
701 precautions to prevent escape prior to licensing. The level of host range testing applied to insects
702 versus insect biocontrol is rather less than with weeds. Many of the 176 species of arthropod
703 BCA released outside the glasshouse in Europe have been released without much host range
704 testing or risk assessment at all. The on-going and catastrophic invasion of the intentionally
705 released predatory harlequin ladybird, *Harmonia axyridis*, shows how significant nontarget
706 impacts can be when things go wrong (Kenis et al., 2017; Roy & Wajnberg, 2008). Nonetheless
707 extensive analyses have demonstrated that nontarget effects impacting native species at the
708 population level are rare when compared with the number of introductions that have occurred
709 (Hajek et al., 2016). As with BCAs targeting pathogens, there are conflicting advantages to
710 modes of action: parasitoids are (sometimes very highly) specialized, which makes them less
711 attractive for commercialization and more vulnerable to counterevolution; but predators have a
712 wider host range with correspondingly greater dangers of unexpected ecological damage (Louda
713 et al., 2003; reviewed by Taylor & Snyder, 2021).

714

715 11 Viruses as management tools against bacteria and fungi

A form of hyperparasitism that is receiving increased attention as a new approach to biological control is the use of viruses to infect and weaken fungal or bacterial plant pathogens. The potential to use mycoviruses for controlling chestnut blight caused by the ascomycete *Cryphonectria parasitica* has been studied for decades and is effective (so far) in some regions but has not proved sufficiently effective in other (Milgroom & Cortesi, 2004). A more recent and very promising example concerns a mycovirus (fungal virus) with a 2 kb genome that converts the necrotrophic fungus *Sclerotinia sclerotiorum* into a beneficial BCA that induces resistance and can also infect and inactivate the pathogenic strains it meets (Zhang et al., 2020).

Bacteria are difficult to control other than by cultural practice and disease resistance if available. Recent studies suggest the potential for bacteriophages to control bacterial diseases (Ahern et al., 2014; Carstens et al., 2019), and indeed the first product—against Pierce's disease—has now reached the market (Table 1): based on a cocktail of four bacteriophages (Das et al., 2015). The use of bacteriophages controlling human disease has been explored since their discovery (Abedon et al., 2011; Furfaro et al., 2018; Sybesma et al., 2018). A challenge with bacteriophages is the need to prepare mixtures of phages specific to each of the component genotypes in the mixture of host bacterial types causing a problem. This also means that resistance is likely to be a major and rapidly arising problem, because of the naturally occurring host–phage coevolutionary race that indeed underlies the need to use mixtures from the start. Thus, there are two points here: (a) specific matching for effectiveness, and (b) the complications of the evolutionary process driven by host–phage matching. The need to use tailored mixtures was an important reason why phage therapy for humans has developed slowly in Western medicine—the Soviet Union block used it, but needed to maintain large banks of phages against every subtype of bacterium they were trying to control. Though this sounds complicated, the

positive side of this is good control of use, because the bacteriophage cocktails used need to be compiled according to need and resistance management can be built in. The negative side is the potential for erratic severe outbreaks. The same considerations apply to mycoviruses. Alternatively, in many of these cases, the narrow host range can be considered to be a biosafety advantage, though there can be advantages in broad host range (Ross et al., 2016).

12 Commercialization

With high development costs and limited targets there have been relatively few market successes and the availability of specific products is often restricted to one or a few countries or limited regions (Table 1) (Cordeau et al., 2016). Few BCAs are as effective as established pesticides. Thus, the market opportunities occur where a gap in activity opens due to consumer choice, safety issues or the evolution of pesticide resistance. However, it seems that biological control can play important roles in part of IPM strategies for reducing input of chemical pesticides and in organic plant production.

In general, commercialization of a biocontrol agent is very challenging and many potential products are never brought to the market. The challenges to successfully commercializing a BCA are many and range from developing the biological production process to raising the capital required for manufacture, distribution and successful marketing (Table 2). As we have already discussed, good control by BCAs has been achieved many times in controlled environments and artificially simplified ecosystems but it has often proved difficult to translate these achievements to commercial or other agricultural settings, whether field, greenhouse, forest or plantation. This is not surprising, because we know that the severity of

761 disease caused by pathogens is subject to environmental influence by factors such as humidity
762 and temperature (the “disease triangle”). Biological control represents adding a third living
763 organism, with its own environmental envelope, to the system. BCAs can be applied in many
764 ways, such as spraying, application to planting material (e.g., seed coating), soil surface mixing,
765 postharvest spray or aerosol application. Determining optimal formulation of a living organism,
766 choice of mode of deployment and design of field trials are also challenges prior to
767 commercialization. Once these issues, including registration, have been solved then there are the
768 issues of being able scale up to a profitable production level with a reliable product that adds
769 sufficient yield and/or quality to give net profit to a grower and is sufficiently nonspecific to
770 allow development costs to be spread over multiple targets. Shelf life is perhaps not a major
771 issue in industrialized agriculture but is clearly an issue in rural communities in developing
772 countries. How BCA stability compares to that of chemical pesticides is an important issue.
773 Despite these challenges, there are a number examples of successful commercialization of
774 biological control products (Table 1) and there are now many companies within the agroindustry
775 that are aiming to market new BCA products. A prospect that we do not address here is the
776 possibility of combining fungicides and BCA in integrated management.

777 The global BCA market, continuously increasing, reached almost $4.0 billion in 2020
778 with a projection towards $10.6 billion by 2027 (Anon, 2020). Several governments are
779 supporting the use of more environmentally friendly agri-inputs especially when we gradually
780 recover from the COVID-19 pandemic. North America, under stringent rules and regulations
781 regarding the use of chemical crop protection products, is currently the largest market for BCA
782 and this is expected to continue throughout the forecast period. Particularly promising as a
783 market is the current situation in South America, with Brazil and Argentina showing an increase

of area under organic farming (Paull & Hennig, 2019) and therefore an amplification of demand for BCA products (Zalles et al., 2019). This is also due to new advances in biological understanding and technologies following from them, as well as increasing investments by the major players in this market (https://www.fortunebusinessinsights.com/industry-reports/biopesticides-market-100073). This trend is likely to be seen in many other parts of the world in due course.

Finally, in Europe the "Farm to Fork" strategy, a new challenge to create sustainable food systems which will reduce dependency on pesticides and antimicrobials, reduce excess fertilization, improve animal welfare, and reverse biodiversity loss, is driving the crop protection market towards a higher use of biological control. The stated aim is to reduce, by 2030, the overall use and risk of chemical pesticides by 50% and the use of more hazardous pesticides by 50% (https://ec.europa.eu/food/sites/food/files/safety/docs/f2f_action-plan_2020_strategy-info_en.pdf) (Zalles et al., 2019).

There is also a need to consider sources of research and development funding in relation to public attitudes. One specific action in the strategy (https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en) is "investing in environmentally-friendly technologies" and large R&D programmes where academia and industry join forces are indeed part of this agenda.

13 Final remarks

Biological control of plant diseases with living organisms is challenging because the biology of at least three organisms has to function effectively in a variable environment. As witnessed by

Table 1, much progress has been made over recent decades but much more development needs to be done for individual diseases before these methods can be considered to be mature and as natural a part of disease management technologies as disease resistance and pesticides are today. At the biological level, scientific progress on understanding ecology and the biological (cellular/molecular) mechanisms governing the outcome of interactions alone and in combination is needed. By understanding these, there will be a rational basis for strain improvement, formulation and delivery, which can result in improved efficacy and stability. The political landscape, especially the green lobby, needs to be realistic about what can be achieved and the risks that need to be addressed. We are ever getting closer to being able to answer the question "what will it take to progress biologicals from 'niche markets' to broad acre crops and industrialized farming?" The pressures for reducing the use of pesticides in farming certainly provides an incentive to do this.

Acknowledgements

Roughly 300 people registered for the BSPP webinar on biological control and well over 200 participated; many contributed to the discussion. We cannot mention all but have addressed points raised by Godfrey Apangu, Ralf-Udo Ehlers, Katia Hougaard, Anna Mangor Iversen, Ruth D'urban-Jackson John Lucas, Janna Macholdt, Makomborero Nyoni, Maryam Rafiqi, Paul Rushton, Phil Taylor, Ffion Thomas and Widodo. We also wish to thank the two exceptionally constructive referees for the comments to the original submitted version of this paper. Finally, Matthew Everatt, DEFRA (Department for Environment, Food & Rural Affairs, UK Government) and Rosalind Noble, BSPP provided the text for Box 1.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed.

References

Abdallah, M. F., De Boevre, M., Landschoot, S., De Saeger, S., Haesaert, G. & Audenaert, K. (2018) Fungal endophytes control *Fusarium graminearum* and reduce trichothecenes and zearalenone in maize. *Toxins*, **10**, 493.

Abedon, S. T., Kuhl, S. J., Blasdel, B. G. & Kutter, E. M. (2011) Phage treatment of human infections. *Bacteriophage*, **1**, 66–85.

Ahern, S. J., Das, M., Bhowmick, T. S., Young, R. & Gonzalez, C. F. (2014) Characterization of novel virulent broad-host-range phages of *Xylella fastidiosa* and *Xanthomonas*. *Journal of Bacteriology*, **196**, 459–471.

Alabouvette, C. (1986) Fusarium-wilt suppressive soils from the Châteaurenard region: review of a 10-year study. *Agronomie*, **6**, 273–284.

