Chapter 22
Combining Biocontrol Agents and Organics Amendments to Manage Soil-Borne Phytopathogens

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22.1 Introduction

A huge amount of agrochemicals are currently used to ensure the health of our crops. Thus, world sales of fungicides reached US$9.91 billion in 2010 and have increased annually by 6.5 % since 1999 (Hirooka and Ishii 2013). In 2013, FAO and WHO published the maximum tolerated levels for residues of 57 different fungicides used in agriculture worldwide (Codex Alimentarius database 2013, www.codexalimentarius.net). The increasing use/misuse of chemicals poses serious collateral problems such as environmental pollution (Ongley 1996), development of pathogen/pest resistance (Sparks 2013; Tupe et al. 2014), residual toxicity towards (micro)organisms (Yoom et al. 2013), and loss of biodiversity (Ghorbani et al. 2008). For example, the emergence of resistant strains of diverse phytopathogens to widely used, chemically based biocides is an increasing problem arising in many areas after the continuous use of these products (Brent and Hollomon 2007). The Fungicide Resistance Action Committee (2013, www.frac.info) periodically reviews the list of resistant plant pathogenic microorganisms, and the number increases after each report release. Indeed, five new pathogen resistances were documented and registered only in 2013. Development of pathogen resistance does not only affect crop production but also human health in two ways: (1) directly, since increasing biocide dosages means more residues potentially enhancing the risk for human (and animal) health and (2) indirectly, because resistance can also be acquired by opportunistic human pathogens (Lelièvre et al. 2013). Moreover, agrochemical treatments are mostly nonspecific and do not only affect target

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pathogens but also other microorganisms which are potentially beneficial to soil/plant health (Ranganathswamy et al. 2013). While problems related to the abuse/misuse of chemically based biocides are evident and perceived by consumers as highly concerning because of their side effects, many crop diseases are currently difficult, if not impossible, to manage without the use of chemicals. Therefore, an urgent need to develop and implement novel plant disease control strategies is highly demanded. Furthermore, these strategies are claimed to fit synonymous concepts such as "eco-friendly," "environmentally friendly," "nature friendly," or even "green," which can be applied at any stage from production to commercialization of a given crop. All these terms have the same meaning, i.e., "not harmful to the environment and to humans." Strategies based on this concept are thus considered healthier and safer than the traditional disease/pest control measures by means of chemical inputs. Nevertheless, according to sustainable agriculture criteria, the interdependence between economic and environmental aspects should not be forgotten. Thus, to attain sustainability a complete ban of chemical inputs is not always possible without compromising the viability of many farms devoted to specific crops in defined geographical areas. In order to achieve this primary goal, research on disease control management must therefore be focused on strategies aiming to avoid, or at least to greatly reduce, the high dependence on chemical inputs by implementing integrated disease management (IDM) frameworks (see, for instance, López-Escudero and Mercado-Blanco 2011). These approaches consist in the combined use of all available countermeasures effective against a given crop disease. The phytopathological challenge thus consists in that the increasing utilization of nonchemical strategies to control plant diseases and pests (i.e., lower dependence of pesticides, fungicides, and soil volatile disinfectants) should affect neither the production of food nor the economic viability of the farming business (Hamblin 1995). Profits derived from these strategies are not only economic and environmental but they also constitute the best approach to confront emerging pathogen(s) resistance(s) derived from the continuous use of fungicides (Brent and Hollomon 2007).

The aim of the present chapter is to provide a brief overview on research efforts devoted to the use of biological control agents (BCAs) and organic amendments (OAs) against soil-borne diseases within IDM strategies. More specifically, we will focus on the ad hoc combination of BCAs and OAs. Furthermore, we have tried to discuss aspects such as how these approaches may influence soil microbial communities or the suitability of using OAs as carriers to develop more stable and effective formulations of BCAs. Finally, even though literature about the combined application of soil amendments and BCAs against soil-borne diseases is abundant, information regarding its implementation in woody plants is very scant. Therefore, we will also discuss whether this control approach is feasible in tree crops and forestry under field conditions. But first, we will briefly present a few general concepts that the reader will find closely associated along the text.

