

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

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1 Running head: COLLINGE et al.

2 Review

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

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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 Health (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-TTM 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 MycostopTM, a Finnish product based on a strain of *Streptomyces griseoviridis*, PolygandrumTM—a *Pythium oligandrum*-based product that was also sold in Europe (Veselý, 1989) and in the USA, GlioGardTM 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

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

4 How to find a new BCA

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

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

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

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

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

Consortia, that is, mixtures of microorganisms, are receiving increasing attention as a 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 AlfSAFE and similar products for controlling aflatoxin contamination using nontoxigenic *Aspergillus flavus* strains to compete with the toxigenic forms

(Amaiike & 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/m² 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).

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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

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

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

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

Induced resistance is a well-studied phenomenon in the laboratory and there are good laboratory examples of this as a mode of action. However, induced resistance will be ineffective against existing high population densities of pathogen. Interaction with target pathogens via 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 *Npr1* (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

promoters for driving the expression of such genes. The use of tissue-specific promoters 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

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

528 Microbiota can increase natural soil suppressiveness against soilborne pathogens
529 particularly when intensive cropping systems (with high inputs of synthetic chemicals, low soil
530 organic matter accumulation, little humification and frequent soil tillage) are the primary reasons
531 for soil depletion (Cook, 1992). Soil microbiota associated with biocontrol can be a key factor in
532 the beneficial influence of agronomic practices on plant health (French et al., 2021). Next-
533 generation sequencing often offers a deeper characterization of the soil microbial community
534 during microbiome manipulation. This may allow more mechanistic understanding of what is
535 happening and the effect on crops in terms of soil suppressiveness and so help to limit
536 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

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

9 Risk assessment

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

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

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

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

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

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

647 However, in some countries, most prominently in Brazil, people from farms are starting
648 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).

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

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

11 Viruses as management tools against bacteria and fungi

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

724 Bacteria are difficult to control other than by cultural practice and disease resistance if
725 available. Recent studies suggest the potential for bacteriophages to control bacterial diseases
726 (Ahern et al., 2014; Carstens et al., 2019), and indeed the first product—against Pierce’s
727 disease—has now reached the market (Table 1): based on a cocktail of four bacteriophages (Das
728 et al., 2015). The use of bacteriophages controlling human disease has been explored since their
729 discovery (Abedon et al., 2011; Furfaro et al., 2018; Sybesma et al., 2018). A challenge with
730 bacteriophages is the need to prepare mixtures of phages specific to each of the component
731 genotypes in the mixture of host bacterial types causing a problem. This also means that
732 resistance is likely to be a major and rapidly arising problem, because of the naturally occurring
733 host–phage coevolutionary race that indeed underlies the need to use mixtures from the start.
734 Thus, there are two points here: (a) specific matching for effectiveness, and (b) the complications
735 of the evolutionary process driven by host–phage matching. The need to use tailored mixtures
736 was an important reason why phage therapy for humans has developed slowly in Western
737 medicine—the Soviet Union block used it, but needed to maintain large banks of phages against
738 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

