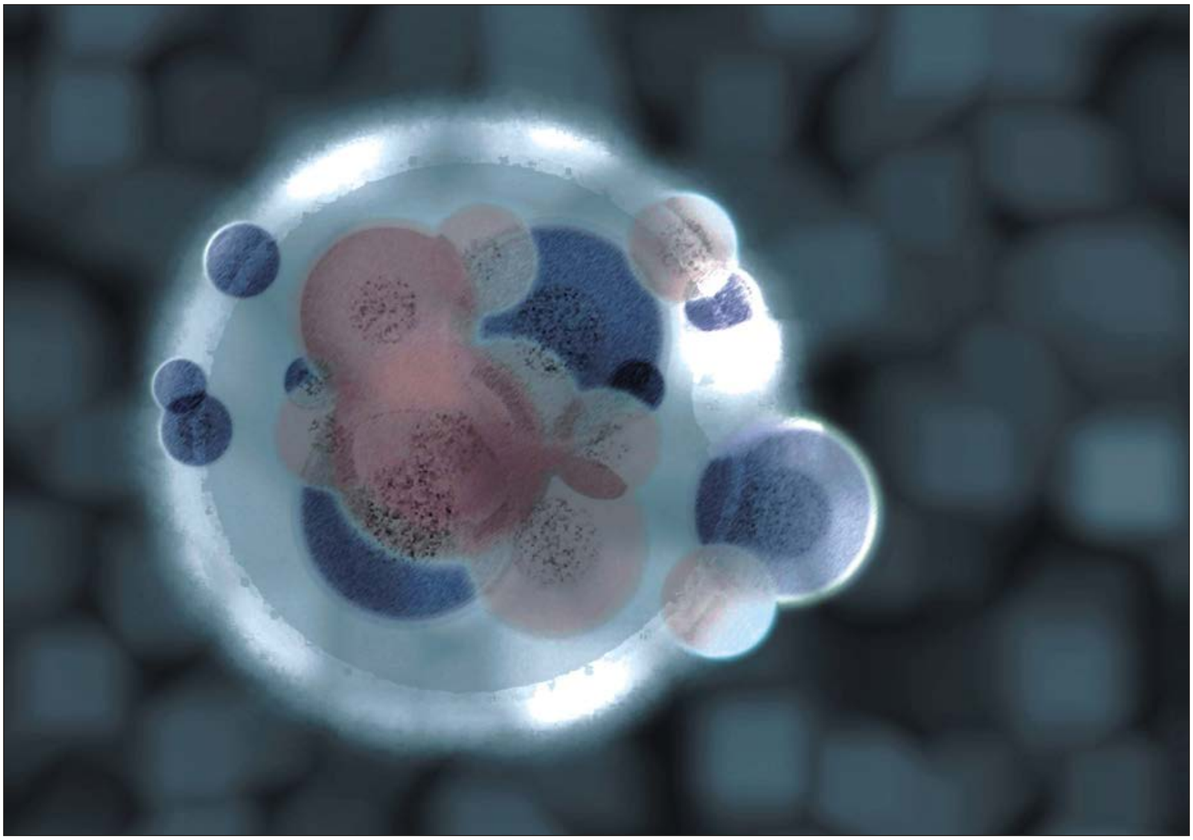

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A Review on Biological Control of Fungal Plant Pathogens Using Microbial Antagonists

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Abstract: The objective of this study was to review the published research works on biological control of fungal plant diseases during past 50 years. Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products every year. Biological control of plant diseases including fungal pathogens has been considered a viable alternative method to chemical control. In plant pathology, the term biocontrol applies to the use of microbial antagonists to suppress diseases. Throughout their lifecycle, plants and pathogens interact with a wide variety of organisms. These interactions can significantly affect plant health in various ways. Different mode of actions of biocontrol-active microorganisms in controlling fungal plant diseases include hyperparasitism, predation, antibiosis, cross protection, competition for site and nutrient and induced resistance. Successful application of biological control strategies requires more knowledge-intensive management. Various methods for application of biocontrol agents include: application directly to the infection court at a high population level to swamp the pathogen, application at one place in which biocontrol microorganisms are applied at one place (each crop year) but at lower populations which then multiply and spread to other plant parts and give protection against pathogens and one time or occasional application that maintain pathogen populations below threshold levels. Commercial use and application of biological disease control have been slow mainly due to their variable performances under different environmental conditions in the field. To overcome this problem and in order to take the biocontrol technology to the field and improve the commercialization of biocontrol, it is important to develop new formulations of biocontrol microorganisms with higher degree of stability and survival. Majority of biocontrol products are applied against seed borne and soil borne fungal pathogens, including the causal agents of seed rot, damping-off and root rot diseases. These products are mostly used as seed treatment and have been effective in protecting several major crops such as wheat, rice, corn, sugar beet and cotton against fungal pathogens. However, in some cases, biocontrol microorganisms have also been tested as spray application on foliar diseases, including powdery mildew, downy mildew, blights and leaf spots. A few post harvest fungal diseases have also been controlled by the use of antagonistic fungi and bacteria. Biocontrol microorganisms are also being used as the form of composts in some plants. Research data and observations in nurseries have shown that addition of composted organic matter to potting mixes results in suppression of soil borne diseases. A significant improvement have been made in different aspects of biological control of fungal plant diseases, but this area still need much more development and investigations to solve the existing problems. In order to have more effective biological control strategies in the future, it is critical to carry out more research studies on some less developed aspects of biocontrol, including development of novel formulations, understanding the impact of environmental factors on biocontrol agents, mass production of biocontrol microorganisms and the use of biotechnology and nano-technology in improvement of biocontrol mechanisms and strategies. Future outlooks of biocontrol of plant diseases is bright and promising and with the growing demand for biocontrol products among the growers, it is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources and approach a sustainable agricultural system.

Key words: Plant diseases, beneficial microorganisms, mechanisms, application, composts, commercialization, future outlook, development

INTRODUCTION

Plant pests (harmful insects, parasitic weeds and pathogens) are among the most important biotic agents causing serious losses and damages to agricultural products. Plant pests need to be controlled to ensure food, feed and fiber production quantitatively and qualitatively. A number of different strategies are

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currently being employed to manage and control plant pests (Agrios, 1988; Baker, 1987; Cook, 1993; Bargabus *et al*., 2002, 2004; Benhamou, 2004; Chisholm *et al*., 2006; Heydari, 2007; Heydari *et al*., 2007; Islam *et al*., 2005; Kloepper *et al*., 2004). Beyond good agronomic and cultural practices, growers often rely heavily on chemical pesticide application (Agrios, 1988; Baker, 1987). However, the environmental pollution caused by excessive use of agrochemicals has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Today, there are strict regulations on chemical pesticide use and there is political pressure to remove the most hazardous chemicals from the market. In addition to the above-mentioned issues, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases (Baker, 1987; Cook, 1993).