22.2 Biological Control Agents

Biological control (biocontrol) emerges as one of the most promising alternatives to chemical control. Biocontrol can be defined as "the reduction of a phytopathogen inoculum amount, or its ability to cause disease, by means of the activities of one or more [micro]organisms (except human being)" (Cook and Baker 1983), or "the use of natural or modified organisms, genes, or gene products to reduce the effects of pests and diseases" (Cook 1988). Besides this main aim, implementation of biocontrol measures can lead to an increase in the number, diversity, and activity of nonpathogenic microbial communities originally present in soils and that can antagonize deleterious microorganisms. Without any doubt, biocontrol tools are environmentally friendly and can be implemented in combination with additional chemical, physical, and/or agronomical measures within IDM frameworks (López-Escudero and Mercado-Blanco 2011). Biological control can be used either as preventive or palliative strategy. Concerning plant diseases, biocontrol mainly relies on the artificial introduction of microbial antagonists, the so-called BCAs, to the targeted pathosystem. Nevertheless, biocontrol can also be based on strategies aiming to the modification of the microbial communities present in a particular agro-ecosystem, and/or their activities, by implementing specific agricultural practices. This can be achieved, for instance, by using suppressive soils (see, for instance, Mazzola 2002) or OAs (see below). The effective utilization of BCAs should be based on a profound knowledge of the mechanisms involved in biocontrol (i.e., competition, antibiosis, mycoparasitism, induction of defense responses, etc.), and on how the BCA performance can be affected by the broad range of (a) biotic factors which are dynamically interacting in any given pathosystem. Among BCAs, the species belonging to the genus *Trichoderma* are one of the most widely used microorganisms as biofungicides (Zaidi and Singh 2013). Characteristics like cosmopolitan distribution, adaptability to different soils, direct antagonism against plant pathogens (through mechanisms such as mycoparasitism, production of a large number of secondary metabolites, and/or competition), plant growth promotion, induction of systemic resistance, enhanced tolerance to abiotic stresses, compost colonization, and decomposition of organic matter (Zaidi and Singh 2013) make these fungi as one of the microorganisms best studied (and utilized) not only as BCA but also as biofertilizers (Woo et al. 2014). *Trichoderma* spp. isolates have thus been used to control pathogens from roots to leaves, either in herbaceous or woody plants (Zaidi and Singh 2013). Besides *Trichoderma*, many beneficial bacteria have been also studied as BCAs, the most frequent genera being *Agrobacterium* (e.g., Kawaguchi and Inoue 2012), *Bacillus* (e.g., Ruano-Rosa et al. 2014), *Pseudomonas* (e.g., Mercado-Blanco and Bakker 2007), and *Streptomyces* (e.g., Weiland 2014). Their biocontrol mechanisms can be antibiosis, competition for (micro) nutrients, colonization for specific sites needed for the pathogen to infect the plant, and/or induction of resistance by activating host plant defense responses (Narayanasami 2013). Many examples in which biocontrol bacteria have been successfully applied are available. However, this topic falls out the scope of this chapter and has been reviewed extensively elsewhere (see, for instance, Compant et al. 2013; Suárez-Estrella et al. 2013). Besides these two groups of microorganisms, mycorrhizal fungi (e.g., Ismail et al. 2013), nonpathogenic fungi (e.g., Abeysinghe 2009), or hypovirulent isolates of mycoviruses (Milgroom and Cortesi 2004) have also been studied and used as BCAs.

22.3 Organic Amendments Specified

The aim of this chapter is not to perform a comprehensive review of all materials considered as OA. We particularly aim to review cases in which such substrates have been used in combination with BCAs (see below). FAO defines Soil Amendment as "those materials that are applied to the soil to correct a major constraint other than low nutrient content" (Food and Agriculture Organization of the United Nations 2010a). The Soil Science Society of America defines OA as "any material such as lime, gypsum, sawdust, compost, animal manures, crop residue, or synthetic soil conditioners that is worked into the soil or applied on the surface to enhance plant growth. Amendments may contain important fertilizer elements, but the term commonly refers to added materials other than those used primarily as fertilizers" (Soil Science Glossary Terms Committee 2008). Organic amendments are used with the objective to improve the physical properties of soil, either directly or by activating living (micro) organisms present in the soil. They include organic materials, sometimes considered as waste, with a highly diverse composition and from a wide range of animal and vegetal origins (Food and Agriculture Organization of the United Nations 2010a). Sphagnum peat, wood chips, grass clippings, straw, compost, manure, biosolids, sawdust, and wood ash are considered, among others, OA (Davis and Whiting 2014). Amendments like charcoal or biochar, a solid carbon-rich product from biomass pyrolysis, will not be considered in this chapter. However, it is worth mentioning that these soil amendments are applied not only as fertilizers but also against foliar and soil-borne diseases. On the effect of biochar application on crop productivity and disease suppression, interested readers can consult, for instance, Atkinson et al. (2010) or Jaiswal et al. (2014).

Organic amendments have been used in many ways in agriculture, mainly as non-synthetic fertilizers. The use of OA contributes to reduce agrochemical inputs, thereby minimizing residues originated from farming activity (Trillas et al. 2006). One of the most interesting and promising applications of OAs relies on their ability to lessen the deleterious effects of pathogen attacks to acceptable thresholds (Boulter et al. 2002). There are many examples describing the successful use of OAs to control pathogens (including bacteria, fungi, and nematodes) (Bailey and Lazarovits 2003), to reduce their incidence (e.g., Borrego-Benjumea et al. 2014), or to isolate OA-residing microorganisms that may be applied against phytopathogens because of their proven antagonistic activity (e.g., Kavroulakis et al. 2010). Concerning the use of OA in plant disease control, Agrios (2005) includes soil amendment within biological control methods since they can stimulate antagonistic microbiota to pathogens present in soil, have an organic origin, and usually harbor beneficial microorganisms. Others, however, consider this approach within the category of farming practices control measures or even as category on its own: soil amendment control (Deepak 2011). Considering these premises (stimulation of soil microbiota, content of beneficial microorganisms, etc.) we consider the use of OA as a biological control strategy.