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

818

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828

829 Data availability statement

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

831

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1355

1356 Figure legends

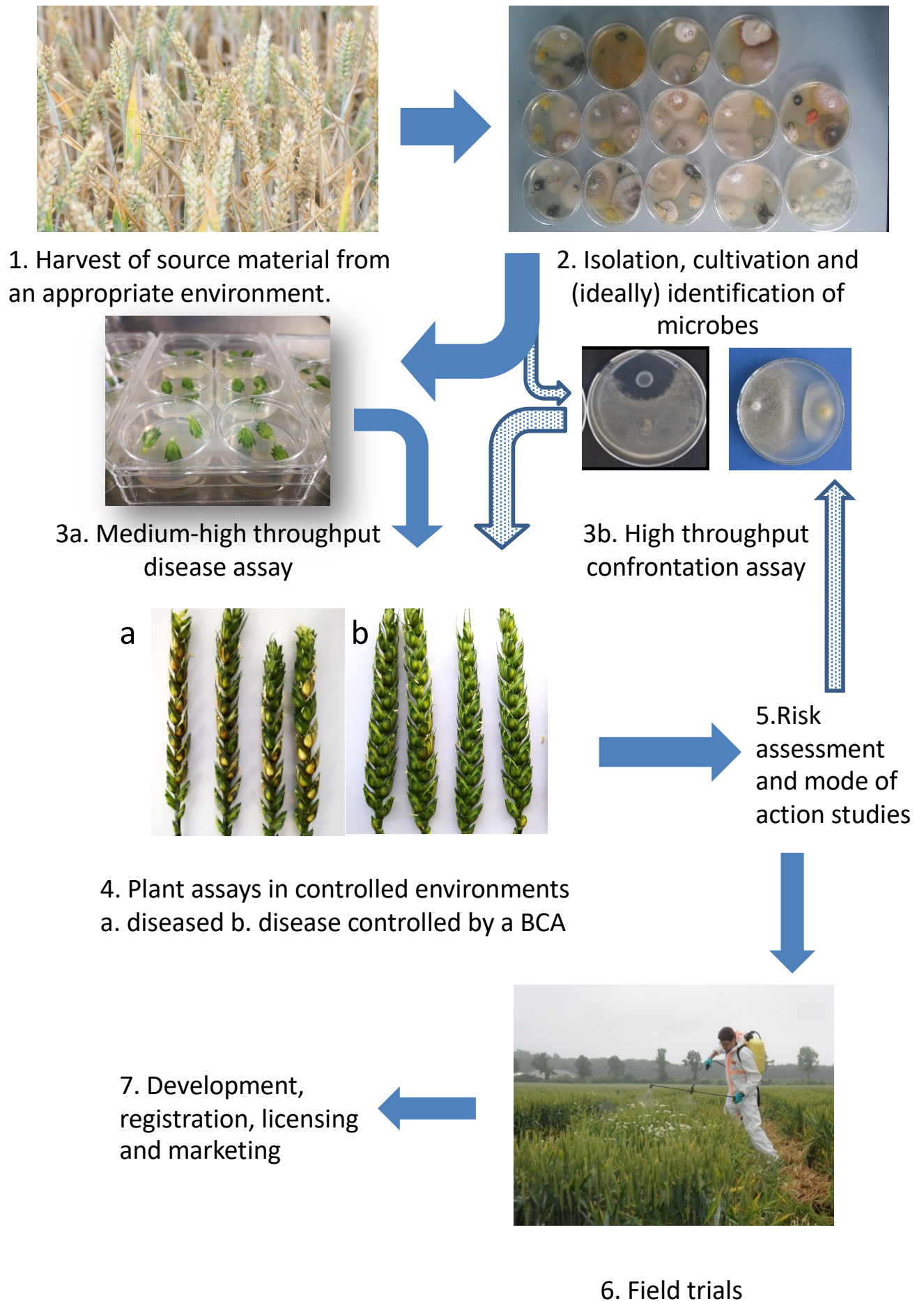
1357 **Figure 1** Two schemes for selecting potential biological control agents (BCAs). (1) Collect
1358 samples from an appropriate environment, e.g., from the habitat where the disease can be a
1359 problem. (2) Isolate, cultivate and (ideally) identify the organisms: risk assessment. (3a) test for
1360 BCA activity in a bioassay involving host, pathogen and BCA. (3b) test for direct activity of
1361 potential BCA against the pathogen in an in vitro system (left *Pseudomonas* and *Rhizoctonia*,
1362 right *Serendipita indica* and *Gaeumannomyces graminis*. (4) Plant assays in controlled
1363 environments (a) diseased (b) disease controlled by a BCA. (5) Risk assessment and mode of
1364 action studies. (6) Field trials. (7) Development, registration, licensing and marketing.