Plant diseases are mostly controlled by the use of chemical pesticides and in some cases by cultural practices (Agrios, 1988; Cook, 1993). However, the widespread use of chemicals in agriculture has been a subject of public concern and scrutiny due to the potential harmful effects on the environment, their undesirable effects on non-target organisms and possible carcinogenicity of some chemicals (Agrios, 1988; Cook, 1993; Heydari, 2007; Heydari *et al*., 2007). Other problems include development of resistant races of pathogens, a gradual elimination and phasing out of some available pesticides and the reluctance of some chemical companies to develop and test new chemicals due to the problems with registration process and cost (Cook, 1993). The need for the development of non-chemical alternative methods to control plant diseases is therefore clear.

Biological control of plant diseases has been considered a viable alternative method to manage plant diseases (Cook, 1993). Biological control is the inhibition of growth, infection or reproduction of one organism using another organism (Cook, 1993; Baker, 1987). Biocontrol is environmentally safe and in some cases is the only option available to protect plants against pathogens (Cook, 1993). Biological control employs natural enemies of pests or pathogens to eradicate or control their population. This can involve the introduction of exotic species, or it can be a matter of harnessing whatever form of biological control exists naturally in the ecosystem. The induction of plant resistance using non-pathogenic or incompatible microorganisms is also a form of biological control (Cook, 1993; Schouten *et al*., 2004). Fungal plant diseases are considered the most important microbial agents causing serious losses in the agriculture annually (Agrios, 1988). Some fungal diseases that have successfully been controlled using biological agents are pathogens of pruning wounds and other cut surfaces, diseases of leaves and flowers, such as powdery mildew, diseases of fruits and vegetables, such as Botrytis and fungal pathogens in the soil (Agrios, 1988; Baker, 1987; Cook, 1993; Heydari, 2007; Heydari *et al*., 2004, 2007; Heydari and Misaghi, 1998, 1999, 2003).

A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people and the environment. To that end, the objectives of this review chapter is to present an advanced survey of the nature and practice of biological control as it is applied to the suppression of plant diseases. In this review, different aspects of biological control of fungal plant diseases including definitions, modes of action, application strategies, current status and future development and outlooks will be discussed.

TERMINOLOGY

The term biological control and its abbreviated synonym biocontrol have been used in different fields of biology, most notably entomology and plant pathology. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations (Cook, 1993). In both fields, the organism that suppresses the pest or pathogen is referred to as the Biological Control Agent (BCA). More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources (Cook, 1993). These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen. While such inputs may mimic the activities of living organisms, non-living inputs should more properly be referred to as biopesticides or biofertilizers, depending on the primary benefit provided to the host plant (Cook, 1993).

The various definitions offered in the scientific literature have sometimes caused confusion and controversy. For example, members of the United States National Research Council took into account modern biotechnological developments and referred to biological control as the use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects and microorganisms, but this definition spurred much subsequent debates and it was frequently considered too broad by many scientists who worked in the field.

Published definitions of biocontrol differ depending on the target of suppression; number, type and source of biological agents and the degree and timing of human intervention (Cook, 1993). Most broadly, biological control is the suppression of damaging activities of one organism by one or more other organisms, often referred to as natural enemies. With regards to plant diseases, suppression can be accomplished in many ways. If grower's activities are considered relevant, cultural practices such as the use of rotations and planting of disease resistant cultivars (whether naturally selected or genetically engineered) would be included in the definition (Cook, 1993).

Because the plant host responds to numerous biological factors, pathogenic and non-pathogenic, induced host resistance might be considered a form of biological control (Cook, 1993). More narrowly, biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens. This may involve the use of microbial inoculants to suppress a single type or class of plant diseases. This may also involve managing soils to promote the combined activities of native soil and plant-associated organisms that contribute to general suppression (Cook, 1993). Most narrowly, biological control refers to the suppression of a single pathogen by a single antagonist, in a single cropping system. Most specialists in the field would concur with one of the narrower definitions presented above.

INTERACTIONS BETWEEN PLANTS AND BENEFICIAL MICROBES

Throughout their lifecycle, plants and pathogens interact with a wide variety of organisms. These interactions can significantly affect plant health in various ways (Agrios, 1988; Bull *et al*., 2002; Katská, 1994; Chisholm *et al*., 2006; Fitter and Garbaye, 1994; McSpadden-Gardener and Weller, 2001). In order to understand the mechanisms of biological control, it is helpful to appreciate the different ways that organisms interact. Note too, in order to interact, organisms must have some form of direct or indirect contact. The types of interactions between plants and microorganisms have been referred to as mutualism, protocooperation, commensalisms, neutralism, competition, amensalism, parasitism and predation (Bankhead *et al*., 2004; Bull *et al*., 2002; Katská, 1994; Chisholm *et al*., 2006; Fitter and Garbaye, 1994; Hoitink and Boehm, 1999). While the terminology has been developed for macroecology, examples of all of these types of interactions can be found in the natural world at both the macroscopic and microscopic level. And, because the development of plant diseases involves both plants and microbes, the interactions that lead to biological control take place at multiple levels of scale (Bull *et al*., 2002; Katská, 1994; Chisholm *et al*., 2006; Fitter and Garbaye, 1994).

From the plant's point of view, biological control may be considered a positive result arising from different specific and non-specific interactions (Cook, 1993; Weller *et al*., 2002). We can begin to classify and functionally delineate the diverse components of ecosystems that contribute to biological control. Mutualism is an association among several species where all of them are benefited from this association (Biermann and Linderman, 1983; Bull *et al*., 2002; Katská, 1994; Chisholm *et al*., 2006; Duchesne, 1994; Fitter and Garbaye, 1994; Garcia-Garrido and Ocampo, 1989; Kerry, 2000). Sometimes, it can be an obligatory relation involving close physical and biochemical contact between two organisms, such as those between plants and mycorrhizal fungi (Bull *et al*., 2002; Katská, 1994; Chisholm *et al*., 2006; Fitter and Garbaye, 1994). However, they are generally facultative and opportunistic.

For example, Rhizobium bacteria reproduce either in the soil or, to a much greater degree, through their mutualistic association with legume plants. These types of mutualism can contribute to biological control, by providing plant with improved nutrition and/or by stimulating host defense mechanism and ability (Chisholm *et al*., 2006; Fitter and Garbaye, 1994). Many of the microorganisms isolated and classified as biocontrol agents (BCA) can be considered facultative mutualists, because host and disease suppression by them will vary depending on the prevailing environmental conditions (Cook, 1993).

Commensalism is also a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited (Fitter and Garbaye, 1994). Most plant-associated microorganisms are assumed to be commensals with regards to the host plant, because their presence, individually or in total, rarely results in positive or negative consequences to the plant (Katská, 1994; Chisholm *et al*., 2006). While the presence of these microorganisms may present a variety of challenges to an infecting pathogen, their absence decreases pathogen infection or disease severity and is indicative of commensal interactions (Cook, 1993).

Biological interactions in which the population density of one species has absolutely no effect on the other are called neutralism (Berg *et al*., 2005; Chisholm *et al*., 2006). Related to biological control, an inability to associate the population dynamics of pathogen with that of another organism would indicate neutralism (Chisholm *et al*., 2006). In contrast, antagonism between organisms results in a negative outcome for one or both. Competition within and between species caused a decreased growth, activity, and/or fecundity of the interacting organisms (Cook, 1993). Biocontrol can occur when non-pathogens compete with pathogens for nutrients and sites in host plant. Direct interactions that benefit one population at the expense of another also affect our understanding of biological control (Cook, 1993).