The effectiveness and consistency of OA in disease suppression are influenced, among other factors, by the target pathosystem and by the own variability (i.e., original sources, chemical characteristics, etc.) of the OA. Indeed, the number of pathosystems is huge and modifications/changes in the composition and characteristics of any given OA can enormously vary as well. Mechanisms of disease suppression displayed by OA can also be diverse. Furthermore, increase of disease incidence after the use of an amendment has been occasionally reported (Noble 2011). Therefore, finding the right application strategy for any OA needs of an in-depth knowledge of (1) the pathosystem, (2) the characteristics of the OA, (3) the environmental (biotic and abiotic) factors present in the site of application, and (4) how multitrophic interactions taking place in this site can be influenced by the addition of the OA, which usually carries a diverse microbiota as well. It has thus been shown that results obtained after OAs application can be highly variable and inconsistent. For instance, household waste-based compost batches usually present lack of uniformity. It is therefore of utmost importance to develop protocols to guarantee reproducible disease suppression results upon application of these amendments (Giotis et al. 2009).

Finally, it is also crucial to pay attention to the original source from which materials employed as OAs are derived since they might even contribute to pathogen spread. Indeed, it has been demonstrated that fresh manure from sheep previously fed in a cotton field affected with *Verticillium dahliae* Kleb., contained and transmitted pathogen propagules (microsclerotia) thereby contributing to the increase of the pathogen population in soil (López-Escudero and Blanco-López 1999).

22.4 Soil-Borne Pathogens: The Specific Target of OA and BCA in Disease Management Strategies

Soils contain a huge amount of organisms, many of them with the capacity to cause diseases in plants, viz., viruses, phytoplasmas, nematodes, protozoa, parasitic phanerogams, fungi, and bacteria. Fungi and oomycetes are likely the most important groups of soil-borne pathogens because of their number, diversity, and crop production losses produced by their attacks (García-Jiménez et al. 2010). For example, some 40 soil-borne pathogens cause important diseases in potato (*Solanum tuberosum* L.) tubers, the fourth main food crop in the world (Fiers et al. 2012). Numerous contributing factors help to understand why soil-borne pathogens are serious biotic constraints for many plants and why their efficient control is so difficult. For instance, many of them are able to produce resistance structures (i.e., microsclerotia, chlamydospores, oospores, etc.) enabling their endurance in soils under adverse situations during prolonged periods of time until favorable conditions allow germination. This is the case of microsclerotia produced by *V. dahliae*, the causal agent of verticillium wilts in many plants (Pegg and Brady 2002). Consequently, plausible management strategies to control these diseases, including biocontrol, should aim to eradicate microsclerotia or to avoid their germination (Antonopoulos et al. 2008). The potential use and efficacy of soil amendments to control *Verticillium* spp., including their effects on microsclerotia viability, have been thoroughly reviewed by Goicoechea (2009). Similarly, *Phytophthora* spp. can develop oospores, thick-walled sexual spores enabling this oomycete to survive under unfavorable conditions (e.g., drought, presence of microbial antagonists, etc.). Furthermore, many species of *Phytophthora* can develop other resistance structures like chlamydospores (Jung et al. 2013). Indeed, control strategies aimed to control these pathogens must take into account the possibility they produce resistance structures.

22.5 Effects of Introduced Inputs on the Microbial Soil Communities

Soils are the reservoir of a huge microbial biodiversity compared with other ecosystems. The use of culture-independent and metagenomics approaches is revealing a much wider diversity in soil microbial communities than that uncovered by traditional culture-dependent methods (Daniel 2005). These communities are not static and their composition, abundance, and activity, as well as the multitrophic interactions established among their constituents, can be affected by a number of (a) biotic factors along time and space. For instance, microbial diversity can be influenced by different stresses (e.g., nutrients shortage, environmental factors, or pH) and man-induced perturbations (e.g., soil management practices) (Decaëns 2010). Management practices like irrigation, tillage, cropping, and fertilizer and pesticide application are considered among the most influential factors affecting the composition of the rhizosphere microbiome (Prashar et al. 2014). Therefore, any (a) biotic input introduced into soils will result in short- and/or long-term changes of the microbial community structure. Since soil microbiota, either deleterious or beneficial, is crucial for plant fitness, potential alterations of its structure and functioning due to introduced inputs (chemical such as fungicides or fertilizers, or biological like OA or microorganisms) must be seriously considered to avoid unexpected side effects for the target crop.

Chemical inputs can affect both the composition and the structure of the soil-inhabiting microbial populations. Moreover, their effects can be different depending on the microbial group. Jacobsen and Hjelmsø (2014) point out that changes in microbial diversity vary according to the type of pesticide used. They provide a comprehensive list of agrochemicals (herbicides, soil fumigants, fungicides, insecticides) with variable effects on the bacterial community composition. For instance, it has been reported that copper decreases acidobacteria abundance, or that methyl bromide increases that of Gram positive bacteria (Jacobsen and Hjelmsø 2014, and references therein).

Introduction of BCAs into soils, either directly (e.g., by application of microbial antagonists formulations) or indirectly (e.g., as part of the microbiota present in OAs), has also a potential impact on indigenous soil microbial communities (Fig. 22.1). A given BCA bioformulation usually consists of a high cell/propagule density of the beneficial microorganism to ensure effective colonization of the plant rhizosphere (Trabelsi and Mhamdi 2013). This strategy provokes, at least transiently, a perturbation of the ecological equilibrium present in soil communities because the "new comer" and the indigenous microbiota must now compete for nutrients and space, which are usually scarce. In this scenario, mechanisms such as antibiosis or production of siderophores (Varma and Chincholkar 2007) deployed by the BCA can play an important role to efficiently displace native microorganisms. Similarly, the latter can use their own weapons to confront the invasion of the artificially introduced BCAs. The soil, and particularly the rhizosphere, becomes a battlefield where a multiplicity of trophic interactions takes place to (re)shape the structure of microbial communities (Raaijmakers et al. 2009). Trabelsi and Mhamdi (2013) compile an extensive number of research works and analyze how introduction of BCAs affects microbial communities. They also stressed the importance of the technique used to study the influence that artificial microbial inoculations have