Alabouvette, C., Olivain, C., Migheli, Q. & Steinberg, C. (2009) Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. *New Phytologist*, **184**, 529–544.

Amaike, S. & Keller, N. P. (2011) *Aspergillus flavus*. *Annual Review of Phytopathology*, **49**,

847 107–133.

848 Amer, S. (2020) *Genetic architecture of wheat yield responses to drought*. Reading: University
849 of Reading. PhD thesis.

850 Anastassiadou, M., Bernasconi, G., Brancato, A., Carrasco Cabrera, L., Greco, L., Jarrah, S., et
851 al. (2020) Review of the existing maximum residue levels for *Pseudomonas* sp. strain
852 DSMZ 13134 according to Article 12 of Regulation (EC) No 396/2005. *EFSA Journal*, **18**,
853 e06234.

854 Anon (2020) Biopesticides market size, share & COVID-19 impact analysis, by type
855 (Bioinsecticide, Biofungicide, Bionematicide, and Others), source (Microbials and
856 Biochemicals), mode of application (Foliar application, seed treatment, soil treatment, and
857 others), crop (cereals, oilseeds, fruits & vegetables, and others), and regional forecast,
858 2020–2027. (Biological, A., ed.). pp. 1–145.

859 Backer, R., Naidoo, S. & van den Berg, N. (2019) The NONEXPRESSOR OF
860 PATHOGENESIS-RELATED GENES 1 (NPR1) and related family: mechanistic insights
861 in plant disease resistance. *Frontiers in Plant Science*, **10**, 102.

862 Baker, K. & Cook, R. J. (1974) *Biological control of plant pathogens*. San Francisco: W.H.
863 Freeman and Company.

864 Bale, J. (2011) Harmonization of regulations for invertebrate biocontrol agents in Europe:
865 progress, problems and solutions. *Journal of Applied Entomology*, **135**, 503–513.

866 Bandyopadhyay, R., Atehnkeng, J., Ortega-Beltran, A., Akande, A., Falade, T. D. O. & Cotty, P.

867 J. (2019) “Ground-truthing” efficacy of biological control for aflatoxin mitigation in
868 farmers’ fields in Nigeria: from field trials to commercial usage, a 10-year study. *Frontiers*
869 *in Microbiology*, **10**. DOI: 10.3389/fmicb.2019.02528.

870 del Barrio-Duque, A., Ley, J., Samad, A., Antonielli, L., Sessitsch, A. & Compant, S. (2019)
871 Beneficial endophytic bacteria-*Serendipita indica* interaction for crop enhancement and
872 resistance to phytopathogens. *Frontiers in Microbiology*, **10**, 2888.

873 Benítez, T., Rincón, A. M., Limón, M. C. & Codón, A. C. (2004) Biocontrol mechanisms of
874 *Trichoderma* strains. *International Microbiology*, **7**, 249–260.

875 Bennett, A. J., Leifert, C. & Whipps, J. M. (2003) Survival of the biocontrol agents
876 *Coniothyrium minitans* and *Bacillus subtilis* MBI 600 introduced into pasteurised,
877 sterilised and non-sterile soils. *Soil Biology and Biochemistry*, **35**, 1565–1573.

878 Blaya, J., López-Mondéjar, R., Lloret, E., Pascual, J. A. & Ros, M. (2013) Changes induced by
879 *Trichoderma harzianum* in suppressive compost controlling Fusarium wilt. *Pesticide*
880 *Biochemistry and Physiology*, **107**, 112–119.

881 Brannen, P. M. & Kenney, D. S. (1997) Kodiak®—a successful biological-control product for
882 suppression of soil-borne plant pathogens of cotton. *Journal of Industrial Microbiology*
883 *and Biotechnology*, **19**, 169–171.

884 Briese, D. (2006) Host specificity testing of weed biological control agents: initial attempts to
885 modernize the centrifugal phylogenetic method. In: *Proceedings of the fifth California*
886 *conference on biological control*. *2006*. [publisher?], pp. 32–39.

887 Brozova, J. (2002) Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant
888 protection. *Plant Protection Science*, **38**, 29–35.

889 Caradus, J. R. & Johnson, L. J. (2020) *Epichloë* fungal endophytes – from a biological curiosity
890 in wild grasses to an essential component of resilient high performing ryegrass and fescue
891 pastures. *Journal of Fungi*, **6**. 322

892 Carmona-Hernandez, S., Reyes-Pérez, J. J., Chiquito-Contreras, R. G., Rincon-Enriquez, G.,
893 Cerdan-Cabrera, C. R. & Hernandez-Montiel, L. G. (2019) Biocontrol of postharvest fruit
894 fungal diseases by bacterial antagonists: a review. *Agronomy*, **9**, 121.

895 Carstens, A. B., Djurhuus, A. M., Kot, W. & Hansen, L. H. (2019) A novel six-phage cocktail
896 reduces *Pectobacterium atrosepticum* soft rot infection in potato tubers under simulated
897 storage conditions. *FEMS Microbiology Letters*, **366**, fnz101

898 Carstens, A. B., Djurhuus, A. M., Kot, W., Jacobs-Sera, D., Hatfull, G. F. & Hansen, L. H.
899 (2018) Unlocking the Potential of 46 New Bacteriophages for Biocontrol of *Dickeya*
900 *solani*. *Viruses*, **10**, 621.

901 Catskă, V., Afifi, A. F. & Vancura, V. (1975) The effect of volatile and gaseous metabolites of
902 swelling seeds on germination of fungal spores. *Folia Microbiologica*, **20**, 152–156.

903 Chen, S., Pan, L., Liu, S., Pan, L., Li, X. & Wang, B. (2021) Recombinant expression and
904 surface display of a zearalenone lactonohydrolase from *Trichoderma aggressivum* in
905 *Escherichia coli*. *Protein Expression and Purification*, **187**, 105933.

906 Cheng, C., Li, D., Qi, Q., Sun, X., Anue, M. R., David, B. M., et al. (2020) The root endophytic

907 fungus *Serendipita indica* improves resistance of Banana to *Fusarium oxysporum* f. sp.
908 *cubense* tropical race 4. *European Journal of Plant Pathology*, **156**, 87–100.

909 Chin-A-Woeng, T. F. C., Bloemberg, G. V. & Lugtenberg, B. J. J. (2003) Phenazines and their
910 role in biocontrol by *Pseudomonas* bacteria. *New Phytologist*, **157**, 503–523.

911 Collinge, D. B., Jørgensen, H. J. L., Latz, M. A. C., Manzotti, A., Ntana, F., Rojas, E. C., et al.
912 (2019) Searching for novel fungal biological control agents for plant disease control among
913 endophytes. In: Hodkinson, T. R., Doohan, F. M., Saunders, M. and Murphy, B. R. (eds.).
914 *Endophytes: for a growing world.* Cambridge: Cambridge University Press, pp. 25–51.

915 Collinge, D. B., Jørgensen, H. J. L., Lund, O. S. & Lyngkjær, M. F. (2010) Engineering
916 pathogen resistance in crop plants – current trends and future prospects. *Annual Review of*
917 *Phytopathology*, **48**, 269–291.

918 Collinge, D. B. & Sarrocco, S. (2022) Transgenic approaches for plant disease control: status and
919 prospects 2021. *Plant Pathology*, **71**, 207–225.

920 Conrath, U., Beckers, G. J. M., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., et al. (2006)
921 Priming: getting ready for battle. *Molecular Plant-Microbe Interactions*, **19**, 1062–1071.

922 Constantin, M. E., de Lamo, F. J., Vlieger, B. V., Rep, M. & Takken, F. L. W. (2019)
923 Endophyte-mediated resistance in tomato to *Fusarium oxysporum* is independent of ET,
924 JA, and SA. *Frontiers in Plant Science*, **10**, 979.

925 Cook, J. R. (1992) Wheat root health management and environmental concern. *Canadian*
926 *Journal of Plant Pathology*, **14**, 76–85.

927 Cook, R. & Baker, K. (1983) *The nature and practice of biological control of plant pathogens*.
928 St Paul, MN, USA: American Phytopathological Society.

929 Cook, R. & Papendick, R. (1972) Influence of water potential of soils and plants on root disease.
930 *Annual Review of Phytopathology*, **10**, 349–374.

931 Cook, R. J. (2007) Take-all decline: model system in the science of biological control and clue to
932 the success of intensive cropping. In: Vincent, C., Goettel, M. S. and Lazarovits, G. (eds.)
933 *Biological control: a global perspective*. Wallingford: CAB International, pp. 399–414.

934 Cordeau, S., Triolet, M., Wayman, S., Steinberg, C. & Guillemin, J.-P. (2016) Bioherbicides:
935 dead in the water? A review of the existing products for integrated weed management.
936 *Crop Protection*, **87**, 44–49.

937 Das, M., Bhowmick, T. S., Ahern, S. J., Young, R. & Gonzalez, C. F. (2015) Control of Pierce's
938 disease by phage. *PLoS One*, **10**, e0128902.

939 De Corato, U. (2020) Soil microbiota manipulation and its role in suppressing soil-borne plant
940 pathogens in organic farming systems under the light of microbiome-assisted strategies.
941 *Chemical and Biological Technologies in Agriculture*, **7**, 17.