Parasitism is also a symbiotic relation in which two organisms coexist over a prolonged period of time (Cook, 1993; Chisholm *et al*., 2006; Lo *et al*., 1997). In this type of interaction, one organism, usually the physically smaller (parasite) benefits and the other (host) is harmed. The activities of various hyperparasites, for example those agents that parasitize plant pathogens, can result in biocontrol (Lo *et al*., 1997). Another interesting contribution to biocontrol is when host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens through the stimulation of host defense systems (Cook, 1993). Finally predation refers to the hunting and killing of one organism by another for consumption and sustenance. While the term predator typically refers to animals that feed at higher trophic levels in the macroscopic world, it has also been applied to the actions of microorganisms such as protists and mesofauna, e.g. fungal feeding nematodes and microarthropods, that consume pathogen biomass for sustenance (Cook, 1993).

Biological control can result in various forms of these types of interactions, depending on the environmental conditions within which they occur. Significant biological control, as was described above, generally arises from manipulating mutualisms between microorganisms and their plant hosts or from manipulating antagonisms between microbes and pathogens (Bull *et al*., 2002; Katská, 1994; Chisholm *et al*., 2006; Fitter and Garbaye, 1994).

MECHANISMS OF BIOLOGICAL CONTROL

Since biological control is a result of many different types of interactions among microorganisms, scientists have concentrated on characterization of mechanisms occurring in different experimental situations (Audenaert *et al*., 2002; De Meyer and Hofte, 1997; Elad and Baker, 1985; Heydari *et al*., 1997; Homma *et al*., 1989; Howell *et al*., 1988; Islam *et al*., 2005; Meziane *et al*., 2005; Ryu *et al*., 2004; Van Dijk and Nelson, 2000). In all cases, pathogens are antagonized by the presence and activities of other microorganisms that they encounter.

Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the biocontrol active microorganisms. In this type of interaction, Hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of mechanism because the activities of no other organism would be required to exert a suppressive effect (Harman *et al*., 2004; Linderman, 1994). In contrast, indirect antagonism is resulted from the activities that do not involve targeting a pathogen by a biocontrol active microorganism. Improvement and stimulation of plant host defense mechanism by non-pathogenic microorganisms is the most indirect form of antagonism (Kloepper *et al*., 1980; Lafontaine and Benhamo, 1996; Leeman *et al*., 1995; Maurhofer *et al*., 1994; Silva *et al*., 2004). While many studies have concentrated on the establishment of the importance of specific mechanisms of biocontrol to particular pathosystems, all of the mechanisms described below are likely to be operating to some extent in all natural and managed ecosystems.

The most effective biocontrol active microorganisms studied appear to antagonize plant pathogens employing several modes of actions (Cook, 1993). For example, pseudomonads known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defenses (Kloepper *et al*., 1980; Lafontaine and Benhamou, 1996; Leeman *et al*., 1995; Maurhofer *et al*., 1994; Silva *et al*., 2004). Additionally, DAPG-producers bacterial antagonists can aggressively colonize roots, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of plant through competition for organic nutrients. However, the most important modes of actions of biocontrol active microorganisms are as follows:

Mycoparasitism: In Hyperparasitism, the pathogen is directly attacked by a specific biocontrol agent (BCA) that kills it or its propagules. Four major groups of hyperparasites have generally been identified which include hypoviruses, facultative parasites, obligate bacterial pathogens and predators. An example of hypoparasites is the virus that infects *Cryphonectria parasitica*, the fungal causal agent of chestnut blight, which causes hypovirulence, a reduction in pathogenicity of the pathogen. This phenomenon has resulted in the control of chestnut blight in many places (Milgroom and Cortesi, 2004). However, the interaction of virus, fungus, tree and environment determines the success or failure of hypovirulence.

In addition to hypoviruses several fungal hypoparasites have also been identified including those that attack sclerotia (e.g., *Coniothyrium minitans*) or others that attack fungal hyphae (e.g. *Pythium oligandrum*). In some cases, a single fungal pathogen can be attacked by multiple hyperparasites. For example, *Acremonium alternatum*, *Acrodontium crateriforme*, *Ampelomyces quisqualis*, *Cladosporium oxysporum* and *Gliocladium virens* are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Milgroom and Cortesi, 2004).

In contrast to hyperparasitism, microbial predation is more general, non-specific and generally provides less predictable levels of disease control. Some biocontrol agents exhibit predatory behavior under nutrient-limited conditions. Such as *Trichoderma*, a fungal antagonist that produces a range of enzymes that are directed against cell walls of pathogenic fungi. However, when fresh bark is used in composts, *Trichoderma* sp. does not directly attack the plant pathogen, *Rhizoctonia solani*. But, in decomposing bark, the concentration of readily available cellulose decreases and this activates the chitinase genes of *Trichoderma* sp. Which, in turn, produce chitinase to parasitize *R. solani* (Benhamou and Chet, 1997).

Antibiosis: Many microbes produce and secrete one or more compounds with antibiotic activity (Homma *et al*., 1989; Howell and Stipanovic, 1980; Islam *et al*., 2005; Leclére *et al*., 2005; Shahraki *et al*., 2009; Shanahan *et al*., 1992; Thomashow *et al*., 1990; Thomashow and Weller, 1988). In a general definition antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. It has been shown that some antibiotics produced by microorganisms are particularly effective against plant pathogens and the diseases they cause (Homma *et al*., 1989; Howell and Stipanovic, 1980; Islam *et al*., 2005; Shanahan *et al*., 1992; Thomashow *et al*., 1990, 2002; Thomashow and Weller, 1988). In all cases, the antibiotics have been shown to be particularly effective at suppressing growth of the target pathogen *in vitro* and/or *in situ* conditions. An effective antibiotic must be produced in sufficient quantities (dose) near the pathogen. *In situ* production of antibiotics by several different biocontrol agents has been studied (Thomashow *et al*., 1990). While several procedures have been developed to ascertain when and where biocontrol agents may produce antibiotics detecting expression in the infection court is difficult because of the heterogenous distribution of plant-associated microbes and the potential sites of infection (Thomashow *et al*., 1990).

However, in some cases, the relative importance of antibiotic production by biocontrol bacteria has been demonstrated. For example, mutant strains incapable of producing phenazines (Thomashow and Weller, 1988) or phloroglucinols (Keel *et al*., 1989) have been shown to be equally capable of colonizing the rhizosphere, but much less capable of suppressing soil borne root diseases than the corresponding wild-type and complemented mutant strains. Many biocontrol strains have been shown to produce multiple antibiotics which can suppress one or more pathogens (Homma *et al*., 1989; Howell and Stipanovic, 1980; Islam *et al*., 2005; Shanahan *et al*., 1992; Thomashow *et al*., 1990; Thomashow and Weller, 1988). The ability of production of several antibiotics probably results in suppression of diverse microbial competitors and plant pathogens.