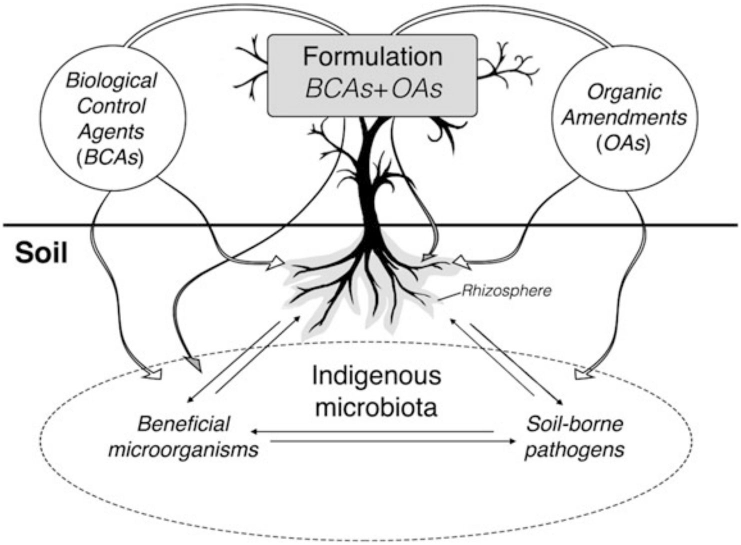

Fig. 22.1 Effects that the introduction of biological control agents (BCAs) and/or organic amendments (OAs) have in soil microbial communities networks (see main text for details)

in soils. For instance, the true impact of BCA introductions may vary depending on whether fatty acid methyl esters or terminal restriction fragment length polymorphism methodologies are used. They also conclude that the effects on plant growth and health are not necessarily a direct consequence of the introduced BCA, but they can be related to induction or repression of the resident microbial populations upon BCA inoculation. Therefore, synergistic and/or antagonistic interactions can take place after BCA inoculation, and they may endure for short and/or long periods of time.

Soil amendments, particularly OA, have the capability to modify soil characteristics such as concentration of nutrients (e.g., P, K, Fe), pH, NO_3 content, organic material, and structure. Since these traits are decisively shaping the structure of the soil-resident microbiota, there is no doubt that OA addition into soil will eventually affect microbial communities and their activity (Fig. 22.1). For instance, Yao et al. (2006) reported the influence that compost treatment had over soil microbial composition in apple (*Malus domestica*) orchards. Overall, they found differences in bacterial and fungi soil activities (measured as soil respiration) and community composition between non-treated and compost-treated soils. In their experiments, soil treated with compost showed the highest respiration rate and cumulative CO_2 production after 10 months, although these parameters eventually decreased and reached normal levels. Similarly, Giotis et al. (2009) observed that the incorporation of organic matter increased soil microbial activity and/or the number of microbial antagonists. Doan et al. (2014) also demonstrated that the nature of OAs has important consequences on soil microbial abundance and diversity. Finally, Gu et al. (2009) studied how long-term chemical fertilization (N-, P-, and K-based fertilizer) and farmyard manure affected soil microbial biomass (expressed as mg kg^{-1} of N and C) and diversity of bacterial communities in paddy soils. They observed that OA resulted in highest soil microbial biomass and diversity of bacterial communities. Moreover, combining OA with N, P, or K, increased microbial biomass and enhanced bacterial diversity compared to those observed with chemical fertilizers alone. The interested reader can consult many works on this particular subject (e.g., Liu et al. 2009; Zhang et al. 2012).

Modification of soil microbial communities and their implication in disease control has also been reported when different control measures are combined. Thus, effective control of Verticillium wilt of cotton due to changes in the fungal structure of rhizosphere soil (reducing fungal diversity) was observed after long-term (three growing seasons) greenhouse pot experiments when a combination of a bioorganic fertilizer (amino acid fertilizer from rapeseed meal fermentation), pig manure compost, and *Bacillus subtilis* was used (Luo et al. 2010). Larkin (2008) combined an aerated compost tea amendment, microorganisms (*B. subtilis*, *Trichoderma virens*, and *T. harzianum*), and even crop rotation to analyze how these inputs altered microbial populations and their activity in the soil. Results showed that different combinations of these treatments not only modified the soil microbial community characteristics but also reduced soil-borne diseases (stem canker and black scurf, caused by *Rhizoctonia solani,* and common scab, caused by *Streptomyces scabiei*) in potato. These authors support the idea that using a combination of treatments within an integrated soil management strategy yields better outcomes than the application of single management approaches. Related to this, Zhao et al. (2011) also observed that application of different formulations such as BIO I (pig manure compost, canola cake fermentation material, *Penicillium* sp., and *Aspergillus* sp.) significantly altered the soil microbial community structure, thereby suppressing Fusarium wilt of melon (*Cucumis melo* L.) effectively. In summary, evidence that inputs like BCAs and OAs can modify microbial community structures and that these changes can persist for a long time is available. However, the actual contribution of each component still remains to be unraveled.

