Review

A review of the non-target effects of fungi used to biologically control plant diseases

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Abstract

Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. Fungal biological control agents have several mechanisms of action that allow them to control pathogens, including mycoparasitism, production of antibiotics or enzymes, competition for nutrients and the induction of plant host defences. While effective in the control of plant diseases, these mechanisms may pose risks to non-target species including mycorrhizal and saprophytic fungi, soil bacteria, plants, insects, aquatic and terrestrial animals, and humans. Non-target effects including mycoparasitism of mycorrhizae, reduction in plant root colonisation by mycorrhizal fungi, disorders in commercial mushrooms and nodulation by Rhizobium spp., and changes in plant growth have been associated with fungal biological control agents, such as Trichoderma spp. Also, the genera Trichoderma and Gliocladium have been linked to respiratory disorders and shellfish toxicity in humans, respectively. Biological control agents, such as Pythium oligandrum, Talaromyces flavus, Coniothyrium minitans and Ampelomyces quisqualis have modes of action which may pose risks to non-target fungi, bacteria, plants and animals. There is need for future research into ecological impacts associated with the release of any biological agent and methods of determining possible non-target effects. Adequate monitoring and the use of molecular techniques to identify and follow the movement of biological control agents are needed to examine and mitigate negative biological impacts.

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1. Introduction

Plant diseases are of significant concern due to the intimate relationship between plant health and the welfare of people, animals, and the environment. The ability to provide adequate food and fibre is becoming increasingly strained and continued improvement in sustainable plant disease management is required to help meet these demands (Harrington, 1995; Nene, 1996). Plant diseases often substantially reduce quality and quantity of agricultural commodities (Seitz et al., 1982; Alderman et al., 1996). Also, infestation by micro-organisms in the field or in post-harvest storage can affect the health of humans and livestock, especially if the contaminating organism produces toxic residues in or on consumable products (Diekman and Green, 1992; Nelson et al., 1993; Cheeke, 1995). Control of plant disease is vital in plant agriculture and food storage, yet, modern methods of disease management, while effective in the control of plant pathogens,
may negatively impact non-target organisms (Gray Jr. et al., 1994; Elbert et al., 2000).

Commercially produced, registered products, such as fungicides, are frequently recommended for plant disease management. These chemicals have an established history of controlling many economically important diseases, despite the development of tolerance or resistance in pest organisms (Godet and Limpert, 1998; Gossen and Rimmer, 2001). In most countries, products used for disease management are subject to tests of efficacy and assessments of risks to non-target organisms. This risk can be defined as the combination of the agent’s toxicity and the probability of exposure of non-target organisms (Cook et al., 1996; Hintz et al., 2001). In pest control situations, risk assessment may be performed to determine the effect of an agent on the target pest population while identifying potential detrimental effects on the environment and non-target species (Teng and Yang, 1993).

Despite government regulation, there is concern over the risk of non-target effects of pesticides, which has sparked an interest in technologies that reduce dependency on synthetic, chemical pesticides. One such technology is biological control, or the release of micro-organisms to control a specific target species (Whipps, 1997). Biological control agents (BCAs) are perceived to have specific advantages over synthetic fungicides, including fewer non-target and environmental effects, efficacy against fungicide-resistant pathogens, reduced probability of resistance development (Cook, 1988), and use in organic farming situations where synthetic fungicides are restricted (Anonymous, 1999; Harman, 2000; Toree et al., 2001).

2. Target effects of biological control agents

The ecological role of fungi has been exploited in recent years to control plant pathogens in agricultural, horticultural, and forestry systems. Many fungi possess mechanisms that allow them to efficiently cure or prevent both foliar and root diseases caused by pathogenic fungi. One of the strategies used to control pathogens is mycoparasitism (Harman, 2000), whereby a species or strain of fungus directly attacks and feeds on other fungi (Kendrick, 1992). Another mechanism involves the production of antibiotics or enzymes that inhibit the growth or reduce the competitive ability of other organisms (Howell and Stipanovic, 1983; Simon et al., 1988; Dunlop et al., 1989; Ghisalberti and Sivasithamparam, 1991; Harman et al., 1993; Howell et al., 1993; Harman, 2000). Control may also be achieved through competition for space and resources with highly competitive BCAs quickly colonising plant surfaces, creating an effective ‘living barrier’ to subsequent pathogen invasion (Cook, 1988). Another mechanism is the mobilisation of nutrients in the soil, a process that makes compounds in the soil more available for plant uptake, resulting in increased general health and disease resistance (Harman, 2000). BCAs may also induce changes in the plant that increase disease resistance similar to the phenomena of induced and systemic acquired resistance (Handelsman and Stabb, 1996; Harman, 2000).