Metabolite production: Many biocontrol active microorganisms produce other metabolites that can interfere with pathogen growth and activities. Lytic enzymes are among these metabolites that can break down polymeric compounds, including chitin, proteins, cellulose, hemicellulose and DNA (Anderson *et al*., 2004; Howell *et al*., 1988; Loper and Buyer, 1991; Ordentlich *et al*., 1988; Press *et al*., 2001; Wilhite *et al*., 2001). Studies have shown that some of these metabolites can sometimes directly result in the suppression of plant pathogens. For example, control of *Sclerotium rolfsii* by *Serratia marcescens* appeared to be mediated by chitinase expression (Ordentlich *et al*., 1988). It seems more likely that antagonistic activities of these metabolites are indicative of the need to degrade complex polymers in order to obtain carbon nutrition. Microorganisms that show a preference in colonizing and suppression of plant pathogens might be classified as biocontrol agents. For example, Lysobacter and Myxobacteria that produce lytic enzymes have been shown to be effective against some plant pathogenic fungi (Bull *et al*., 2002).

Studies have shown that some products of lytic enzyme activity may have indirect efficacy against plant pathogens. For example, oligosaccharides derived from fungal cell walls have been shown to induce plant host defenses (Howell *et al*., 1988). It is believed that the effectiveness of the above compounds against plant pathogens is dependent on the composition and carbon and nitrogen sources of the soil and rhizosphere. For example, in post-harvest disease control, addition of chitosan which is a non-toxic and biodegradable polymer of beta-1, 4-glucosamine produced from chitin by alkaline deacylation stimulated microbial degradation of pathogens (Benhamou, 2004). Amendment of plant growth substratum with chitosan suppressed the root rot caused by *Fusarium oxysporum* f. sp. radicis-lycopersici in tomato (Lafontaine and Benhamou, 1996).

In addition to the above-mentioned metabolites, other microbial byproducts may also play important roles in plant disease biocontrol (Phillips *et al*., 2004). For example, Hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations (Ramette *et al*., 2003). The production of HCN by certain fluorescent pseudomonads is believed to be effective against plant pathogens. Results of some research studies in this regard have shown that *P. fluorescens* CHA0, an antagonistic bacterium, produces antibiotics including siderophores and HCN, but suppression of black rot of tobacco caused by *Thielaviopsis basicola* appeared to be due primarily to HCN production. In another study Howell *et al*. (1988) reported that volatile compounds such as ammonia produced by *Enterobacter cloacae* were involved in the suppression of cotton seedling damping-off caused by *Pythium ultimum*.

Competition: The nutrient sources in the soil and rhizosphere are frequently not sufficient for microorganisms. For a successful colonization of phytosphere and rhizosphere a microbe must effectively compete for the available nutrients (Elad and Baker, 1985; Keel *et al*., 1989; Loper and Buyer, 1991). On plant surfaces, host-supplied nutrients include exudates, leachates, or senesced tissue. In addition to these, nutrients can also be obtained from waste products of other organisms such as insects and the soil. This is a general believe that competition between pathogens and non-pathogens for nutrient resources is an important issue in biocontrol (Elad and Baker, 1985; Keel *et al*., 1989; Loper and Buyer, 1991). Ii is also believed that competition for nutrients is more critical for soil borne pathogens, including *Fusarium* and *Pythium* species that infect through mycelial contact than foliar pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs (Elad and Baker, 1985; Keel *et al*., 1989; Loper and Buyer, 1991). Results of a study by Anderson *et al*. (1988) revealed that production of a particular plant glycoprotein called agglutinin was correlated with potential *of Pseudomonas putida* to colonize the root system. *P. putida* mutants deficient in this ability exhibited reduced capacity to colonize the rhizosphere and a corresponding reduction in Fusarium wilt suppression in cucumber (Tari and Anderson, 1988).

It has been shown that non-pathogenic plant-associated microrganisms generally protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow. For example, effective catabolism of nutrients in the spermosphere has been identified as a mechanism contributing to the suppression of *Pythium ultimum* by *Enterobacter cloacae* (Van Dijk and Nelson, 2000; Kageyama and Nelson, 2003). At the same time, these microbes produce metabolites that are effective in suppression of pathogens. These microbes colonize the sites where water and carbon-containing nutrients are most readily available, such as exit points of secondary roots, damaged epidermal cells and nectaries and utilize the root mucilage.

Competition for rare but essential micronutrients, such as iron, has also been shown to be important in biological disease control. Iron is extremely limited in the rhizosphere, depending on soil pH. In highly oxidized and aerated soil, iron is present in ferric form (Kageyama and Nelson, 2003; Shahraki *et al*., 2009), which is insoluble in water and the concentration may be extremely low. This very low concentration can not support the growth of microorganisms. To survive in such environment, organisms were found to secrete iron-binding ligands called Siderophores having high ability to obtain iron from the micro-organisms (Shahraki *et al*., 2009). Almost all microorganisms produce siderophores, of either the catechol type or hydroxamate type (Kageyama and Nelson, 2003).

A direct correlation was established in vitro between siderophore synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of *F. oxysporum* (Elad and Baker, 1985). It was shown that mutants incapable of producing some siderophores, such as pyoverdine, were reduced in their capacity to suppress different plant pathogens (Keel *et al*., 1989; Loper and Buyer, 1991). The increased efficiency in iron uptake of the commensal microorganisms is thought to be a critical factor in their root colonization ability which is a major factor in biocontrol performance of bacterial antagonists. Induction of resistance: Plants actively respond to a variety of environmental stimulating factors, including gravity, light, temperature, physical stress, water and nutrient availability and chemicals produced by soil and plant associated microorganisms (Audenaert *et al*., 2002; De Meyer and Hofte, 1997; Kloepper *et al*., 1980; Leeman *et al*., 1995; Moyne *et al*., 2000; Vallad and Goodman, 2004; Van Loon *et al*., 1998; Van Peer and Schippers, 1992; Van Wees *et al*., 1997). Such stimuli can either induce or condition plant host defenses through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. Induction of host defenses can be local and/or systemic in nature, depending on the type, source and amount of stimulation agents (Audenaert *et al*., 2002; De Meyer and Hofte, 1997; Kloepper *et al*., 1980; Leeman *et al*., 1995; Moyne *et al*., 2000; Vallad and Goodman, 2004;

Van Loon *et al*., 1998; Van Peer and Schippers, 1992; Van Wees *et al*., 1997).

Recently, plant pathologists have begun to characterize the determinants and pathways of induced resistance stimulated by biological control agents and other non-pathogenic microorganisms (Audenaert *et al*., 2002; Moyne *et al*., 2000; Vallad and Goodman, 2004). The first pathway called Systemic Acquired Resistance (SAR), is mediated by Salicylic Acid (SA), a chemical compound which is usually produced after pathogen infection and typically leads to the expression of Pathogenesis-related (PR) proteins (Vallad and Goodman, 2004). These PR proteins include a variety of enzymes some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death (Vallad and Goodman, 2004).