22.6 Use of OAs in Integrated Disease Management Frameworks

Once we have introduced BCA and OA, tools that can be used on their own to control soil-borne diseases, we will now focus our attention on examples showing the potential that the ad hoc combination of BCA and OA has to effectively confront soil phytopathogens. Actually, this strategy has not yet been sufficiently explored, but promising results can be expected within IDM frameworks. It seems to be a general opinion among researchers that the effective control of a disease by means of a single BCA is difficult to achieve. Some authors have thus proposed alternatives such as the use of better adapted microorganisms, e.g., those from the same ecological niche where they will be applied (Ruano-Rosa and López-Herrera 2009), or the combination of BCAs (Xu et al. 2011), especially when they display complementary modes of action against the target pathogen. Examples of the successful use of combinations of BCAs, either fungus–fungus (Abo-Elyousr et al. 2009; Ruano-Rosa and López-Herrera 2009) or fungus–bacterium (Roberts et al. 2005; Ruano-Rosa et al. 2014), are available. Nevertheless, the limited efficacy observed for many available BCAs encourages the search for alternative and sustainable disease control approaches (Boukaew et al. 2013) which usually intend the combination of different control methods fitting IDM framework criteria.

Even though it falls out of the scope of this chapter, we would like to briefly mention that OAs can also be applied in combination with disease control strategies such as crop rotation (Larkin 2008) or soil solarization (Melero-Vara et al. 2011). For instance, soil solarization effects can be improved and/or enhanced by the addition of OAs because of the decomposition of organic matter increases heat generation and production of volatile compounds toxic for pathogenic (and beneficial) soil microbiota (Pokharel 2011). Interested readers can find excellent examples in the literature on the combination of these approaches, even including BCAs, to improve soil-borne pathogen control (e.g., Israel et al. 2005; Porras et al. 2007; Joshi et al. 2009; Melero-Vara et al. 2011; Domínguez et al. 2014). As mentioned in the previous section, implementation of these control measures (alone or in combination) can also greatly alter soil-resident microbial communities, including beneficial microorganisms that can be important for the health and fitness of the target crop (Israel et al. 2005; Porras et al. 2007; Larkin 2008).

22.6.1 Organic Carriers as Physical Support to Deliver BCAs

Selection of beneficial microorganisms that could be applied to a crop either as BCAs, biofertilizers, or for bioremediation purposes, is an arduous process that needs to take into account many factors (e.g., pathogen antagonism range, compatibility between BCAs, stress tolerance, plant growth promoting ability, environmental and human health risk assessment, etc.). A detailed evaluation and proper knowledge of beneficial traits displayed by the selected microbe will greatly determine its potential success when introduced into target agro-ecosystems. After this long process, the production, formulation, storage, and effective application of the selected microorganism usually represent additional bottlenecks prior to the implementation of a successful biocontrol strategy (Alabouvette and Steinberg 2006). Antagonistic microorganisms must therefore be formulated and applied in a way enabling the successful colonization and endurance in the targeted ecological niche (soil, rhizosphere, etc.) (El-Hassan and Gowen 2006; Nakkeeran et al. 2006). This has been recently well documented by Bashan et al. (2014), who comprehensively reviewed recent advances in plant growth promoting bacteria (PGPB) inoculant technology. In our opinion, most of the considerations addressed by these authors for PGPB could be also applied to microorganisms aimed to be used in biological control. In fact, microbe-mediated biocontrol is an indirect way to promote plant growth (Hayat et al. 2010). According to Bashan et al. (2014), two main factors contribute to the success of a PGPB-based formulation: (1) the own capabilities of the bacteria and (2) the technology used to deliver it. For instance, the introduction of any PGPB (or BCA) lacking an appropriate support (carrier) may lead to a rapid decline of its population level after inoculation. This means that its biocontrol potential might not be deployed regardless how powerful the beneficial traits have been previously demonstrated. Moreover, since native soil microbial communities are often better adapted than inoculated (artificially introduced) microorganisms, some advantages should be given to the inoculum once it is formulated.

We use the term “carrier” as any type of physical support, either organic or inorganic employed to develop a suitable formulation to be effectively applied in a given agro-ecosystem. A large number of carriers can be found as part of a bioformulation. Regarding inorganic carriers talc, kaolin, clay, perlite, or vermiculite among others (e.g., El-Hassan and Gowen 2006) and more recently microencapsulation (Kim et al. 2012) are being widely used. Peats and composts are among the most commonly used organic carriers. However, many others are available, even combinations of several of them. The abundance of organic carriers is reflected by the extensive bibliography available on this topic (see Table 22.1 for some examples).