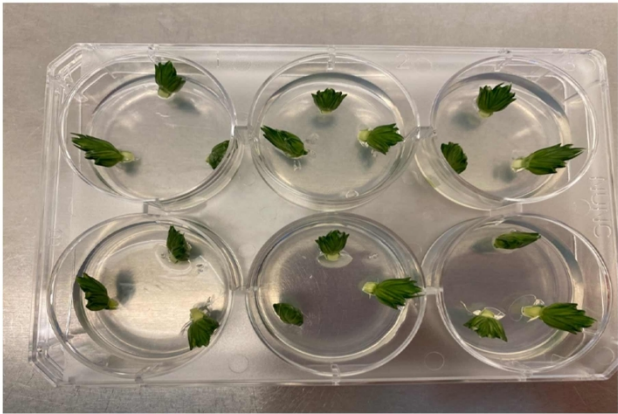
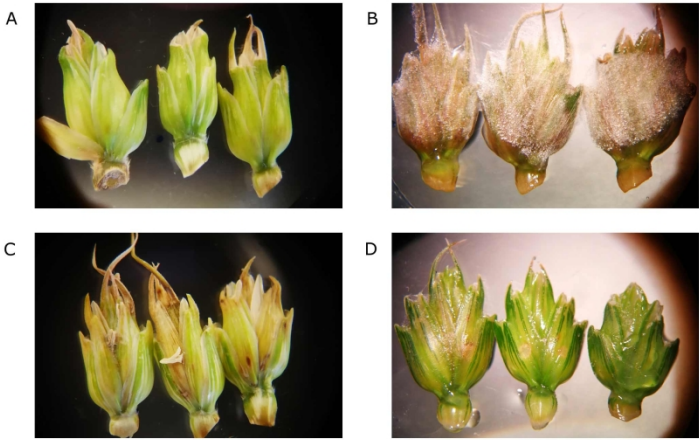
1365 **Figure 2** High-throughput assay for Fusarium head blight using detached spikelets (Rojas et al
1366 2020a). (a) Water control, (b) *Fusarium graminearum* (Fg) control, (c) Fg + *Pseudozyma*
1367 *floculosa*, (d) Fg + *Penicillium olsonii*, (e) setup using large well plates.

1368 **Figure 3** Endophytic colonization of wheat root by *Trichoderma gamsii* T6085 7 days
1369 postinoculation: arrows indicate intracellular (dashed line) and intercellular (continuous line)
1370 colonization by *T. gamsii* T6085 hyphae. Fungal cells were detected with WGA-Alexa Fluor 488
1371 (green channel): the plant cell wall was detected with FM4-64 dye (red channel) by confocal
1372 microscopy. (Photography: Sabrina Sarrocco & Marie Dufresne.)