Several review articles discuss the role of fungi in the control of specific pathogens (Handelsman and Stabb, 1996; Wilson, 1997; Bailey and Lumsden, 1998; Harman and Björkman, 1998; Harman, 2000). Interactions between BCAs and plant pathogens are often beneficial for disease management in field, greenhouse, and forestry systems. However, the micro-organisms involved may have detrimental impacts on other organisms present in the ecosystem to which they are applied.

3. Non-target effects of biological control agents

Cook et al. (1996) reviewed the potential non-target effects of BCAs including competitive displacement, allergenicity, toxicity, and pathogenicity. Competitive displacement occurs when a BCA expels or replaces native non-target species through contention for space or nutrients. Allergenicity may occur in humans or animals that develop sensitivities to spores or formulations of the BCAs, whereas the release of antibiotics or alkaloids, which may aid in the control of diseases, may have toxic effects on non-target species. Finally, the potential of a BCA to infect organisms other than the target pests is of most concern from a risk management perspective (Cook et al., 1996). An additional factor to consider upon the release of a BCA is the potential for the agent to reproduce and spread to non-target environments where it may have unanticipated effects on native organisms (Simberloff and
Stirling, 1996a) Since some BCAs are hypovirulent forms of plant pathogens that compete with virulent isolates, there is a possibility that gene transfer may occur between isolates, resulting in a gain of virulence in the BCA and loss of biological control abilities (Gullino Jr., et al., 1995).

Since many BCAs target fungal pathogens, the most likely non-target effect of BCAs is a reduction in the diversity and/or abundance of other fungi in an ecosystem. This may have significant environmental impacts because fungi are involved in the cycling of nutrients due to their ability to release enzymes that permit the metabolism of complex organic molecules that are unavailable to many organisms, such as lignin (Orth et al., 1993). The disruption of native fungi in soils due to the release of a BCA may also have ecological effects on plants that form symbiotic relationships (mycorrhizae) with fungi. Mycorrhizal fungi grow within plant roots and penetrate cortical cells or form a mantle on the outer surface of the roots while growing between cortical cells (Kendrick, 1992; Smith et al., 1994). Mycorrhizal associations provide several benefits to plants including increased shoot and/or root growth (Huang et al., 1985; Sai, 1987; Koide et al., 1988; Wacker et al., 1990; Li et al., 1991; Lu and Koide, 1994; McAllister et al., 1994b; Ruiz-Lozano et al., 1995), the transfer of nutrients, such as phosphorus, from soil to plant (Bolan et al., 1987; Li et al., 1991; Jakobsen et al., 1999; Marchiner and Dell, 1994; Perez-Moreno and Read, 2000), increased disease resistance (Perrin, 1990; Wacker et al., 1990; Morin et al., 1999) and drought tolerance (Ruiz-Lozano et al., 1995). Disruption of soil fungi by BCAs may also affect some insects that form complex relationships with fungal species, e.g. leaf-cutting ants raising fungivorous microorganisms in underground gardens. These ants supply the fungi with food, remove invading pathogens and gain sustenance from the fungal hyphae (Cazin Jr., et al., 1989; Bass and Cherrett, 1996; Thorn, 1997).

Several species of fungi commonly found in soils control plant diseases by preying on pathogenic soil micro-organisms, such as nematodes (Janssen et al., 2000), pathogenic fungi (Foley and Deacon, 1986; Huang and Kokko, 1987; Falk et al., 1995; Ibbar et al., 1996; Madi et al., 1997; Benhamou et al., 1999) or insects (Goettel et al., 1995). Released BCAs have the potential to disrupt entire ecosystems through changes in the native soil community, some of which may be reflected in direct or indirect impacts on plants and animals.