Table 22.1 Examples of studies where organic amendments (OAs) were combined with biological control agents (BCAs) against soil-borne diseases

Organic amendment	Biological control agent	Disease/host (Pathogen)	Reference[a]
Wheat bran, peat moss	*Tricoderma harzianum*	Allium white-rot (*Sclerotium cepivorum*)	Avila et al. (2006)[a]
Vermicompost, neem cake	*T. harzianum*	Brinjal Fusarium wilt (*Fusarium solani* f. sp. *melongenae*)	Bhadauria et al. (2012)
Vineyard pruning wastes	*T. harzianum*	Fusarium wilt (*Fusarium oxysporum* f. sp. *melonis*)	Blaya et al. (2013)[a]
Pig manure compost, canola cake	*Bacillus subtilis*	Cucumber Fusarium wilt (*F. oxysporum* f. sp. *cucumerinum*)	Cao et al. (2011)[a]
Fresh chicken manure	*Trichoderma asperellum, Trichoderma atroviride*	Strawberry charcoal rot (*Macrophomina phaseolina*)	Domínguez et al. (2014)[b]
Sawdust, potato processing wastes, and rice straw	*T. harzianum, Penicillium oxalicum, Chaetomium globosum*	Legumes Fusarium wilt (*F. oxysporum*)	Haggag and Saber (2000)[a]
Cow dung	*T. harzianum*	Foot rot of lentil (*F. oxysporum* and *Sclerotium rolfsii*)	Hannan et al. (2012)
Amino acid fertilizer (from rapeseed meal fermentation)	*Bacillus pumilus*	Cucumber Damping-off disease (*Rhizoctonia solani*)	Huang et al. (2012)[a]
Farm yard manure, compost, poultry manure, press mud, vermicompost, and neem cake	*Pseudomonas fluorescens*	Tomato damping-off (*Pythium aphanidermatum*)	Jayaraj et al. (2007)
Farm yard manure, and poultry manure	*Trichoderma viride*	Tomato damping-off (*Pythium* spp., *R. solani*, *Phytophthora* spp., *Fusarium* spp.)	Joshi et al. (2009)
Amino acid fertilizer (from rapeseed meal fermentation), pig manure compost	*B. subtilis*	Cotton Verticillium wilt (*Verticillium dahliae*)	Lang et al. (2012)[a]
Neem cake and Farm yard manure	*T. viride*, *P. fluorescens*, *B. subtilis*	Physic nut collar and root rot (*Lasiodiplodia theobromae*)	Latha et al. (2011)
Pig manure compost/ microbe-hydrolyzed rapeseed cake	*Brevibacillus brevis*, *Streptomyces rochei*	Tobacco bacterial wilt (*Ralstonia solanacearum*)	Liu et al. (2013)[a]
Compost	*Pisolithus tinctorius*, *Scleroderma verrucosum*	Oak decline (*P. cinnamomi*)	Moreira et al. (2007)

(continued)

Table 22.1 (continued)

Organic amendment	Biological control agent	Disease/host (Pathogen)	Reference[a]
Mustard oil cake	*P. fluorescens*, *Glomus sinuosum*, *Gigaspora albida*	French bean root rot (*R. solani*)	Neeraj and Singh (2011)
Compost from agricultural waste (from cork, grape and olive marc, and spent mushroom)	*T. asperellum*	Cucumber (*R. solani*)	Trillas et al. (2006)
Olive mill wastes	*Bacillus amyloliquefaciens*, *Burkholderia cepacia*	Olive Verticillium wilt (*V. dahliae*)	Vitullo et al. (2013)
Pig manure compost, canola cake	*B. amyloliquefaciens*	Banana Fusarium wilt (*F. oxysporum* f. sp. *cubense*)	Wang et al. (2013)[a]
Pig manure, rice straw	*B. amyloliquefaciens*	Tomato Bacterial wilt (*R. solanacearum*)	Wei et al. (2011)[a]
Pig manure compost, canola cake	*Paenybacillus polymyxa*, *T. harzianum*	Watermelon Fusarium wilt (*F. oxysporum* f. sp. *nevium*)	Wu et al. (2009)
Compost (pig manure, rice straw, residues from medicine, alcohol, and vinegar production)	*T. harzianum*	Cucumber Fusarium wilt (*F. oxysporum* f. sp. *cucumerinum*)	Yang et al. (2011)[a]
Commercial organic fertilizer (pig manure compost, canola cake)	*P. polymyxa*, *B. subtilis*, *Penicillium* sp., *Aspergillus* sp.	Melon Fusarium wilt (*F. oxysporum* f. sp. *melonis*)	Zhao et al. (2011)[a]

[a] Studies in which the OA was used as a carrier of the BCA
[b] Additional control treatment was used in combination with OA+BCA

The development of carriers based on organic matter emerges as an excellent alternative for a more effective application of disease control treatments based on OA plus BCA (OA+BCA) combinations. Indeed, the own nature of this type of carriers provide an adequate nutrient reservoir to the BCA thereby enhancing its survival in a hostile environment such as soil. For example, it is well known that the widely used BCA *Trichoderma* spp. must not be applied in the stage of spores (conidia) if not supported by a suitable carrier. This is due to the high sensitivity to soil fungistasis showed by these asexual reproductive structures (Pan et al. 2006). Hence, the application of *Trichoderma*-based formulations can fail if spores (even at the stage of early germination) are applied to the soil without an adequate nutrients supply (Yang et al. 2011). A number of examples in which OA+BCA combinations performed better than single OA treatments are available. For instance, Zhao et al. (2011) developed different formulations using as a carrier an organic fertilizer supplemented with different BCAs (see in Table 22.1). The carrier did not show any disease suppressive effect by itself but in combination with the BCAs resulted in a suitable formulation that effectively controlled Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* in melon.