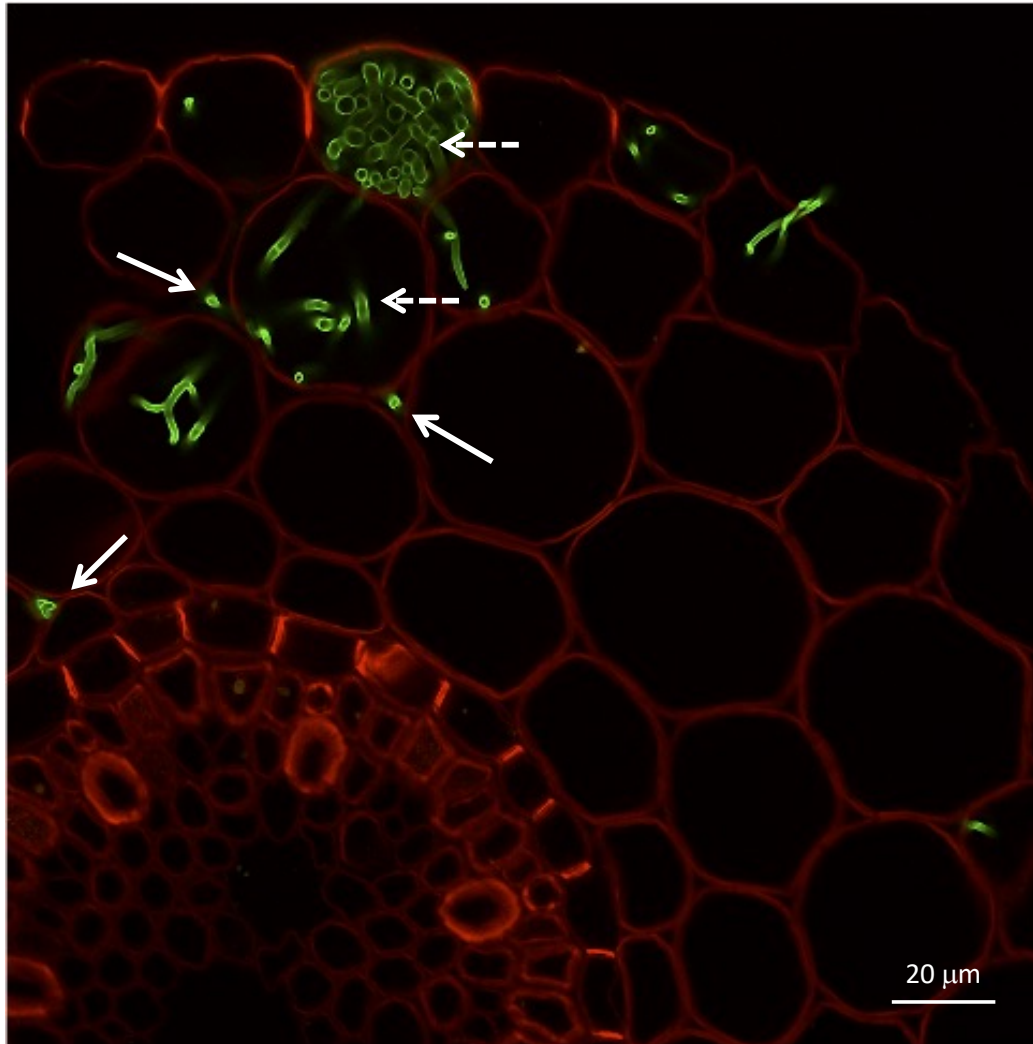
1373 **Figure 4** (a) Healthy powdery mildew colony on courgette (zucchini, *Cucurbita pepo*) leaves. h:
1374 hyphae; d: developing conidium on conidiophore; m: mature conidium (b) *Ampelomyces* sp.
1375 growing on the mycelium of powdery mildew and suppressing conidial production. p: pycnidia;
1376 h: mildew hypha; c: tip of mildew conidiophore. Note the absence of mildew spores: all mildew
1377 conidiophores are surrounded by *Ampelomyces* pycnidia. (Photography: Michael Shaw from
1378 surface strips on transparent sticky tape; pictures edited to remove air bubbles.)

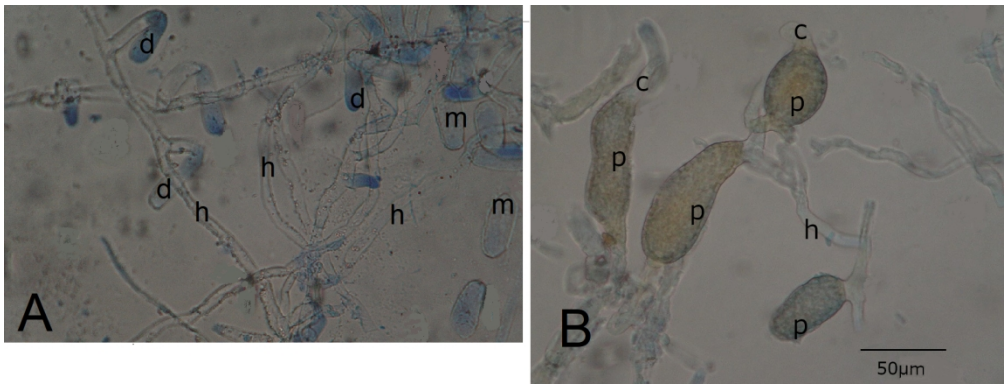
Figure 1 Schemes for selecting potential BCAs





629x891mm (100 x 100 DPI)