3.1. Trichoderma spp.

Trichoderma spp. have been developed into several commercial biological control products used in field crop and greenhouse systems (Harman, 2000) and are known to control numerous soil-borne diseases, such as those caused by Pythium ultimum Trin. (Naseby et al., 2000), Sclerotinia sclerotiorum (Lib.) de Bary (Ibhar et al., 1996) and Fusarium oxysporum Schlechter.end. (Sivan and Chet, 1993). Trichoderma aureoviride Rifai can form coiled structures of hyphae around the hyphae of filamentous pathogens. This coiling is considered characteristic of mycoparasitism and is often followed by penetration of the cell wall and dissolution of the cytoplasm as indicated by increased cellular vacuolisation in the host (Calvet et al., 1989). The penetration of Trichoderma harzianum Rifai into the cell wall of other fungi is attributed to the production of enzymes that catalyse the breakdown of chitin, a primary component of fungal cell walls (Zeilinger et al., 1999). Penetration is followed by the release of antibiotics that permeate the perforated hyphae and prevent resynthesis of the host cell wall (Lorito et al., 1996). After penetration of the cell wall, T. harzianum can cause dissolution of the cytoplasm and grow within the empty host hyphae (Ibhar et al., 1996).

T. harzianum has been shown to penetrate the resting spores and subtending hyphae of the arbuscular mycorrhizal species, Glomus intraradices Schenck and Smith, resulting in dissolution of the hyphal and spore cytoplasm. This penetration was followed by the growth of T. harzianum in the hyphae of Glomus mosseae (Nicol. and Ger.) Gerl. and Trappe, eventually leading to the rupture of the host (Rousseau et al., 1996). Metabolites produced by T. harzianum may also play a role in mycoparasitism because changes in spore protoplasm and cell wall integrity occurred prior to penetration by the T. harzianum, weakening or causing death of spores or hyphae of G. mosseae and facilitating penetration by the mycoparasite. Perforations in the cell wall indicated that chitinase enzymes were released by T. harzianum to weaken the cell wall and assist the penetration by the mycoparasite (Rousseau et al., 1996). Similarly, Trichoderma koningii Oudemans was shown to reduce germination.
Mycoparasitism is not always involved in interactions between mycorrhizal fungi and Trichoderma spp. For example, interaction between G. mosseae and Trichoderma pseudokoningii has been shown to have a negligible or even stimulatory effect on the growth and propagation of mycorrhizal fungi. In fact, the presence of T. pseudokoningii increased the number of asexual spores produced by G. mosseae, and increased the time to vegetative sporulation (McAllister et al., 1992). This stimulation appears to be dependent on the order of inoculation of the fungi. Percent colonisation of roots by arbuscular mycorrhizal species was reduced only when plants were simultaneously inoculated with T. punctiforme and G. mosseae, or T. pseudokoningii was inoculated 2 weeks prior to T. punctiforme. The ability of T. harzianum to impedes colonisation of plant roots by mycorrhizal species has also been shown (McAllister et al., 1999). The presence of T. harzianum has been also associated with decreased colonisation of soybean (Glycine max) roots by G. mosseae. However, even if T. punctiforme did not affect colonisation by G. mosseae, T. pseudokoningii did not affect colonisation by G. mosseae. Thus, if the antagonistic effect of the mycorrhizal fungus on plant growth, reflected in increased growth, remains when the two species are grown together in culture (Calvet et al., 1992). In contrast, the antagonistic effect of the mycorrhizal fungus on the growth and propagation of mycorrhizal fungi. In fact, the presence of T. pseudokoningii increased the number of asexual spores produced by G. mosseae, and increased the time to vegetative sporulation (Calvet et al., 1992).
an enzyme associated with the mycorrhizal-mediated release of inorganic phosphate from soil (Dighton, 1983; Jha et al., 1992), increased in the rhizosphere of corn roots inoculated with a community of arbuscular mycorrhizal fungi isolated from test soil. In the presence of T. harzianum strain T-12, the level of phosphatase significantly decreased in the rhizosphere plants inoculated with a population of mycorrhizal fungi compared to mycorrhizal plants grown without T. harzianum. However, T. harzianum increased the levels of phosphatase in the rhizosphere of plants colonised by only G. mosseaem or G. deserticola (Vázquez et al., 2000), implying that the effect is dependent on the mycorrhizal species. The presence of T. harzianum in the rhizosphere of corn colonised by G. deserticola reduced the levels of trehalase, an enzyme indicative of a symbiotic relationship since it catalyses the breakdown of a sugar commonly associated with symbioses between plants and micro-organisms (Melior, 1992).