942 Degenkolb, T., Fog Nielsen, K., Dieckmann, R., Branco-Rocha, F., Chaverri, P., Samuels, G. J.,
943 et al. (2015) Peptaibol, secondary-metabolite, and hydrophobin pattern of commercial
944 biocontrol agents formulated with species of the *Trichoderma harzianum* complex.
945 *Chemistry & Biodiversity*, **12**, 662–684.

946 Dilla-Ermita, C. J., Lewis, R. W., Sullivan, T. S. & Hulbert, S. H. (2021) Wheat genotype-

947 specific recruitment of rhizosphere bacterial microbiota under controlled environments.
948 *Frontiers in Plant Science*, **12**, 718264.

949 Dimakopoulou, M., Tjamos, S. E., Antoniou, P. P., Pietri, A., Battilani, P., Avramidis, N., et al.
950 (2008) Phyllosphere grapevine yeast *Aureobasidium pullulans* reduces *Aspergillus*
951 *carbonarius* (sour rot) incidence in wine-producing vineyards in Greece. *Biological*
952 *Control*, **46**, 158–165.

953 Dorner, J. W. & Lamb, M. C. (2006) Development and commercial use of afla-Guard®, an
954 aflatoxin biocontrol agent. *Mycotoxin Research*, **22**, 33-38.

955 Droby, S., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E. E., et al. (2002)
956 Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent
957 *Candida oleophila*. *Phytopathology*, **92**, 393–399.

958 Dubey, M. K., Jensen, D. F. & Karlsson, M. (2014) An ATP-binding cassette pleiotropic drug
959 transporter protein is required for xenobiotic tolerance and antagonism in the fungal
960 biocontrol agent *Clonostachys rosea*. *Molecular Plant-Microbe Interactions*, **27**, 725–732.

961 Edenborn, S. L., Johnson, L. M. K., Edenborn, H. M., Albarran-Jack, M. R. & Demetrion, L. D.
962 (2018) Amendment of a hardwood biochar with compost tea: effects on plant growth,
963 insect damage and the functional diversity of soil microbial communities. *Biological*
964 *Agriculture & Horticulture*, **34**, 88–106.

965 Ehlers, R.-U. (2011) Regulation of biological control agents and the EU policy support action
966 REBECA. In: Ehlers, R.-U. (ed.). *Regulation of biological control agents*. Dordrecht:
967 Springer Netherlands, pp. 3–23.

968 Eberl, L. & Vandamme, P. (2016) Members of the genus *Burkholderia*: good and bad guys.
969 *F1000Res*, **5**, F1000 Faculty Rev-1007.

970 EMPHASIS (2016) White paper: the regulatory framework for biological control agents.
971 EMPHASIS Project, H2020 Grant Agreement n. 634179. Available at:
972 http://www.emphasisproject.eu/upload/deliverables/file/White_Paper_Update.pdf
973 [Accessed date?].

974 Falk, S. P., Gadoury, D. M., Cortesi, P., Pearson, R. C. & Seem, R. C. (1995a) Parasitism of
975 *Uncinula necator* cleistothecia by the mycoparasite *Ampelomyces quisqualis*
976 *Phytopathology*, **85**, 794–800.

977 Falk, S. P., Gadoury, D. M., Pearson, R. C. & Seem, R. C. (1995b) Partial control of grape
978 powdery mildew by the mycoparasite *Ampelomyces quisqualis*. *Plant Disease*, **79**, 483–
979 490.

980 Farag, M. A., Zhang, H. & Ryu, C.-M. (2013) Dynamic chemical communication between plants
981 and bacteria through airborne signals: induced resistance by bacterial volatiles. *Journal of*
982 *Chemical Ecology*, **39**, 1007–1018.

983 Fravel, D. R. (2005) Commercialization and implementation of biocontrol. *Annual Review of*
984 *Phytopathology*, **43**, 337–359.

985 French, E., Kaplan, I., Iyer-Pascuzzi, A., Nakatsu, C. H. & Enders, L. (2021) Emerging strategies
986 for precision microbiome management in diverse agroecosystems. *Nature Plants*, **7**, 256–
987 267.

988 Friesen, T. L., Stukenbrock, E. H., Liu, Z. H., Meinhardt, S., Ling, H., Faris, J. D., et al. (2006)
989 Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature*
990 *Genetics*, **38**, 953–956.

991 Furfaro, L. L., Payne, M. S. & Chang, B. J. (2018) Bacteriophage therapy: clinical trials and
992 regulatory hurdles. *Frontiers in Cellular and Infection Microbiology*, **8**, 376.

993 Gardener, B. B. M. & Fravel, D. R. (2002) Biological control of plant pathogens: research,
994 commercialization, and application in the USA. *Plant Health Progress*, **3**, 17.

995 Gerin, D., Pollastro, S., Raguseo, C., De Miccolis Angelini, R. M. & Faretra, F. (2018) A ready-
996 to-use single- and duplex-TaqMan-qPCR assay to detect and quantify the biocontrol agents
997 *Trichoderma asperellum* and *Trichoderma gamsii*. *Frontiers in Microbiology*, **9**, 2073.

998 Gill, S. S., Gill, R., Trivedi, D. K., Anjum, N. A., Sharma, K. K., Ansari, M. W., et al. (2016)
999 *Piriformospora indica*: Potential and significance in plant stress tolerance. *Frontiers in*
1000 *Microbiology*, **7**, 332.

1001 Grondona, I., Rodríguez, A., Gómez, M. I., Camacho, R., Llobell, A. & Monte, E. (2004)
1002 TUSAL®, a commercial biocontrol formulation based on *Trichoderma*. *IOBC/wprs*
1003 *Bulletin*, **27**, 285–288.

1004 Großkopf, T. & Soyer, O. S. (2014) Synthetic microbial communities. *Current Opinion in*
1005 *Microbiology*, **18**, 72–77.

1006 Hajek, A. E., Hurley, B. P., Kenis, M., Garnas, J. R., Bush, S. J., Wingfield, M. J., et al. (2016)
1007 Exotic biological control agents: a solution or contribution to arthropod invasions?

Biological Invasions, **18**, 953–969.

Harman, G. E. (2000) Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease*, **84**, 377–393.

Harman, G. E. (2006) Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, **96**, 190–194.

Hilbert, M., Voll, L. M., Ding, Y., Hofmann, J., Sharma, M. & Zuccaro, A. (2012) Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. *New Phytologist*, **196**, 520–534.

Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Lipka, V., et al. (2011) Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. *Plant Physiology* **156**, 726–740.

Jalaluddin, M. & Jenkyn, J. F. (1996) Effects of wheat crop debris on the sporulation and survival of *Pseudocercosporella herpotrichoides*. *Plant Pathology*, **45**, 1052–1064.

Jensen, D. F., Dubey, M., Jensen, B. & Karlsson, M. (2021) *Clonostachys rosea* to control plant diseases. In: Köhl, J. and Ravensberg, W. J. (eds.). *Microbial bioprotectants for plant disease management*. Cambridge, UK: Burleigh Dodds Science Publishing, pp. 429–471.

Jensen, D. F., Karlsson, M. & Lindahl, B. D. (2017) Fungal–fungal interactions: from natural ecosystems to managed plant production, with emphasis on biological control of plant diseases. In: Dighton, J. and White, J. F. (eds.). *The Fungal Community – Its Organization*

and Role in the Ecosystem. Boca Raton: CRC Press, pp. 549–562.

Jensen, D. F., Karlsson, M., Sarrocco, S. & Vannacci, G. (2016) Biological Control using microorganisms as an alternative to disease resistance. In: Collinge, D. B. (ed.) *Plant pathogen resistance biotechnology*. New York and London: Wiley Blackwell, pp. 341–363.

Jensen, D. F., Knudsen, I. M. B., Lübeck, M., Mamarabadi, M., Hockenhull, J. & Jensen, B. (2007) Development of a biocontrol agent for plant disease control with special emphasis on the near commercial fungal antagonist *Clonostachys rosea* strain 'IK726'. *Australasian Plant Pathology*, **36**, 95–101.

Jørgensen, H. J. L., Collinge, D. B., Rojas, E. C., Latz, M. A. C., Manzotti, A., Ntana, F., et al. (2020) Plant endophytes. In: *Encyclopaedia of Life Sciences*. Wiley. DOI: 10.1002/9780470015902.a0028893.

Junaid, J. M., Dar, N. A., Bhat, T. A., Bhat, A. H. & Bhat, M. A. (2013) Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *International Journal of Modern Plant & Animal Science*, **1**, 39–57.

Karlsson, M., Atanasova, L., Jensen, D. F. & Zeilinger, S. (2018) Necrotrophic mycoparasites and their genomes. In: Heitman, J., Howlett, B., Crous, P., Stukenbrock, E., James, T. and Gow, N. (eds.) *The fungal kingdom*. Washington DC: ASM Press, pp. 1005–1026.

Karlsson, M., Durling, M. B., Choi, J., Kosawang, C., Lackner, G., Tzelepis, G. D., et al. (2015) Insights on the evolution of mycoparasitism from the genome of *Clonostachys rosea*. *Genome Biology and Evolution*, **7**, 465–480.

1049 Kenis, M., Hurley, B. P., Hajek, A. E. & Cock, M. J. W. (2017) Classical biological control of
1050 insect pests of trees: facts and figures. *Biological Invasions*, **19**, 3401–3417.

1051 Kerr, A. (2016) Biological control of crown gall. *Australasian Plant Pathology*, **45**, 15–18.

1052 Kerr, A. & Bullard, G. (2020) Biocontrol of crown gall by *Rhizobium rhizogenes*: challenges in
1053 biopesticide commercialisation. *Agronomy*, **10**, 1126.