Second pathway, called Induced Systemic Resistance (ISR), is mediated by Jasmonic Acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria (Audenaert *et al*., 2002; De Meyer and Hofte, 1997; Kloepper *et al*., 1980; Leeman *et al*., 1995; Moyne *et al*., 2000; Van Loon *et al*., 1998; Van Peer and Schippers, 1992; Van Wees *et al*., 1997). Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic and some bacterial pathogens take advantage of this to overcome the SAR. For example, pathogenic strains of *Pseudomonas syringae* produce coronatine, which is similar to JA, to overcome the SA-mediated pathway (Vallad and Goodman, 2004). Since the various host-resistance pathways can be activated to variable degrees by different microorganisms and insect feeding, it is therefore possible that multiple stimuli are constantly being received and processed by the plant. Thus, the magnitude and duration of host defense induction will likely vary over time. Only if induction can be controlled, i.e., by overwhelming or synergistically interacting with endogenous signals, will host resistance be increased (Audenaert *et al*., 2002; De Meyer and Hofte, 1997; Kloepper *et al*., 1980; Leeman *et al*., 1995; Moyne *et al*., 2000).

Some strains of root-colonizing microorganisms have been identified as potential elicitors of plant host defenses. For example, some biocontrol active strains of *Pseudomonas* sp. and *Trichoderma* sp. are known to strongly induce plant host defenses (Haas and Defago, 2005; Harman *et al*., 2004). In other instances, inoculation with Plant Growth Promoting Rhizobacteria (PGPR) have been shown to be effective in controlling multiple diseases caused by different fungal pathogens, including anthracnose (*Colletotrichum lagenarium*). A number of chemical elicitors of SAR and ISR such as salicylic acid, siderophore, lipopolysaccharides and 2, 3-butanediol may be produced by the PGPR strains upon inoculation (Ryu *et al*., 2004; Van Loon *et al*., 1998).

A substantial number of microbial products have been reported to elicit host defenses, indicating that host defenses are likely stimulated continually during the plant's lifecycle (Ryu *et al*., 2004; Van Loon *et al*., 1998). These inducers include lipopolysaccharides and flagellin from Gram-negative bacteria; cold shock proteins of diverse bacteria; transglutaminase, elicitins and a-glucans in Oomycetes; invertase in yeast; chitin and ergosterol in all fungi; and xylanase in Trichoderma (Ryu *et al*., 2004). These findings indicate that plants would detect the composition of their plant-associated microbial communities and respond to changes in the quantity, quality and localization of many different signals. The importance of such interactions is indicated by the fact that further induction of host resistance pathways, by chemical and microbiological inducers, is not always effective in improving plant health or productivity in the field (Vallad and Goodman, 2004).

METHODS OF APPLICATION OF ANTAGONISTS

Overall application: Successful application of biological control strategies requires more knowledge-intensive management (Baker, 1987; Cook, 1993; Heydari *et al*., 2004; Shah-Smith and Burns, 1997). Understanding when and where biological control of plant pathogens can be profitable, requires an appreciation of its place within integrated pest management systems (Cook, 1993; Heydari *et al*., 2004; Shah-Smith and Burns, 1997).

In general, the foundation of a sound pest and disease management program in an annual cropping system begins with cultural practices that alter the farm landscape to promote crop health (Cook, 1993; Heydari *et al*., 2004; Shah-Smith and Burns, 1997). These include crop rotations that limit the availability of host material used by plant pathogens (Cook, 1993). Proper use of tillage can disrupt pathogen life cycles and prepare seed beds of optimal moisture and bulk density. Careful management of soil fertility and moisture can also limit plant diseases by minimizing plant stress (Cook, 1993). In nurseries and greenhouses environmental control can be more tightly regulated in terms of temperature, light, moisture and soil composition, but the design of such systems cannot wholly eliminate disease problems (Paulitz and Belanger, 2001).

The second layer of defense against pests consists of the quality of crop germplasm. Breeding for pathogen resistance including fungal pathogens contributes substantially to crop success in most regions (Cook, 1993). Newer technologies that directly incorporate genes into crop genomes, commonly referred to as genetic modification or genetic engineering, are bringing new traits into crop. Other technologies, such as seed washing, testing for pathogens and treatments are also used to keep germplasm pathogen-free. In perennial cropping systems, such as orchards and forests, germplasm quality may be more important than cultural practices, because rotation and tillage cannot be used as regularly (Agrios, 1988; Cook, 1993). Upon these two layers, growers can further reduce pathogen pressure by considering both biological and chemical inputs.

Biologically based inputs such as microbial fungicides can be used to interfere with pathogen activities. Registered biofungicides are generally labeled with short reentry intervals and pre-harvest intervals, giving greater flexibility to growers who need to balance their operational requirements and disease management goals. When living microorganisms are introduced, they may also augment natural beneficial populations to further reduce the damage caused by targeted pathogens (Cook, 1993; Heydari *et al*., 2004; Shah-Smith and Burns, 1997).

Applying to the infection site: Application directly to the infection court at a high population level to swamp the pathogen (inundate application), seed coating and treatment with antagonistic fungi and bacteria, e.g., *Trichoderma harzianum* and *Psudomonas fluorescens* (Cook, 1993; Heydari and Misaghi, 2003; Heydari *et al*., 2004), antagonists applied to fruit for protection in storage, e.g., *Pseudomonas fluorescens* (De Capdeville *et al*., 2002; El-Ghaouth *et al*., 2000; Janisiewicz and Korsten, 2002; Janisiewicz and Peterson, 2004) and application to soil at the site of seed placement (Heydari and Misaghi, 2003). These types of applications are the most commonly used procedures which have resulted in the successful control of several fungal plant pathogens.

One place application: in this procedure, biocontrol microorganisms are applied at one place (each crop year), but at lower populations which then multiply and spread to other plant parts and give protection (augmentative application) against fungal pathogens. An Example of this method is Plant Growth Promoting Rhizobacteria (PGPR) and atoxigenic *Aspergillus flavus* on wheat seed scattered on the soil to spread to cotton flowers where they displace aflatoxin producing strains of *A. flavus* and fungal antagonists added to soil (Islam *et al*., 2005; Kloepper *et al*., 2004).

Occasional application: One time or occasional application maintains pathogen populations below threshold levels. In theory, parasites of the pathogen, or hypovirulent (disease carrying) strains of the pathogen, might be used and not require yearly repetition (e.g., hypovirulent strains of the chestnut blight pathogen) in which host plant is inoculated with attenuated strains of pathogenic that protects the host plant against the virulent strains of pathogen (Milgroom and Cortesi, 2004).

BIOCONTROL OF DIFFERENT FUNGAL PATHOGENS

Microorganisms naturally present in the plants ecosystem will help reduce disease potential or disease damage, but only if they are allowed to grow vigorously (Cook, 1993). They accomplish these tasks by competing with the pathogens for food sources, producing metabolites that inhibit the growth of the pathogens and physically eliminating the pathogens from the plant by occupying the space and sites first. Microorganisms not naturally present in plant environment can be introduced in an attempt to control diseases (Cook, 1993). This can be done by application of organic materials that contain natural microbial populations such as composts or natural microbial populations added to them including natural organic fertilizers with microbial supplements. In both cases, the products must be applied prior to disease development as they are preventive and not curative (Baker, 1987; Cook, 1993). Natural organic fertilizers should be used for their nutritional value (nitrogen and potassium) and not for any possible secondary effects.

Fungal plant pathogens are very diverse and cause diseases on different parts of plants such as root, stem, leaf, fruit, etc. In this section, application of biological control strategies for controlling fungal diseases on different parts of plants will be discussed.