The use of organic-based carriers in OA+BCA control strategy has two main beneficial outcomes. On the one hand, recycling organic material (i.e., pruning remains) may help farmers to deal with waste derived from their activity. For instance, this is an urgent need in some Mediterranean countries in the case of olive (*Olea europaea* L.) mill waste management, an important by-product from olive oil industry activity (Papasotiriou et al. 2013). On the other hand, some organic-based carriers such as specific composts from agriculture wastes have been demonstrated to be effective on its own in the control of a number of soil-borne pathogens (Trillas et al. 2006). For instance, Papasotiriou et al. (2013) have demonstrated that the use of olive mill waste compost reduced *V. dahliae* microsclerotia germination as well as the number of hyphae per germinated microsclerotium *in planta*. Likewise, Alfano et al. (2011) have shown that the use of composted olive mill waste has in vivo suppressive effect against *Fusarium oxysporum* f. sp. *lycopersici* and *Pythium ultimum* [the causal agents of Fusarium wilt and damping off on tomato (*Solanum lycopersicum* Mill) seedling, respectively]. Both suppression by competition (nutrients and/or space) and antagonistic effect due to microorganisms inhabiting the compost are likely involved in the suppressive effect.

22.6.2 Combining OAs with BCAs

Disease management strategies are obviously focused on the improvement of the crop's health. However, application of OA+BCA combinations can provide additional beneficial effects to the crop (i.e., better plant development, enhanced yield, plant growth, etc.). This is a consequence of the fertilizing properties of OAs, which can release chemical substances with similar or better outcomes than synthetic fertilizers (Ding et al. 2013). Furthermore, it is well known that some BCAs have the capability to promote plant growth by means of a number of direct mechanisms (Lugtenberg and Kamilova 2009). The interested reader can consult excellent reviews on this topic (i.e., Kaewchai et al. 2009; Tailor and Joshi 2014).

A number of studies dealing with the use of OA+BCA combinations and their effects on the plant growth, crop yield, and/or on the soil microbial community structure, besides its effectiveness against pathogens, are available (Table 22.1). Nevertheless, we would like to differentiate between two types of OA+BCA combinations depending on whether they are applied as joint formulations (i.e., blended and/or composted mixtures prior to application, marked in Table 22.1) or as individual treatments that are subsequently applied (either at the same time or not) upon introduction in the target crop/field. *Trichoderma* spp. and a number of bacterial genera are, once again, the most widely used BCAs in this control strategy.

For instance, Bhadauria et al. (2012) reported that application of *T. harzianum* (as seed treatment) plus soil treatment with neem (*Azadirachta indica* A. Juss.) cake was an effective treatment to reduce Fusarium wilt incidence (*Fusarium solani* f. sp. *melongenae*) in brinjal (eggplant, *Solanum melongena* L.) plants. Moreover, this combined treatment reduced the amount of pathogen propagules and did not produce unwanted residues what makes it an excellent eco-friendly strategy for the management of this disease. Likewise, the addition of *T. harzianum* to compost (see Table 22.1) improved the biocontrol effectiveness and induced changes in the biotic (e.g., changes in bacterial community composition) and abiotic (pH modification) characteristics of this AO (Blaya et al. 2013). Jayaraj et al. (2007) used different OAs (farmyard manure, leaf compost, poultry manure, press mud, vermicompost, and neem cake) combined with *P. fluorescens* to control damping-off (*Pythium aphanidermatum*) in tomato. In this case, OAs were incorporated into soil prior to planting while the BCA was applied as seed treatment using a formulation (see Table 22.1). Results showed an enhancement of *P. fluorescens* rhizosphere population as well as a reduction of the disease incidence caused by this oomycete.

Taking into account the expected advantages of mixing BCAs (combination of complementary modes of action) mentioned above, Liu et al. (2013) developed a bioorganic fertilizer using an OA as a carrier (see Table 22.1). They observed better suppression of the bacterial pathogen *Ralstonia solanacearum* in tobacco (*Nicotiana tabacum* L.) plants pot experiments when a formulation containing two BCAs were applied in combination with compost (see Table 22.1). In addition to the enhanced disease suppressive effect, they also found increased plant growth probably due to a synergistic effect derived from the combination of BCAs with the compost. Considering the benefits achieved by the combination OAs and BCAs, a progressive substitution of chemically based fungicides seems to be a practicable strategy (De Ceuster and Hoitink 1999).

22.7 Can OA+BCA Combinations Be a Feasible Disease Control Approach in Woody Plants?

Trees and woody crops are of utmost importance for the life of the planet. For instance, forests cover around 31 % of the world's land surface (Food and Agriculture Organization of the United Nations 2010b), providing many important goods (e.g., wood, paper, etc.) and playing essential roles in processes such as nutrients and water cycling and storage. Trees are also crucial to prevent soil erosion, to mitigate the effects of climate change acting as carbon dioxide sink, and to support microbial, animal, and plant biodiversity in many areas. Therefore, the health of forests and woody agro-ecosystems is of particular relevance.