1054 Keyser, C. A., Jensen, B. & Meyling, N. V. (2016) Dual effects of *Metarhizium* spp. and
1055 *Clonostachys rosea* against an insect and a seed-borne pathogen in wheat. *Pest*
1056 *Management Science*, **72**, 517–526.

1057 Khalil, S. & Alsanius, B. W. (2006) Biochemical characterization of biocontrol agents used for
1058 control of root pathogens. *Communications in Agricultural and Applied Biological*
1059 *Sciences*, **71**, 979–984.

1060 Khatabi, B., Molitor, A., Lindermayr, C., Pfiffi, S., Durner, J., Von Wettstein, D., et al. (2012)
1061 Ethylene supports colonization of plant roots by the mutualistic fungus *Piriformospora*
1062 *indica*. *PLoS One*, **7**, e35502.

1063 Kim, D.-R., Jeon, C.-W., Cho, G., Thomashow, L. S., Weller, D. M., Paik, M.-J., et al. (2021)
1064 Glutamic acid reshapes the plant microbiota to protect plants against pathogens.
1065 *Microbiome*, **9**, 244.

1066 Kiss, L. (2003) A review of fungal antagonists of powdery mildews and their potential as
1067 biocontrol agents. *Pest Management Science*, **59**, 475–483.

1068 Knudsen, I. M. B., Hockenhull, J., Jensen, D. F., Gerhardson, B., Hökeberg, M., Tahvonen, R., et

al. (1997) Selection of biological control agents for controlling soil and seed-borne diseases in the field. *European Journal of Plant Pathology*, **103**, 775–784.

Köhl, J., Postma, J., Nicot, P., Ruocco, M. & Blum, B. (2011) Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. *Biological Control*, **57**, 1–12.

Kosawang, C., Karlsson, M., Vélëz, H., Rasmussen, P. H., Collinge, D. B., Jensen, B., et al. (2014) Zearalenone detoxification by zearalenone hydrolase is important for the antagonistic ability of *Clonostachys rosea* against mycotoxigenic *Fusarium graminearum*. *Fungal Biology*, **118**, 364–373.

Kunz, S. (2004) Entwicklung von "Blossom-Protect"- ein Hefepräparat zur Reduktion von Blüteninfektionen durch Feuerbrand [Development of "Blossom-Protect" - a yeast preparation for the reduction of blossom infections by fire blight]. In: *Ecofruit - 11th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 3 February to 5 February 2004 at Weinsberg/Germany*. [publisher?], pp. 108–112.

Kurose, D., Furuya, N., Tsuchiya, K., Tsushima, S. & Evans, H. C. (2012) Endophytic fungi associated with *Fallopia japonica* (Polygonaceae) in Japan and their interactions with *Puccinia polygoni-amphibii* var. *tovariae*, a candidate for classical biological control. *Fungal Biology*, **116**, 785–791.

Kwak, Y.-S., Bakker, P. A. H. M., Glandorf, D. C. M., Rice, J. T., Paulitz, T. C. & Weller, D. M. (2009) Diversity, virulence, and 2,4-diacetylphloroglucinol sensitivity of

Gaeumannomyces graminis var. *tritici* isolates from Washington State. *Phytopathology*, **99**, 472–479.

Lahdenperä, M. L., Simon, E. & Uoti, J. (1991) Mycostop - A novel biofungicide based on *Streptomyces* bacteria. In: Beemster, A. B. R., Bollen, G. J., Gerlagh, M., Ruissen, M. A., Schippers, B. and Tempel, A. (eds.) *Developments in agricultural and managed forest ecology*. Elsevier, pp. 258–263.

Lahlali, R., Peng, G., McGregor, L., Gossen, B. D., Hwang, S. F. & McDonald, M. (2011) Mechanisms of the biofungicide Serenade (*Bacillus subtilis* QST713) in suppressing clubroot. *Biocontrol Science and Technology*, **21**, 1351–1362.

Lana, U. G. d. P., Amanda, N. G. T., Aguiar, F. M., Gomes, E. A. & Valicente, F. H. (2019) Quality evaluation of *Bacillus thuringiensis*-based biopesticides produced on farm system. *Boletim de Pesquisa e Desenvolvimento.* Embrapa Milho e Sorgo.

Lasinio, G. J., Pollice, A., Pappalettere, L., Vannacci, G. & Sarrocco, S. (2021) A statistical protocol to describe differences among nutrient utilization patterns of Fusarium spp. and *Trichoderma gamsii*. *Plant Pathology*. **70**, 1146–1157

Latz, M. A. C., Jensen, B., Collinge, D. B. & Jørgensen, H. J. L. (2018) Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. *Plant Ecology and Diversity*, **11**, 555.

Latz, M. A. C., Jensen, B., Collinge, D. B. & Jørgensen, H. J. L. (2020) Identification of two endophytic fungi that control Septoria tritici blotch in the field, using a structured screening approach. *Biological Control*, **141**, 104128.

1111 Latz, M. A. C., Kerrn, M. H., Sørensen, H., Collinge, D. B., Jensen, B., Brown, J. K. M., et al.
1112 (2021) Succession of the fungal endophytic microbiome of wheat is dependent on tissue-
1113 specific interactions between host genotype and environment. *Science of the Total*
1114 *Environment*, **759**, 143804.

1115 Laur, J., Ramakrishnan, G. B., Labbé, C., Lefebvre, F., Spanu, P. D. & Bélanger, R. R. (2017)
1116 Effectors involved in fungal–fungal interaction lead to a rare phenomenon of
1117 hyperbiotrophy in the tritrophic system biocontrol agent–powdery mildew–plant. *New*
1118 *Phytologist*, **217**, 713–725.

1119 Li, X.-Z., Song, M.-L., Yao, X., Chai, Q., Simpson, W. R., Li, C.-J., et al. (2017) The effect of
1120 seed-borne fungi and *Epichloë* endophyte on seed germination and biomass of *Elymus*
1121 *sibiricus*. *Frontiers in Microbiology*, **8**, 2488.

1122 Lorito, M., Woo, S. L., Harman, G. E. & Monte, E. (2010) Translational research on
1123 *Trichoderma*: From 'omics to the field. *Annual Review of Phytopathology*, **48**, 395–417.

1124 Louda, S. M., Arnett, A. E., Rand, T. A. & Russell, F. L. (2003) Invasiveness of some biological
1125 control insects and adequacy of their ecological risk assessment and regulation.
1126 *Conservation Biology*, **17**, 73–82.

1127 Luna, E., Bruce, T. J. A., Roberts, M. R., Flors, V. & Ton, J. (2012) Next-generation systemic
1128 acquired resistance. *Plant Physiology*, **158**, 844–853.

1129 Ma, L. J., van der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M. J., Di Pietro, A.,
1130 et al. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in
1131 *Fusarium*. *Nature*, **464**, 367–373.

1132 Macías-Rodríguez, L., Guzmán-Gómez, A., García-Juárez, P. & Contreras-Cornejo, H. A.
1133 (2018) *Trichoderma atroviride* promotes tomato development and alters the root exudation
1134 of carbohydrates, which stimulates fungal growth and the biocontrol of the phytopathogen
1135 *Phytophthora cinnamomi* in a tripartite interaction system. *FEMS Microbiology Ecology*,
1136 **94**, doi: 10.1093/femsec/fiy137.

1137 Mahoney, A. K., Yin, C. & Hulbert, S. H. (2017) Community structure, species variation, and
1138 potential functions of rhizosphere-associated bacteria of different winter wheat (*Triticum*
1139 *aestivum*) cultivars. *Frontiers in Plant Science*, **8**, 132.

1140 Manzotti, A., Bergna, A., Burow, M., Jørgensen, H. J. L., Cernava, T., Berg, G., et al. (2020)
1141 Insights into the community structure and lifestyle of the fungal root endophytes of tomato
1142 by combining amplicon sequencing and isolation approaches with phytohormone profiling.
1143 *FEMS Microbiology Ecology*, **96**, iy137.

1144 Martínez-Diz, M. d. P., Díaz-Losada, E., Díaz-Fernández, Á., Bouzas-Cid, Y. & Gramaje, D.
1145 (2020) Protection of grapevine pruning wounds against *Phaeomoniella chlamydospora* and
1146 *Diplodia seriata* by commercial biological and chemical methods. *Crop Protection*,
1147 105465.

1148 Matarese, F., Sarrocco, S., Gruber, S., Seidl-Seiboth, V. & Vannacci, G. (2012) Biocontrol of
1149 Fusarium head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*.
1150 *Microbiology*, **158**, 98–106.

1151 Mauro, A., Garcia-Cela, E., Pietri, A., Cotty, P. J. & Battilani, P. (2018) Biological control
1152 products for aflatoxin prevention in Italy: commercial field evaluation of atoxigenic

Aspergillus flavus active ingredients. *Toxins*, **10**, 30.

Mcquilken, M. P., Gemmell J. & Lahdenperä M-L. (2001) *Gliocladium catenulatum* as a potential biological control agent of damping-off in bedding plants. *Journal of Phytopathology*, **149**, 171–178.

Medeiros, H. A. d., Araújo Filho, J. V. d., Freitas, L. G. d., Castillo, P., Rubio, M. B., Hermosa, R., et al. (2017) Tomato progeny inherit resistance to the nematode *Meloidogyne javanica* linked to plant growth induced by the biocontrol fungus *Trichoderma atroviride*. *Scientific Reports*, **7**, 40216.