The majority of research on biocontrol of fungal diseases have focused on soil borne diseases rather than foliar or post harvest. According to the results of numerous research projects, several fungal and bacterial biocontrol agents have been used as seed and soil application to reduce the incidence of plant diseases caused by soil borne fungal pathogens (Cook, 1993; Heydari, 2007; Heydari *et al*., 2004; Heydari and Misaghi, 2003; Lo *et al*., 1995, 1996, 1997; McSpadden-Gardener, 2001; Naraghi *et al*., 2004; Ramette *et al*., 2003; Scheuerell *et al*., 2005). Since many plant pathogens can spread readily in the foliar parts, control of these diseases requires both suppression of initial plant infection and reduction of the infection rate (Lo *et al*., 1997). Granular applications of strain 1295-22 of *Trihoderma harzianum* has been shown to significantly inhibit disease severity of some plant diseases during the initial stage of disease development, most likely by reducing levels of the pathogen inoculum in the soil (Lo *et al*., 1995, 1996, 1997). It is apparent, therefore, that soil applications alone cannot effectively control the foliar phases of this disease.

Additives have been commonly used with fungicides to improve efficacy and they also may enhance the ability of biocontrol agents to reduce plant diseases. For example, it was reported that seed treatment using 10% Pelgel with solid matrix priming markedly enhanced the efficacy of Trichoderma strains to control *Pythium* sp. on various crops (Lo *et al*., 1997). Research has indicated that for control of multiple fungal plant diseases, greater control was obtained when Triton X-100 was included than when no additives, Pelgel, or Tween 20 were used (Lo *et al*., 1997). The use of specific surfactants with Trichoderma strains seems essential to obtain levels of control equivalent to those achieved with chemical fungicides. Detergents such as Triton X-100 may have several functions in biocontrol systems. They may slow the growth of pathogens more than that of the biocontrol agents or they may enhance wetting and adhesion of spores to infection courts (Lo *et al*., 1997). In preliminary experiments, both Tween 20 and Triton X-100 slowed the growth of both *T. harzianum* and the pathogens, but the ratio of the growth rates of *T. harzianum* and pathogens was greater with Triton X-100 than with Tween 20 (Lo *et al*., 1997).

Living orgarisms, in addition to yielding a large quantity of biomass of the bioprotectant fungus, must perform effectively in each application. To examine this, different spore formulations of *Trihoderma harzianum* were compared in a study for controlling plant diseases (Lo *et al*., 1996). It was found that all formulations provided equivalent levels of control, indicating that the method of spore production may not be a key factor in the efficacy of this fungal biocontrol agent in controlling these diseases (Lo *et al*., 1996). To predictably and successfully use biological control agents for fungal disease control, it is critical that their biology and ecology be more completely understood. Therefore, effective antagonists must become established in plant ecosystems and remain active against target pathogens during periods favorable for plant infection.

Broadcast application of granules of Trichoderma to control plant diseases has resulted in establishment of stable and effective populations of plants in soils (Lo *et al*., 1995, 1996, 1997). Similarly, it was shown that the populations of *T. harzianum* in soils treated with spray applications were as high as those in soils treated with granular formulations (Lo *et al*., 1996). Population levels of strain 1295-22 in about 5×10 5 cfu g^{-1} of soil significantly reduced Pythium blight, root rot and brown patch diseases (Lo *et al*., 1997). However, spray applications, even though resulted in numerically similar levels of root colonization, did not provide the same benefit. This may reflect the differences in inoculum potential of granules versus spray applications. Granules are applied as a several-millimeter-diameter particle that is completely colonized by the fungus. Conidial inoculum, on the other hand, is much smaller and would therefore be expected to possess lower inoculum potential than the granular formulation (Lo *et al*., 1997).

Conversely, in greenhouse and field experiments, it was found that *Trihoderma harzianum* significantly reduced some foliar phases of plant diseases when spray applications of conidial suspensions containing Triton X-100 were used (Lo *et al*., 1995, 1996, 1997). Weekly spray applications were as effective as the standard (monthly) fungicide applications. These results indicate that the efficacy of *T. harzianum* against plant diseases, especially those involving secondary infections, is very strongly affected by the method of application (Lo *et al*., 1997).

The ability to survive on the plant phylloplane is also a desirable trait for strains of fungal and bacterial antagonists used as biocontrol agents against foliar diseases (Lo *et al*., 1997). Spray applications of strain 1295-22 of *T. harzianum* has resulted in disease suppressive population levels on leaf (Lo *et al*., 1997). These populations were sufficient to suppress Pythium root rot, brown patch and dollar spot over the entire season. Thus, *T. harzianum* 1295-22 may possess a measure of phylloplane competence on the plants. The ideal biocontrol strategy attempts to introduce or promote the activity of biocontrol agents only when and where they are needed or are most effective and minimizes wasteful application of inoculum to non-target habitats (Lo *et al*., 1997). Thus, for effective delivery, it is necessary to consider plant–pathogen–antagonist interactions in terms of time and space.

Pythium, Rhizoctonia and Sclerotinia are important soil borne pathogens of many plant species and their survival structures in soil serve as primary inoculum. Consequently, suppression of the initial inoculum will be the first step in managing these pathogens (Lo *et al*., 1997). The granular application of biocontrol agents should be followed by monthly spray applications to suppress foliar phases of these diseases. Inhibition of the secondary infection and dissemination of these pathogens is also important for disease management (Lo *et al*., 1997). Monthly spray applications of *T. harzianum* could provide a second step in protection of plant foliage from attack by preventing these pathogens from initially infecting leaves and by reducing the spread of disease or other methods of inoculum dissemination. Finally, results of Lo *et al*. (1997) study have indicated that it will be necessary to apply weekly sprays for highly effective control of these pathogens under severe disease conditions.

In addition to Trihoderma and other fungal antagonists, several antagonistic bacterial species including *Pseudomonas fluorescens*, *P. putida*, *P. aerofaciens*, *Burkholderia cepacia*, *Bacillus subtillis*, *B. Polymyxa* and *B. cerrues* have also been used successfully in biological control of different soil borne fungal diseases (Heydari *et al*., 1997, 2004, 2007; Heydari and Misaghi, 2003; Kloepper *et al*., 2004; Leeman *et al*., 1995; Shahraki *et al*., 2009; Shishido *et al*., 2005; Weller and Cook, 1983; Zaki *et al*., 1998). By application of these bacterial antagonists, various fungal pathogens including *Rhizoctonia solani*, *Fusarium moxysporium*, *F. solani*, *Verticillium dahliae*, *Gaummannomuces graminis* and soil borne diseases caused by them such as seed rot, damping-off, root rot, vascular wilt and take-all have been biologically controlled on major agricultural crops including cotton, sugar beet, wheat, rice and different vegetables.

Although the majority of biological control research have been concentrated on soil borne fungal diseases, a number of studies have focused on fungal pathogens causing diseases and disorders in above-ground parts of plants (Kessel *et al*., 2005; Khodakaramian *et al*., 2008; Kovach *et al*., 2000; Milgroom and Cortesi, 2004; Smith *et al*., 1993). For example, Anderson *et al*. (2004) studied the possibility of biological control of fungal pathogens in the phylosphere and proposed that it may be possible to reduce the incidence and development of these diseases using fungal and bacterial antagonists.