Many soil-borne pathogens affect woody plants causing serious constraints in economically relevant tree crops and forestry. Among them, species of the genera

Fusarium, *Verticillium*, *Phytophthora*, *Pythium*, *Armillaria*, *Rosellinia*, or *Heterobasidion* can be highlighted as extremely damaging (García-Jiménez et al. 2010). The utilization of BCAs to control these pathogens when affecting woody plants has been investigated in a number of pathosystems (see Pliego and Cazorla 2012, and references therein). The same accounts for the use of OAs although to a lesser extent (Noble and Conventry 2005). Remarkably, however, a search in the literature reveals that, to the best of our knowledge, the combination of BCAs and OAs as a disease control strategy has been implemented in woody plants at a negligible level compared to that in arable crops or seedlings (Table 22.1). A number of reasons could explain why biocontrol strategies in general, and BCA +OA combinations in particular, have been less (or seldom) applied in these particular agro-ecosystems. Thus, it is plausible to think that factors such as large biomass, anatomy, longevity, and/or particularities of tree crops and forests management make it more difficult to develop effective biological control measures against diseases affecting woody plants. For instance, regarding to soil-borne pathogens, large root systems of trees can undergo repeated infection events from pathogen's propagules present in soil. Infection events can then take place either in the same season or in successive ones that contribute to complicate the application of effective biocontrol strategies, including OA+BCA combinations. Pliego and Cazorla (2012) have particularly stressed that the large root systems developed by trees greatly hamper the effectiveness of BCA treatments. Likewise, López-Escudero and Mercado-Blanco (2011) have emphasized the difficulty to control *V. dahliae* in olive because of the pathogen's location within the vascular system, a site always difficult to be reached by chemical or biological treatments. Nevertheless, and in spite of these difficulties, biocontrol measures are feasible for woody plants. For instance, application of BCAs can be done with seedlings, in pots under controlled conditions, and/or during the nursery propagation stage. Thus, Vitullo et al. (2013) focused on pot-growing olive plants at nursery conditions with the aim to guarantee the production of healthy plants. These authors achieved positive results in the control of *V. dahliae* by mixing *Bacillus amyloliquefaciens* and *Burkholderia cepacia* with olive mill waste. However, the important step forward yet to be taken is the application of biocontrol strategies (including OA+BCA with the advantages discussed above) at large scale and under field conditions (tree orchards, forests). The relevant question still to be answered is whether application of BCAs, OAs, and/or OA+BCAs combinations can be done in an economically efficient way considering the particularities of trees (and woody plants in general).

Disease control measures that can be implemented together with OA+BCA combinations (see above) have to confront the idiosyncrasies of woody plants as well, and their potential success can be reduced compared to when they are applied to herbaceous crops. For instance, it is known that efficiency of soil solarization decreases at deep soil layers (López-Herrera et al. 2003). Thus, deep root systems usually developed by trees are less accessible to physically-, chemically- and/or biologically based disease control measures.

A promising alternative to be used in woody plants are endophytic microorganisms adapted to colonize and endure for long periods of time within plant tissues.

Among the agro-biotechnological applications that bacterial and fungal endophytes pose, their potential as BCAs are yet insufficiently explored (Mercado-Blanco and Lugtenberg 2014). However, effective control of Verticillium wilt of olive has been achieved in nursery-propagated plants by the olive root endophyte *P. fluorescens* PICF7 (Prieto et al. 2009) or against poplar canker (caused by three pathogens viz. *Cytospora chrysosperma*, *Phomopsis macrospora*, and *Fusicoccum aesculi*) by using the endophyte *Bacillus pumillus* (Ren et al. 2013). Considering the advantages discussed above, the use of AO (endophytic)+BCA combinations may constitute and interesting approach to be used in the control of diseases affecting woody plants.

22.8 Conclusions

The growing public concern about the undesirable effects derived from an overzealous use of agrochemicals, mainly fungicides and herbicides, has encouraged the search for more environmentally friendly plant disease control alternatives. Chemical inputs have caused, among other side effects, the development of plant pathogen resistance and hazard to animal and human health. For a number of reasons, many plant pathologists have devoted their research efforts to seek novel alternatives for the effective control of phytopathogens that, in addition, aim to diminish the risk of undesirable effects. The implementation of IDM strategies encompassing, among others, measures such as the combined use of BCA and OA likely constitutes the best option towards the success in plant disease management. It must be emphasized that the application of any soil-borne pathogen control method, either individually or combined with other(s), may result in major changes affecting not only the structure and physical–chemical characteristics of the soil but also the indigenous microbiota residing therein. These changes can have a profound influence on the pathogen control process, even determining the success or failure of the strategy used. Obviously, the introduction of OA, BCA, or OA+BCA combinations into a given agro-ecosystem also provokes major changes (Fig. 22.1), which should be studied and understood in detail.

A crucial step for the success of biocontrol strategies is the way the BCA is applied or delivered. Indeed, the choice of the most appropriate carrier when developing a BCA-based formulation is of utmost importance. The carrier should not only serve as nutrients supply but also be a proper support enabling microorganisms to have long shelf lives and to cope with the adverse, highly competing conditions they have to face soon after they are released into the target site (soil, rhizosphere, seeds, etc.). The development of OA-based carriers constitutes an excellent approach because they can simultaneously enhance the survival rate of the BCA, antagonize the target pathogen, and act as plant fertilizers.

To our knowledge, studies combining BCAs and OAs to control diseases of woody plants are scant. Several factors may explain this circumstance and have been briefly presented in this chapter. Nevertheless, the combination of OA and

BCA emerges as an interesting approach yet to be explored. BCAs displaying endophytic lifestyle also offer a number of advantages (e.g., adaptation to live within the plant tissue, plant growth promotion, etc.) to be exploited as well. Promising results have been obtained from these environmentally friendly tools under controlled conditions (i.e., greenhouse, nursery-production stage). The challenge now is to better understand and exploit the benefits of combining them as well as to develop correct strategies for their efficient use in agro-ecosystems and forestry.

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