Milgroom, M. G. & Cortesi, P. (2004) Biological control of chestnut blight with hypovirulence: a critical analysis. *Annual Review of Phytopathology*, **42**, 311–338.

Miljaković, D., Marinković, J. & Balešević-Tubić, S. (2020) The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*, **8**, 1037.

Mostafa, M. A. & Gayed, S. K. (1952) Effect of *Trichoderma* metabolites on growth of cotton plants. *Nature*, **169**, 359–360.

Mukherjee, P. K., Horwitz, B. A., Herrera-Estrella, A., Schmoll, M. & Kenerley, C. M. (2013) *Trichoderma* research in the genome era. *Annual Review of Phytopathology*, **51**, 105–129.

Nga, N. T. T., Giau, N. T., Long, N. T., Lübeck, M., Shetty, N. P., de Neergaard, E., et al. (2010) Rhizobacterially induced protection of watermelon against *Didymella bryoniae*. *Journal of Applied Microbiology*, **109**, 567–582.

1173 Ntana, F., Johnson, S. R., Hamberger, B., Jensen, B., Jørgensen, H. J. L. & Collinge, D. B.
1174 (2022) Regulation of tomato specialised metabolism after establishment of symbiosis with
1175 the endophytic fungus *Serendipita indica*. *Microorganisms*, 10, **194**

1176 Nunes, C. A. (2012) Biological control of postharvest diseases of fruit. *European Journal of*
1177 *Plant Pathology*, **133**, 181–196.

1178 Oerke, E. C. (2006) Crop losses to pests. *Journal of Agricultural Science*, **144**, 31–43.

1179 Oldroyd, G. E. D. & Staskawicz, B. J. (1998) Genetically engineered broad-spectrum disease
1180 resistance in tomato. *Proceedings of the National Academy of Sciences of the UNited*
1181 *States of America*, **95**, 10300–10305.

1182 Panka, D., Jeske, M. & Troczynski, M. (2013) Occurrence of *Neotyphodium* and *Epichloë* fungi
1183 in meadow fescue and red fescue in Poland and screening of endophyte isolates as
1184 potential biological control agents. *Acta Scientiarum Polonorum*, *Hortorum Cultus*, **12**,
1185 67–83.

1186 Papavizas, G. C. (1981) Biological control in crop production. In: *Beltsville Symposia in*
1187 *Agricultural Research*. Totowa, New Jersey, p. 461.

1188 Parke, J. L. & Gurian-Sherman, D. (2001) Diversity of the *Burkholderia cepacia* complex and
1189 implications for risk assessment of biological control strains. *Annual Review of*
1190 *Phytopathology*, **39**, 225–258.

1191 Paull, J. & Hennig, B. (2019) New World map of organic agriculture: Australia is 51%. *Acres*
1192 *Australia*, **101**, 35–36.

1193 Penyalver, R., Vicedo, B. & López, M. M. (2000) Use of the genetically engineered
1194 *Agrobacterium* strain K1026 for biological control of crown gall. *European Journal of*
1195 *Plant Pathology*, **106**, 801–810.

1196 Ponsone, M. L., Chiotta, M. L., Combina, M., Dalcero, A. & Chulze, S. (2011) Biocontrol as a
1197 strategy to reduce the impact of ochratoxin A and *Aspergillus* section Nigri in grapes.
1198 *International Journal of Food Microbiology*, **151**, 70–77.

1199 Pratt, J. E. (1999) PG suspension for the control of Fomes root rot of pine. In: *Information note*.
1200 Bristol: Forestry Commission.

1201 Pratt, J. E., Niemi, M. & Sierota, Z. H. (2000) Comparison of three products based on
1202 *Phlebiopsis gigantea* for the control of *Heterobasidion annosum* in Europe. *Biocontrol*
1203 *Science and Technology*, **10**, 467–477.

1204 Rabiey, M. & Shaw, M. W. (2016) *Piriformospora indica* reduces fusarium head blight disease
1205 severity and mycotoxin DON contamination in wheat under UK weather conditions. *Plant*
1206 *Pathology*, **65**, 940–952.

1207 Rabiey, M., Ullah, I. & Shaw, M. W. (2015) The endophytic fungus *Piriformospora indica*
1208 protects wheat from fusarium crown rot disease in simulated UK autumn conditions. *Plant*
1209 *Pathology*, **64**, 1029–1040.

1210 Rabiey, M., Ullah, I., Shaw, L. J. & Shaw, M. W. (2017) Potential ecological effects of
1211 *Piriformospora indica*, a possible biocontrol agent, in UK agricultural systems. *Biological*
1212 *Control*, **104**, 1–9.

1213 Ramírez-Valdespino, C. A., Casas-Flores, S. & Olmedo-Monfil, V. (2019) *Trichoderma* as a
1214 model to study effector-like molecules. *Frontiers in Microbiology*, **10**, 1030.

1215 Ray, P., Guo, Y., Kolape, J. & Craven, K. D. (2018) Non-targeted colonization by the
1216 endomycorrhizal fungus, *Serendipita vermifera*, in three weeds typically co-occurring with
1217 switchgrass. *Frontiers in Plant Science*, **8**, 2236.

1218 Reiss, A. & Jørgensen, L. N. (2017) Biological control of yellow rust of wheat (*Puccinia*
1219 *striiformis*) with Serenade®ASO (*Bacillus subtilis* strain QST713). *Crop Protection*, **93**,
1220 1–8.

1221 Rishbeth, J. (1963) Stump protection against *Fomes annosus*. *Annals of Applied Biology*, **52**, 63–
1222 77.

1223 Roberts, M. R. & Taylor, J. E. (2016) Exploiting plant induced resistance as a route to
1224 sustainable crop production. In: Collinge, D. B. (ed.) *Biotechnology for plant disease*
1225 *control*. New York and London: Wiley Blackwell, pp. 319–339.

1226 Rojas, E. C., Jensen, B., Jørgensen, H. J. L., Latz, M. A. C., Esteban, P., Ding, Y., et al. (2020a)
1227 Selection of fungal endophytes with biocontrol potential against Fusarium head blight in
1228 wheat. *Biological Control*, **144**, 104222.

1229 Rojas, E. C., Sapkota, R., Jensen, B., Jørgensen, H. J. L., Henriksson, T., Jørgensen, L. N., et al.
1230 (2020b) Fusarium head blight modifies fungal endophytic communities during infection of
1231 wheat spikes. *Microbial Ecology*, **79**, 397–408.

1232 Ross, A., Ward, S. & Hyman, P. (2016) More is better: selecting for broad host range

bacteriophages. *Frontiers in Microbiology*, **7**, 1352.

Roy, H. & Wajnberg, E. (2008) From biological control to invasion: the ladybird *Harmonia axyridis* as a model species. *BioControl*, **53**, 1–4.

Ryan, A. D. & Kinkel, L. L. (1997) Inoculum density and population dynamics of suppressive and pathogenic streptomyces strains and their relationship to biological control of potato scab. *Biological Control*, **10**, 180–186.

Sabri, M., Benkirane, R., Habbadi, K., Sadik, S., Ou-Zine, M., Diouri, M., et al. (2021) Phages as a potential biocontrol of phytobacteria. *Archives of Phytopathology and Plant Protection*, **54**, 1277–1291.

Santhanam, P., Labbé, C., Fietto, L. G. & Bélanger, R. R. (2021) A reassessment of flocculosin-mediated biocontrol activity of *Pseudozyma flocculosa* through CRISPR/Cas9 gene editing. *Fungal Genetics and Biology*, **153**, 103573.

Sarrocco, S., Esteban, P., Vicente, I., Bernardi, R., Plainchamp, T., Domenichini, S., et al. (2020) Straw competition and wheat root endophytism of *Trichoderma gamsii* T6085 as useful traits in the biocontrol of Fusarium head blight. *Phytopathology*, **111**, 1129–1136.

Sarrocco, S., Matarese, F., Moncini, L., Pachetti, G., Ritieni, A., Moretti, A., et al. (2013) Biocontrol of Fusarium head blight by spike application of *Trichoderma gamsii*. *Journal of Plant Pathology*, **S1**, 19–27.

Sarrocco, S., Mauro, A. and Battilani, P. (2019) Use of competitive filamentous fungi as an alternative approach for mycotoxin risk reduction in staple cereals: state of art and future

perspectives. *Toxins*, **11**, 701.

Sarrocco, S. & Vannacci, G. (2018) Preharvest application of beneficial fungi as a strategy to prevent postharvest mycotoxin contamination: A review. *Crop Protection*, **110**, 160–170.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N. & Nelson, A. (2019) The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, **3**, 430–439.

Schlatter, D., Kinkel, L., Thomashow, L., Weller, D. & Paulitz, T. (2017) Disease suppressive soils: new insights from the soil microbiome. *Phytopathology*, **107**, 1284–1297.

Shaw, R., Schaffner, U. & Marchante, E. (2016) The regulation of biological control of weeds in Europe – an evolving landscape. *EPPO Bulletin*, **46**, 254–258.

Shrivastava, S. & Varma, A. (2014) From *Piriformospora indica* to Rootonic: A review. *African Journal of Microbiology Research*, **8**, 2984.