In another study, biological control of powdery mildew disease on different crops using antagonistic fungi was investigated and it was found that biocontrol-active microorganisms can potentially be applied against this very important foliar diseases. *Botrytis cinera* which is the causal agent of gray mold on many plants (Agrios, 1988) was successfully controlled by the use of biocontrol-active microorganism on strawberry (Kovach *et al*., 2000). In another study conducted by Smith *et al*. (1993) biological control of cotton leak of cucumber caused by a fungal foliar pathogen was studied. It was found that *Bacillus cerrues,* a bacterial antagonist was capable of reducing the incidence of the disease significantly (Smith *et al*., 1993).

Another example of using biocontrol-active microorganisms against foliar fungal pathogen is the study in which chestnut blight was successfully controlled by the virus that infects *Cryphonectria parasitica*, the fungal causal agent of the disease through the mechanism of hypovirulence, a reduction in pathogenicity of the pathogen. This phenomenon has resulted in control of the chestnut blight in many places (Milgroom and Cortesi, 2004). However, the interactions of virus, fungus, tree and environment play very important role in the success of disease control.

In addition to soil borne and foliar diseases some studies have also tested the efficacy of biocontrol-active microorganisms on post harvest fungal pathogens which cause losses to fruits and vegetables during post harvest and storage periods (Janisiewicz and Korsten, 2002). Spray applications of fungal and bacterial antagonists have resulted in significant reduction in the infection caused by some fungal pathogens in the storage.

THE USE OF COMPOST AS BIOFERTILIZER

Research data and observations in nurseries have shown that addition of composted organic matter to potting mixes results in suppression of soil borne fungal diseases (McKellar and Nelson, 2003; Paulitz and Belanger, 2001). The concentration of suppressive microorganisms in compost amended substrates is very high, but greatly reduced in soils or potting mixes after the amendment (McKellar and Nelson, 2003; Paulitz and Belanger, 2001). As a result, predictive disease suppression models have been developed based on the composition and concentration of microbial biomass.

The effectiveness of composts in suppression of soil borne diseases is dependent on heat kill, organic matters decomposition, recolonization of compost by suppressive microorganisms following heat kill and physical and chemical factors (McKellar and Nelson, 2003). Although previous works have focused on plant soil borne diseases, current research indicates that potting mixes containing composted organic materials which also have been inoculated with *Trichoderma hamatum* can be effective as a biocontrol alternative to foliar fungicides; however, the mechanism of this systemic type of induced resistance is not yet understood (McKellar and Nelson, 2003). Although the growers have traditionally relied on aged pine bark and composted biosolids to provide the potential for disease suppression, research indicates that composted animal manure have the potential to replace some of these components, but a consistent quantity and quality of these materials will need to be incorporated (McKellar and Nelson, 2003; Paulitz and Belanger, 2001). The maturity (stability) of the composted manure and its salinity largely determine its ability to induce suppression.

COMMERCIALIZATION OF BIOCONTROL

Commercial use and application of biological disease control have been slow mainly due to their variable performances under different environmental conditions in the field (Fravel, 2005; Mercier and Lindow, 2001; Paulitz and Belengar, 2001; Wang *et al*., 2003). Many biocontrol agents perform well in the laboratory and green house conditions but fail to do so in the field. This problem can only be solved by better understanding of the environmental parameters that affect biocontrol agents (Fravel, 2005; Mercier and Lindow, 2001; Paulitz and Belengar, 2001; Wang *et al*., 2003). In addition to this problem, there has also been relatively little investment in the development and production of commercial formulation of biocontrol-active microorganisms probably due to the cost of developing, testing, registering and marketing of these products (Heydari *et al*., 2007; Ardakani *et al*., 2009).

Biological control agents are generally formulated as wetable powders, dusts, granules and aqueous or oil-based liquid products using different mineral and organic carriers (Ardakani *et al*., 2009).

Currently in the market, a number of biologically based products are being sold for the control of fungal plant diseases (Ardakani *et al*., 2009). A growing number of companies are also developing new products that are in the process of registration. Many of these companies are small, privately owned firms with a limited product-line. Others are publicly traded and have substantial capitalization values. In addition, larger companies with more diverse product lines that include a variety of agrochemicals and biotechnological products have played a significant role in the development and marketing of products for the control of plant pathogens (Ardakani *et al*., 2009).

Biocontrol products are either marketed as stand-alone products or formulated as mixtures with other microbials. Some products with biocontrol properties may not be registered, but are sold instead as plant strengtheners or growth promoters without any specific claims regarding disease control (Ardakani *et al*., 2009). To help improve the global market perception of biopesticides as effective products, the biopesticide Industry Alliance is establishing a certification process to ensure industry standards for efficacy, quality and consistency. To improve commercial use and application of biological disease control it is extremely important to emphasize and concentrate on several factors including training of growers, formulation of biocontrol microorganisms and studying the role of environmental factors.

FUTURE OUTLOOK

Biological control really developed as an academic discipline during the 1970s and is now a mature science supported in both the public and private sector. Research related to biological control is published in many different scientific journals, particularly those related to plant pathology and entomology. Additionally, there are some academic journals specifically devoted to this disipline. In the United States, research funds for the biological control are provided primarily by several USDA programs. These include the Section 406 programs, regional IPM grants, Integrated Organic Program, IR-4 and several programs funded as part of the National Research Initiative (Bloom *et al*., 2003). Monies also exist to stimulate the development of commercial ventures through the small business innovation research programs. Such ventures are intended to be conduits for academic research that can be used to develop new companies (Spadaro and Gullino, 2005).

Much has been learned from the biological control research conducted over the past forty years. But, in addition to learning the lessons of the past, biocontrol researchers need to look forward to define new and different questions, the answers to which will help facilitate new biocontrol technologies and applications. Currently, fundamental advances in computing, molecular biology, analytical chemistry and statistics have led to new research aimed at characterizing the structure and functions of biocontrol agents, pathogens and host plants at the molecular, cellular and ecological levels (Spadaro and Gullino, 2005). Some of the research criteria that will advance our understanding of biological control and the conditions under which it can be most fruitfully applied are as follows:

Ecology of antagonistic microbes: Ecological factors play very important roles in the performance and activity of biocontrol-active microorganisms. In this regard, the following criteria need to be clarified and studied:

- The distribution of fungal pathogens and their antagonists in the environment
- The optimum conditions in which biocontrol microorganisms exert their suppressive capacities
- The response of native and introduced populations to different management practices
- The determinants factor of successful colonization and expression of biocontrol traits

- The components and dynamics of plant host defense induction

Application methods: In regard with application strategies still there are some areas which should be investigated and developed for the enhancement of the effectiveness of biocontrol microorganisms. These areas are as follows:

- The search for more effective strains or strain variants for current applications
- The use of genetic engineering of microbes and plants for enhancing biocontrol application methods
- The development of proper formulations to enhance activities of known biocontrol agents

Introducing new strains and mechanisms: Since fungal plant pathogens are very diverse and their pathogenicity is different on host plants, it is therefore very important to look for new and novel biocontrol microorganisms with different mechanisms. In this regard, the following criteria need further investigation:

- The use of previously uncharacterized microbes as biological control agents
- Study on the roles of other genes and gene products which are involved in pathogen suppression
- The efficacy of using novel strain combinations in comparison with individual agents
- Study on the signal molecules of plant and microbial origin which regulate the expression of biocontrol traits by different agents

Integrated pest management: Since the ultimate goal of biological control of plant diseases is to assist the growers to combat and control plant pathogens in the field which is the real agricultural environment, it is therefore important to practically integrate biocontrol strategies into agricultural system. In this regard, the following criteria should be considered and followed carefully:

- Selection of production systems that can most benefit from biocontrol for disease management
- Application and use of biocontrol strategies which best fit with other IPM system components
- Development of effective biocontrol-cultivar combinations by plant breeders

Research and development: Nowadays, growers are interested in reducing dependence on chemical inputs, so biological controls (defined in the narrow sense) can be expected to play an important role in Integrated Pest Management (IPM) systems (Jacobsen *et al*., 2004). Good agricultural practices (GAP) including appropriate site selection, crop rotations, tillage, fertility and water management, provide the foundation for successful pest management by providing a fertile growing environment for the crop. The use of disease-resistant varieties, developed through conventional breeding or genetic engineering, provides the next line of defense. However, such measures are not always sufficient to be productive or economically sustainable. In such cases, the next step would be to deploy biorational controls of diseases. These include BCAs, introduced as inoculants or amendments, as well as active ingredients directly derived from natural origins and having a low impact on the environment and non-target microorganisms (Guetsky *et al*., 2001; Jacobsen *et al*., 2004).

If these foundational options are not sufficient to ensure plant health and/or economically sustainable production, then less specific and less harmful synthetic chemical toxins can be used to ensure productivity and profitability. With the growing interest in reducing chemical inputs, companies involved in the manufacturing and marketing of BCAs should experience continued growth. However, stringent quality control measures must be adopted so that farmers get quality products. New, more effective and stable formulations also will need to be developed.

Most fungal pathogens are susceptible to one or more biocontrol strategies, but practical implementation on a commercial scale has been constrained by a number of factors. Cost, convenience, efficacy and reliability of biological controls are important considerations, but only in relation to the alternative disease control strategies. Cultural practices (e.g., good sanitation, soil preparation and water management) and host resistance can go a long way towards controlling many diseases, so biocontrol should be applied only when such agronomic practices are insufficient for effective disease control. As long as petroleum is cheap and abundant, the cost and convenience of chemical pesticides will be difficult to surpass. However, if the infection court or target pathogen can be effectively colonized using inoculation, the ability of the living organism to reproduce could greatly reduce application costs.

In general, although, regulatory and cultural concerns about the health and safety of specific classes of pesticides are the primary economic drivers promoting the adoption of biological control strategies in urban and rural landscapes (Timms-Wilson *et al*., 2004). Self-perpetuating biological controls (e.g., hypovirulence of the chestnut blight pathogen) are also needed for control of diseases in forested and rangeland ecosystems where high application rates over larger land areas are not economically feasible. In terms of efficacy and reliability, the greatest successes in biological control have been achieved in situations where environmental conditions are most controlled or predictable and where biocontrol agents can preemptively colonize the infection court (Fravel, 2005). Monocyclic, soilborne and post-harvest fungal diseases have been controlled effectively by biological control agents that act as bioprotectants (i.e., preventing infections). Specific applications for the high value crops targeting specific diseases (e.g., downy mildew, powdery mildew and several other fungal diseases) have also been adopted (Kessel *et al*., 2005). As research unravels the various conditions needed for successful biocontrol of different fungal diseases, the adoption of BCAs in IPM systems is bound to increase in the years ahead.

CONCLUSIONS

Due to the serious environmental and health problems that wide spread use of chemical pesticides has created in the world, search for alternative safe methods is unavoidable. Biological control of plant diseases has been the subject of numerous research projects in recent years (Bargabus *et al*., 2004; Benhamou, 2004; Chisholm *et al*., 2006; Heydari, 2007; Islam *et al*., 2005). There is a growing demand for biologically based pest management practices. Recent surveys of both conventional and organic growers indicate an interest in using biocontrol products suggesting that the market potential of biocontrol products will increase in the future (Joshi and Gardener, 2006). Application of different biological control strategies has been successful in the greenhouse industry and continues to increase (Jacobsen *et al*., 2004). An upswing in commercial interests has also developed in the past few years and prospects for increased growth are positive. The Biopesticide Industry Alliance has formed and it is now actively promoting the value and efficacy of biopesticides (including those that control fungal plant pathogens). Clearly, the future success of the biological control industry will depend on innovative business management, product marketing, extension education and research (Timms-Wilson *et al*., 2004; Joshi and Gardener, 2006).

Increased demand for organic products in home gardening activities by using non-chemical methods has enlarged the market for biocontrol products. The field of plant pathology will contribute substantially to making the 21st century the ages of biotechnology by the development of innovative biocontrol strategies. A variety of research questions remain to be fully answered about the nature of biological control and the means to most effectively manage it under production conditions. Advanced molecular techniques are now being used to characterize the diversity, abundance and activities of microbes that live in and around plants, including those that significantly impact plant health (Joshi and Gardener, 2006). Still, much remains to be learned about the microbial ecology of both plant pathogens and their microbial antagonists in different agricultural systems. Fundamental work remains to be done on characterizing the different mechanisms by which organic amendments reduce plant disease including those caused by fungal pathogens. More studies on the practical aspects of mass production and formulation need to be undertaken to make new biocontrol products stable, effective, safer and more cost-effective.

Fungal pathogens are among the most important factors that cause serious damages and losses to plants. Harmful impacts of the chemical pesticides on the environment and non-target organisms have clearly been documented. The need for the development of non-chemical alternative strategies to protect plants against plant diseases including fungal pathogens is therefore clear. Biological control using fungal and bacterial antagonists to manage plant diseases seems to be a promising alternative strategy and have successfully been applied to control some diseases on different plants and crops. Biocontrol strategies may also be used to manage other plant diseases including foliar ones. Some of the important factors that affect the efficacy of microbial biocontrol agents in controlling plant diseases which should carefully be considered include method of application, formulation of biocontrol microorganisms and timing of application. Various composts and organic amendments as other means of biological control have also been tested on some plants and proven to be promising.

There are many products composed of living organisms, primarily bacteria and fungi, being sold that claim they will increase plant health. However, for any material to be considered a biological fungicide the Environmental Protection Agencies and Organizations must register it (Bloom *et al*., 2003). This registration indicates that the safety of the product to humans, non-humans (fish for example) and the environment has been determined. Materials that have not been approved should be used with caution.

Complete elimination of chemical pesticides for controlling plant pests and diseases in modern agriculture may be impossible, but a logical reduction in their application is absolutely feasible. To have a sustainable agricultural system with minimum contamination and risks to the environment, a combination of all available methods should be applied to manage pest problems and this can be achieved by Integrated Pest Management (IPM). Implementation of IPM strategies may be the safest solution for management of pest problems including fungal diseases in every cropping system and with no doubt biological control is one of the most important components of Integrated Pest Management which can lead us toward a sustainable agricultural system in the future.

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