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					
<i>Agrobacterium radiobacter</i>	Crown gall caused by <i>Agrobacterium tumefaciens</i>	Antibiosis and competition in wounds	Australia 1988, USA 2000, Turkey 2005	K84 or K1026 Galltrol, NOGALL® (Becker Underwood)	Kerr and Bullard (2020)
<i>Bacillus amyloliquefaciens</i> (formerly <i>B. subtilis</i>) QST 713	Many, e.g., yellow rust, <i>Pythium</i> , clubroot; bacteria	Antibiosis (lipopeptides), induced resistance	Global c.2005	Serenade (Bayer Crop Science) ^a	Reiss and Jørgensen (2017), Lahlali et al. (2011)
<i>Bacillus subtilis</i> GB03	Cotton wilts caused by <i>Rhizoctonia</i> and <i>Fusarium</i>	Antibiosis and competition	USA mid-1990s	Kodiak® (Gustafson, USA)	Brannen and Kenney (1997), Miljaković et al. (2020)

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

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

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

<i>Pythium oligandrum</i> M1	Grey mould and <i>Sclerotinia</i>	Mycoparasitism, induced resistance	EU c.2001	Polyversum® (Gowan), Polygandrum (Plant Production Institute, Slovakia)	Brozova (2002)
<i>Trichoderma afroharzianum</i> 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)
<i>Trichoderma asperellum</i> ICC012+	Soilborne pathogens and grapevine	Competition for space and nutrients	EU	Radix soil (Isagro), Remedier (Gowan) and others	Martínez-Diz et al. (2020), Gerin et al. (2018)
<i>Trichoderma gamsii</i> ICC080	trunk diseases	mycoparasitism			
<i>T. asperellum</i> T25+	Soilborne pathogens	Competition for space and nutrients,	EU 2009	Tusal (Certis Europe)	Grondona et al. (2004)
<i>Trichoderma atroviride</i> T11		mycoparasitism, antibiosis			

<i>Trichoderma guizhouense</i> 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)
<i>Trichoderma harzianum</i> + <i>Trichoderma polysporum</i>		Competition for space, mycoparasitism	Sweden	BinabT® (not authorized for as BCA in EU)	Khalil and Alsanius (2006)
<i>T. harzianum</i> T22	Root diseases	Competition in rhizosphere, mycoparasitism, CWDE, antibiosis, induced resistance	USA 1990, EU	Root Shield® (Bioworks), Trianium-P (Koppert)	Blaya et al. (2013)
<i>T. harzianum</i> 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)	
<i>Trichoderma</i> <i>simmonsii</i> CBS 134706 (IBT 41406, G.J.S. 08- 134)	Soilborne fungal plant pathogens		EU	Trichosan® (Vitalin Pflanzengesundhei t GmbH,D-Ober- Ramstadt)	Degenkolb et al. (2015)
<i>Serendipita</i> <i>indica</i> (syn. <i>Piriformospora</i> <i>indica</i>)	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 (<i>Xyella</i> <i>fastidiosa</i>)	Parasitism	California	XylPhi-PD, Wilbur-Ellis	Das et al. (2015)
Bacteriophage (presumably a cocktail but not stated)	<i>Xanthomonas</i> <i>campestris</i> pv. <i>vesicatoria</i> , <i>Xanthomonas</i> <i>citri</i> pv. <i>citri</i> ,	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/

	<i>Pseudomonas syringae</i> pv. <i>tomato</i> *, <i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> , 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 <i>Glomus intraradices</i> , <i>Funneliformis</i> (<i>Glomus</i>) <i>mosseae</i> , <i>T. a</i>	Not specified	Biostimulant	Italy	Coveron, Hello Nature	https://www.hello-nature.com/int/product/coveron-leguminose/

atroviride and

PGPR

^a<https://cropsscience.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	
Delivery systems	Compatibility with existing technologies	Mix with other products	Requirements too stringent (e.g., -20°C)
	Seed treatment (seed coating, biopriming, etc.)	Use of existing equipment	No suitable mixes
	Incorporation in growth substrates, spray application for upper part of plants	Growth substrate, incorporation method	Specialist equipment needed
	Drench, broadcast, in furrow	Use of existing equipment or specialist development	
Regulatory and industrial approval	Dusting, spraying, vector dispersal	As above	Incompatible with biome in the medium
	Risk assessment (EU, EPA, etc.)	Scenarios	
	Field performance – GEP efficacy	Scale and scope of testing	
	Ecology of the BCA and antagonist	A research-intensive part of the development	Not quite good enough
			Unfavourable pathogen interactions

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