Silva, K. J. P., Mahna, N., Mou, Z. & Folta, K. M. (2018) NPR1 as a transgenic crop protection strategy in horticultural species. *Horticulture Research*, **5**, 15.

St Martin, C. C. G. (2015) Potential of compost tea for suppressing plant diseases. *CAB Reviews*, **9**, 1–38.

Stenberg, J. A., Sundh, I., Becher, P. G., Björkman, C., Dubey, M., Egan, P. A., et al. (2021) When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science*, **94**, 665–676.

Stockwell, V. O. & Stack, J. P. (2007) Using *Pseudomonas* spp. for Integrated Biological

Control. *Phytopathology*, **97**, 244–249.

Sundh, I. & Eilenberg, J. (2021) Why has the authorization of microbial biological control agents been slower in the EU than in comparable jurisdictions? *Pest Management Science*, **77**, 2170–2178.

Sundheim, L. (1982) Control of cucumber powdery mildew by the hyperparasite *Ampelomyces quisqualis* and fungicides. *Plant Pathology*, **31**, 209–214.

Sybesma, W., Rohde, C., Bardy, P., Pirnay, J.-P., Cooper, I., Caplin, J., et al. (2018) Silk route to the acceptance and re-implementation of bacteriophage therapy—Part II. *Antibiotics*, **7**, 35.

Sztejnberg, A. (1993) *Ampelomyces quisqualis* Aq10, Cncm I-807, For biological control of powdery mildew. US: YISSUM RES DEV CO.

Taylor, J. M. & Snyder, W. E. (2021) Are specialists really safer than generalists for classical biocontrol? *BioControl*, **66**, 9–22.

Teperi, E., Keskinen, M., Ketoja, E. & Tahvonen, R. (1998) Screening for fungal antagonists of seed-borne *Fusarium culmorum* on wheat using in vivo tests. *European Journal of Plant Pathology*, **104**, 243–251.

Tripathi, P., Galla, A., Rabara, R. C. & Rushton, P. J. (2016) Transcription factors that regulate defence responses and their use in increasing disease resistance. In: Collinge, D. B. (ed.) *Plant pathogen resistance biotechnology.* New York and London: Wiley Blackwell, pp. 109–129.

Tronsmo, A., Collinge, D. B., Alabouvette, C. & Jensen, D. F. (2020) Biological control of plant

diseases. In: Tronsmo, A. M., Collinge, D. B., Djurle, A., Munk, L., Yuen, J. and Tronsmo, A. (eds.) *Plant pathology and plant diseases.* Wallingford: CABI, pp. 289–306.

Tuccci, M., Ruocco, M., De Masi, L., De Palma, M. & Lorito, M. (2011) The beneficial effect of Trichoderma spp. on tomato is modulated by the plant genotype. *Molecular Plant Pathology*, **12**, 341–354.

Uma, Bajaj, R., Bhola, D., Singh, S. & Varma, A. (2017) Biotechnological applications of *Piriformospora indica* (*Serendipita indica*) DSM 11827. *Advances in Biotechnology and Microbiology*, **3**, 555616.

Veloso, J., Alabouvette, C., Olivain, C., Flors, V., Pastor, V., García, T., et al. (2016) Modes of action of the protective strain Fo47 in controlling verticillium wilt of pepper. *Plant Pathology*, **65**, 997–1007.

Veselý, D. (1989) Biological control of damping-off pathogens by treating sugar-beet seed with a powdery preparation of the mycoparasite *Pythium Oligandrum* in large-scale field trials. In: Vančura, V. and Kunc, F. (eds.) *Developments in soil science*. Elsevier, pp. 445–449.

Vicente, I., Baroncelli, R., Morán-Diez, M. E., Bernardi, R., Puntoni, G., Hermosa, R., et al. (2020) Combined comparative genomics and gene expression analyses provide insights into the terpene synthases inventory in *Trichoderma*. *Microorganisms*, **8**, 1603.

Vlitos, A. J. & Hooker, W. J. (1951) The influence of sulfur on populations of *Streptomyces scabies* and other streptomycetes in peat soil. *American Journal of Botany*, **38**, 678–683.

Ward, M. G. (2016) Conclusions from the workshop on evaluation and regulation of biological

control agents. *EPPO Bulletin*, **46**, 239–242.

Weindling, R. (1932) *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*, **22**, 837–845.

Weindling, R. (1934) Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology*, **24**, 1153–1179.

Weindling, R. (1941) Experimental consideration of the mold toxins of *Gliocladium* and *Trichoderma*. *Phytopathology*, **31**, 991–1003.

Whipps, J. M. & Lumsden, R. D. (2001) Commercial use of fungi as plant disease biological control agents: status and prospects. In: Butt, T. M., Jackson, C. W. and Magan, N. (eds.) *Fungi as biological control agents*. *Progress*, *problems and potential*. Wallingford: CABI Publishing, pp. 9–22.

Whipps, J. M., Sreenivasaprasad, S., Muthumeenakshi, S., Rogers, C. W. & Challen, M. P. (2008) Use of *Coniothyrium minitan*s as a biocontrol agent and some molecular aspects of sclerotial mycoparasitism. *European Journal of Plant Pathology*, **121**, 323–330.

Xie, J., Xiao, X., Fu, Y., Liu, H., Cheng, J., Ghabrial, S. A., et al. (2011) A novel mycovirus closely related to hypoviruses that infects the plant pathogenic fungus *Sclerotinia sclerotiorum*. *Virology*, **418**, 49–56.

Xu, X. M., Jeffries, P., Pautasso, M. & Jeger, M. J. (2011a) Combined use of biocontrol agents to manage plant diseases in theory and practice. *Phytopathology*, **101**, 1024–1031.

1333 Xu, X. M., Jeffries, P., Pautasso, M. & Jeger, M. J. (2011b) A numerical study of combined use
1334 of two biocontrol agents with different biocontrol mechanisms in controlling foliar
1335 pathogens. *Phytopathology*, **101**, 1032–1044.

1336 Xu, X. M. & Jeger, M. J. (2013) Combined use of two biocontrol agents with different biocontrol
1337 mechanisms most likely results in less than expected efficacy in controlling foliar
1338 pathogens under fluctuating conditions: a modeling study. *Phytopathology*, **103**, 108–116.

1339 Yin, C., Casa Vargas, J. M., Schlatter, D. C., Hagerty, C. H., Hulbert, S. H. & Paulitz, T. C.
1340 (2021) Rhizosphere community selection reveals bacteria associated with reduced root
1341 disease. *Microbiome*, **9**, 86.

1342 Yu, X., Li, B., Fu, Y., Xie, J., Cheng, J., Ghabrial, S. A., et al. (2013) Extracellular transmission
1343 of a DNA mycovirus and its use as a natural fungicide. *Proceedings of the National*
1344 *Academy of Sciences of the United States of America*, **110**, 1452–1457.

1345 Zaki, O., Weekers, F., Thonart, P., Tesch, E., Kuenemann, P. & Jacques, P. (2020) Limiting
1346 factors of mycopesticide development. *Biological Control*, **144**, 104220.

1347 Zalles, V., Hansen, M. C., Potapov, P. V., Stehman, S. V., Tyukavina, A., Pickens, A., et al.
1348 (2019) Near doubling of Brazil's intensive row crop area since 2000. *Proceedings of the*
1349 *National Academy of Sciences of the United States of America*, **116**, 428–435.

1350 Zhang, H., Xie, J., Fu, Y., Cheng, J., Qu, Z., Zhao, Z., et al. (2020) A 2-kb mycovirus converts a
1351 pathogenic fungus into a beneficial endophyte for *Brassica* protection and yield
1352 enhancement. *Molecular Plant*, **13**, 1420–1433.

Żółciak, A., Sikora, K., Wrzosek, M., Damszel, M. & Sierota, Z. (2020) Why does *Phlebiopsis gigantea* not always inhibit root and butt rot in conifers? *Forests*, **11**, 129.

Figure legends

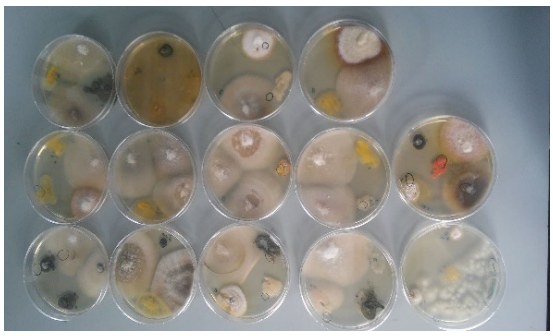
Figure 1 Two schemes for selecting potential biological control agents (BCAs). (1) Collect samples from an appropriate environment, e.g., from the habitat where the disease can be a problem. (2) Isolate, cultivate and (ideally) identify the organisms: risk assessment. (3a) test for BCA activity in a bioassay involving host, pathogen and BCA. (3b) test for direct activity of potential BCA against the pathogen in an in vitro system (left *Pseudomonas* and *Rhizoctonia*, right *Serendipita indica* and *Gaeumannomyces graminis*. (4) Plant assays in controlled environments (a) diseased (b) disease controlled by a BCA. (5) Risk assessment and mode of action studies. (6) Field trials. (7) Development, registration, licensing and marketing.

Figure 2 High-throughput assay for Fusarium head blight using detached spikelets (Rojas et al 2020a). (a) Water control, (b) *Fusarium graminearum* (*Fg*) control, (c) *Fg* + *Pseudozyma floculosa*, (d) *Fg* + *Penicillium olsonii*, (e)setup using large well plates.

Figure 3 Endophytic colonization of wheat root by *Trichoderma gamsii* T6085 7 days postinoculation: arrows indicate intracellular (dashed line) and intercellular (continuous line) colonization by *T. gamsii* T6085 hyphae. Fungal cells were detected with WGA-Alexa Fluor 488 (green channel): the plant cell wall was detected with FM4-64 dye (red channel) by confocal microscopy. (Photography: Sabrina Sarrocco & Marie Dufresne.)

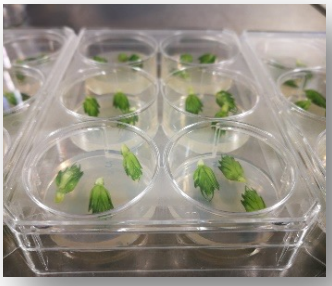
Figure 4 (a) Healthy powdery mildew colony on courgette (zucchini, *Cucurbita pepo*) leaves. h: hyphae; d: developing conidium on conidiophore; m: mature conidium (b) *Ampelomyces* sp. growing on the mycelium of powdery mildew and suppressing conidial production. p: pycnidia; h: mildew hypha; c: tip of mildew conidiophore. Note the absence of mildew spores: all mildew conidiophores are surrounded by *Ampelomyces* pycnidia. (Photography: Michael Shaw from surface strips on transparent sticky tape; pictures edited to remove air bubbles.)

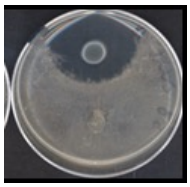
Figure 1 Schemes for selecting potential BCAs

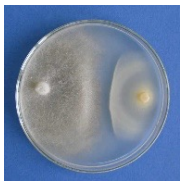


1. Harvest of source material from an appropriate environment.

2. Isolation, cultivation and (ideally) identification of microbes





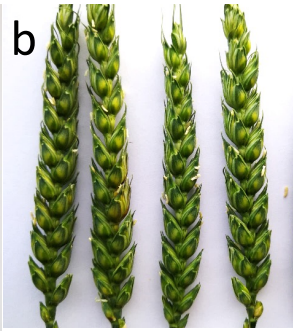


3a. Medium-high throughput disease assay

3b. High throughput confrontation assay

a

b

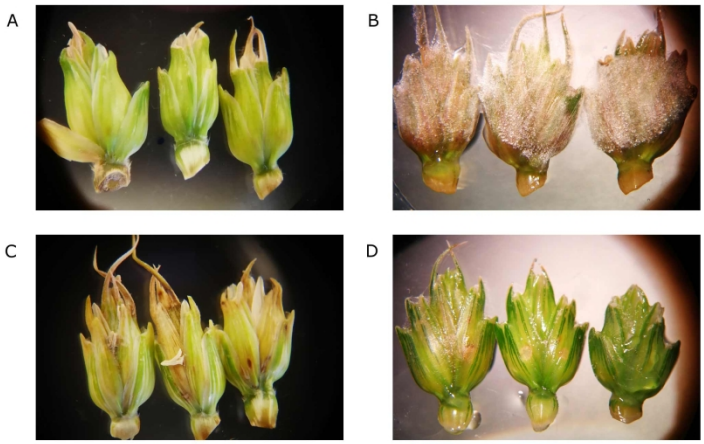


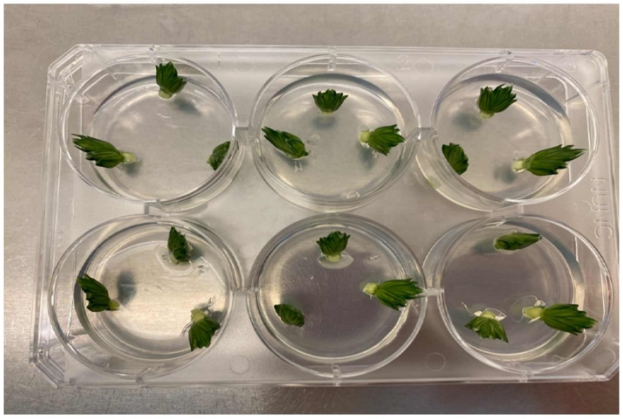
5.Risk assessment and mode of action studies

4. Plant assays in controlled environments
a. diseased b. disease controlled by a BCA

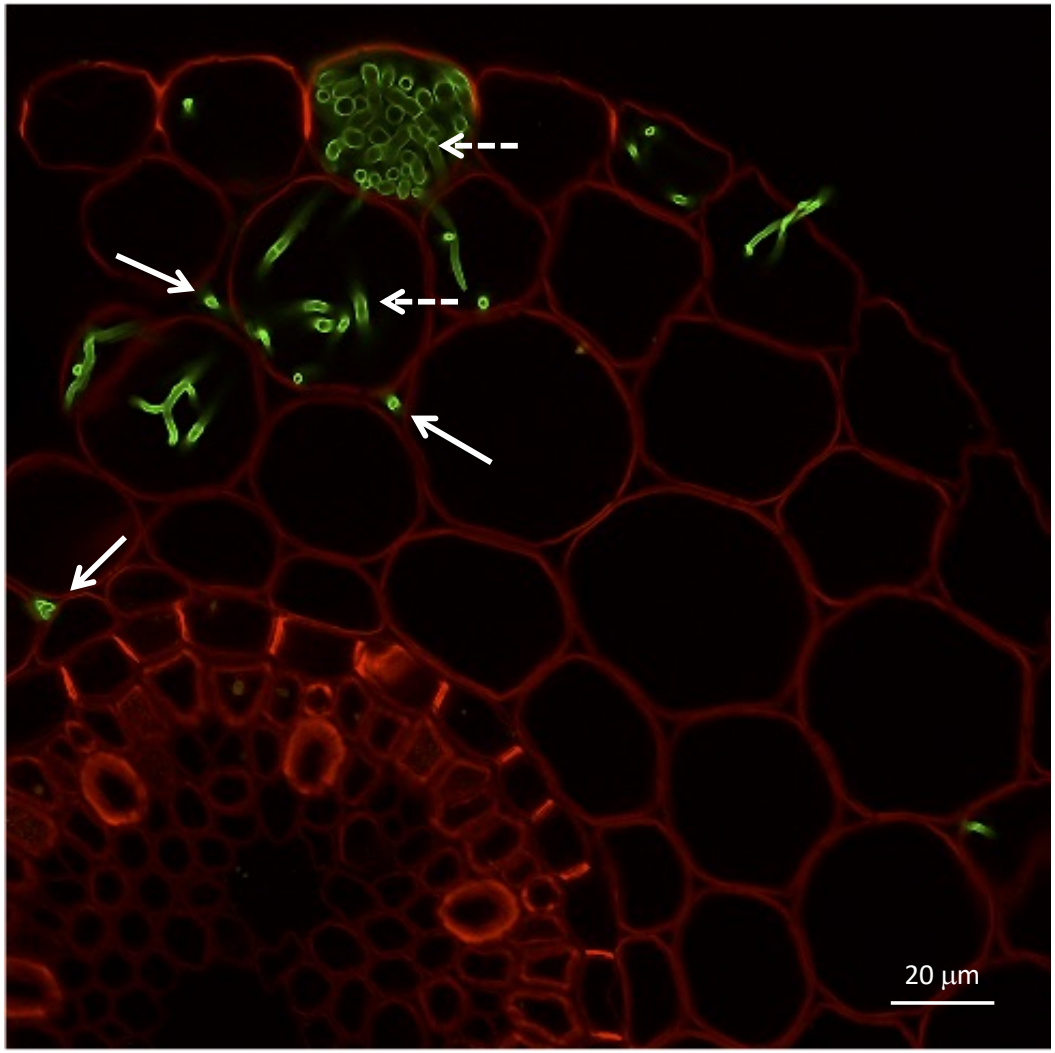
7. Development, registration, licensing and marketing

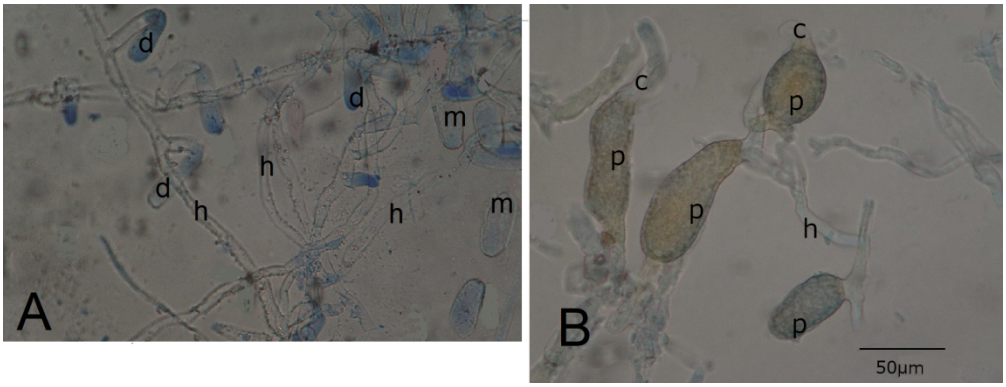
6. Field trials





629x891mm (100 x 100 DPI)


20 µm



323x122mm (144 x 144 DPI)

Table 1 Examples of commercial biological control products for controlling plant disease

Bioactive ingredient(s)	**Target (disease or pathogen)**	**Mechanism(s) and other information**	**Territories approved/marketed**	**Product name (supplier)**	**Reference**
Bacteria					
Agrobacterium radiobacter	Crown gall caused by *Agrobacterium tumefaciens*	Antibiosis and competition in wounds	Australia 1988, USA 2000, Turkey 2005	K84 or K1026 Galltroll, NOGALL® (Becker Underwood)	Kerr and Bullard (2020)
Bacillus amyloliquefaciens (formerly *B. subtilis*) QST 713	Many, e.g., yellow rust, *Pythium,* clubroot*;* bacteria	Antibiosis (lipopeptides), induced resistance	Global c.2005	Serenade (Bayer Crop Science)[a]	Reiss and Jørgensen (2017), Lahlali et al. (2011)
Bacillus subtilis GB03	Cotton wilts caused by *Rhizoctonia* and *Fusarium*	Antibiosis and competition	USA mid-1990s	Kodiak® (Gustafson, USA)	Brannen and Kenney (1997), Miljaković et al. (2020)

Pseudomonas chlororaphis MA342	Many, e.g., Fusarium crown rot	Endophyte in embryo: antibiosis	EU; USA 2001	Cedomon® (Lantmännen BioAgri, SE)	Chin-A-Woeng et al. (2003)
Pseudomonas sp. DSMZ13134	Soilborne pathogens	Competition for space and nutrients, induced resistance	EU 2013	Proradix® SP (Sourcon Padena, DE)	Anastassiadou et al. (2020)
Streptomyces griseoviridis	Many, includes, bacteria, fungi and oomycetes	Antibiosis and competition	Global Finland 1982, USA 1993	Mycostop® (Verdera)	Lahdenperä et al. (1991)
Fungi and oomycetes					
Ampelomyces quisqualis M10	Powdery mildew	Mycoparasitism	Global 1994	AQ10 (CBC Europe)	Sztejnberg (1993)
Aspergillus flavus NRRL 21882	Mycotoxigenic *Aspergillus* spp. on maize	Competition for nutrients and space	USA	Afla-Guard® GR (Syngenta)	Dorner and Lamb (2006)
Aspergillus flavus AF36	*Aspergillus fluvus* on cotton	Competition for nutrients and space	USA 2003	Afla-Guard® (Cicleone Globa)	Junaid et al. (2013)

Aspergillus flavus MUCL 54911	Mycotoxigenic *Aspergillus* spp. on maize	Competition for nutrients and space	Italy	AF-X1 (Pioneer Hi-Breed Italia)	Mauro et al. (2018)
Aureobasidium pullulans DSM 14940 + DSM 14941	Fire blight and postharvest diseases of pome fruits	Competition for space and nutrients, physical barrier against pathogens infection	EU	Blossom Protect (Manica)	Kunz (2004)
Candida oleophila I-182	*Botrytis* spp., *Penicillium* spp. on citrus, pome fruit	Induced resistance	USA 2001	Aspire (Ecogen, Inc.)	Gardener and Fravel (2002), Droby et al. (2002)
Coniothyrium minitans CON/M/91-08	*Sclerotinia sclerotiorum, Sclerotinia minor*	Mycoparasitism of sclerotia	Global 2001	Contans® WG (Bayer)	Whipps et al. (2008)
Gliocladium catenulatum J1446 (current	Soilborne pathogens and grey mould	Competition in rhizosphere, mycoparasitism,	EU 1998	Gliomix® Prestop (Verdera)	Mcquilken et al. (2001)

name *Clonostachys rosea*)		CWDE, antibiosis			
Gliocladium virens GL-21	*Rhizoctonia solani* and *Pythium* spp. on ornamentals, vegetables, cotton		USA 1990	GlioGard™, Soilgard (Thermo Trilogy Corp.)	Gardener and Fravel (2002), Junaid et al. (2013)
Phlebiopsis gigantea	Root and butt rot caused by *Heterobasidion annosum*	Competition (more)	EU 1994	Rotstop (Verdera)	Żółciak et al. (2020), Pratt et al. (2000)
Pseudozyma flocculosa	Powdery mildew on wheat, barley, grapevines, apple and vegetables	Parasitism	USA c.2000	Sporodex (Ecogen, Inc.)	Kiss (2003), Laur et al. (2017)

Pythium oligandrum M1	Grey mould and *Sclerotinia*	Mycoparasitism, induced resistance	EU c.2001	Polyversum® (Gowan), Polygandrum (Plant Production Institute, Slovakia)	Brozova (2002)
Trichoderma afroharzianum CBS 134709 (IBT 41409, G.J.S. 08-137)	Soilborne fungal plant pathogens (mostly food crops)	n/a	EU	Canna® (Canna International BV NL-Breda)	Degenkolb et al. (2015)
Trichoderma asperellum ICC012+ *Trichoderma gamsii* ICC080	Soilborne pathogens and grapevine trunk diseases	Competition for space and nutrients mycoparasitism	EU	Radix soil (Isagro), Remedier (Gowan) and others	Martínez-Diz et al. (2020), Gerin et al. (2018)
T. asperellum T25+ *Trichoderma atroviride* T11	Soilborne pathogens	Competition for space and nutrients, mycoparasitism, antibiosis	EU 2009	Tusal (Certis Europe)	Grondona et al. (2004)

Trichoderma guizhouense CBS 134707 (IBT 41407, G.J.S. 08-135)	Soilborne fungal plant pathogens		USA	Promot WP (JH BiotechInc., Ventura, CA, USA)	Dehenkolb et al. (2015)
Trichoderma harzianum + *Trichoderma polysporum*		Competition for space, mycoparasitism CWDE, antibiosis	Sweden	BinabT® (not authorized for as BCA in EU)	Khalil and Alsanius (2006)
T. harzianum T22	Root diseases	Competition in rhizosphere, mycoparasitism, CWDE, antibiosis, induced resistance	USA 1990, EU	Root Shield® (Bioworks), Trianum-P (Koppert)	Blaya et al. (2013)
T. harzianum CBS 134708	Soilborne fungal plant pathogens		EU	Vitalin (Vitalin Pflanzengesundhei	Degenkolb et al. (2015)

(IBT 41408, G.J.S. 08-136)				t GmbH,D-Ober-Ramstadt)	
Trichoderma simmonsii CBS 134706 (IBT 41406, G.J.S. 08-134)	Soilborne fungal plant pathogens		EU	Trichosan® (Vitalin Pflanzengesundheit GmbH,D-Ober-Ramstadt)	Degenkolb et al. (2015)
Serendipita indica (syn. *Piriformospora indica*)	A wide range of mostly soilborne pathogens	Improves nutrient uptake, but also induces resistance	India	Rootonic: SOM Phytopharma	Shrivastava and Varma (2014), Uma et al. (2017)
Bacteriophage					
Bacteriophage cocktail	Pierce's disease on vine (*Xyella fastidiosa*)	Parasitism	California	XylPhi-PD, Wilbur-Ellis	Das et al. (2015)
Bacteriophage (presumably a cocktail but not stated)	*Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas citri* pv. *citri*,	Parasitism	USA, Hungary	AgriPhage XCV, AgriPhage-Citrus Canker, AgriPhage PST, AgriPhage CMM, AgriPhage-	https://www.agriphage.com/product-info/, https://www.apsbiocontrol.com/products, http://www.erwiphage.com/

	Pseudomonas syringae pv. *tomato**, *Clavibacter michiganensis* subsp. *michiganensis*, soft-rot bacteria of potatoes			Fire Blight, Biolyse-BP, Erwiphage	
Consortium of bacteriophage	Postharvest soft rot of potato	Parasitism	UK	Biolyse-PB, APS biocontrol	https://www.apsbiocontrol.com/products
Consortia					
Consortium comprising *Glomus intraradices*, *Funneliformis* (*Glomus*) *mosseae*, *T. a*	Not specified	Biostimulant	Italy	Coveron, Hello Nature	https://www.hello-nature.com/int/product/coveron-leguminose/

atroviride and PGPR	

[a]https://cropscience.bayer.co.uk/our-products/fungicides/serenade-aso/.

For USA see also Fravel (2005). CWDE, cell wall-degrading enzyme.

Table 2 Challenges and risks during product development

Stage	Challenge	Choices	Risk
Selection of isolate	Access and benefit sharing requirements re. sourcing and future use?	Choose best currently available isolates or search for better	Nagoya protocol
Development	Production	Wet or dry fermentation	Cost effectiveness
	Formulation	Powder, liquid	
	Shelf life	Temperature and humidity during storage, formulation	Requirements too stringent (e.g., −20°C)
	Compatibility with existing technologies	Mix with other products	No suitable mixes
Delivery systems	Seed treatment (seed coating, biopriming, etc.)	Use of existing equipment	Specialist equipment needed
	Incorporation in growth substrates, spray application for upper part of plants	Growth substrate, incorporation method	Incompatible with biome in the medium
	Drench, broadcast, in furrow	Use of existing equipment or specialist development	
	Dusting, spraying, vector dispersal	As above	
Regulatory and industrial approval	Risk assessment (EU, EPA, etc.)	Scenarios	Refusal, or onerous conditions
	Field performance – GEP efficacy	Scale and scope of testing	Not quite good enough
	Ecology of the BCA and antagonist	A research-intensive part of the development	Unfavourable pathogen interactions

Full commercialization	Market size and market introduction	Partners, advisory support, publicity, pricing policy	Market too small to recoup development costs