*Trichoderma* spp. can influence ectomycorrhizae as well as arbuscular mycorrhizae. *Trichoderma viride* Pers. and *Trichoderma polysporum* (Link ex. Pers.) Rifai were strongly antagonistic to the ectomycorrhizal fungus, *Laccaria bicolor* (Maire) Ort. Formation of mycorrhizae was reduced when black spruce seedlings (*Picea mariana* (Mill.) B.S.P.) were inoculated simultaneously with *L. bicolor* and *T. polysporum*. *T. viride* completely prevented colonisation by *L. bicolor* unless the mycorrhizal fungus was allowed to establish before inoculation with the antagonist (Summerbell, 1987).

The antagonistic and mycoparasitic behaviour of *Trichoderma* spp. is not limited to pathogenic or symbiotic fungi. Saprophytic fungi, including cultivated mushrooms, are also at risk. *T. harzianum*, in particular, strain Th2, has been associated with a cropping disorder in the commercial white mushroom, *Agaricus bisporus* (Lange) Imbach, named green mold disease (Mumpini et al., 1998; Mamoun et al., 2000). The symptoms of this disease include malformation and spotting in the fruiting bodies of the white mushrooms (Seaby, 1989). It is important to note that the relationship between *T. harzianum* and *A. bisporus* appears to be highly specialised and dependent on the strain of the antagonist. The Th1 and Th3 strains of *T. harzianum* produced metabolites that inhibited the growth of the commercial mushroom in vitro, but they are not commonly associated with the disease (Mumpini et al., 1998), perhaps due to metabolites produced by *A. bisporus*, which affect spore germination and growth in many strains with the exception of the disease-inducing Th2. In fact, the presence of *A. bisporus* or the addition of metabolites produced by *A. bisporus* actually increased growth (Mamoun et al., 2000) and sporulation of Th2 (Mumpini et al., 1998). It is believed that *A. bisporus* and Th2 can grow simultaneously until contact between mycelia occurs. Once the hyphae of each species meet, sporulation of Th2 is induced and pathogenic behaviour in strain Th2 begins (Mumpini et al., 1998). Since Th2 is not used in biological control formulations (Hermosa et al., 2000), the release of *Trichoderma* spp. proven to be non-pathogenic to commercial fungi should not pose any significant threat.

Mycoparasites may have undesired effects on some pathogenic fungi. For example, when several isolates of *Trichoderma* spp. were applied as foliar sprays to control *S. sclerotiorum*, there was an increase in the number of reproductive structures formed by the pathogen compared to untreated controls (Gerlagh et al., 1989). Thus, the use of *Trichoderma* spp. to control this pathogen may result in increased incidence of the disease and spread of the pathogen.

Some soil bacteria play an important role in the growth and development of plants by altering the availability of nutrients. Bacterial species of the genus *Rhizobium* form symbiotic relationships with legumes, reside in root nodules and fix atmospheric nitrogen to compounds that are more readily available to the plant (Paul and Clark, 1989). Some BCAs have the potential to disrupt this association as was noted in peanut (*Arachis hypogaea* L.) when simultaneous application of *T. viride* and *Rhizobium* spp. caused significant reductions in the number and weight of root nodules. This may be due to the ability of *T. viride* to grow quickly in the soil and colonise plant surfaces, thus preventing the subsequent invasion of roots by the bacteria (Anusuya and Sullia, 1985). Detrimental effects are not always noted as dry bean (*Phaseolus vulgaris* L.) seedlings grown from seeds simultaneously inoculated with *Rhizobium* spp. and *Trichoderma harzianum* (Bon.) Bain showed no difference in the number of root nodules compared with roots treated only with *Rhizobium* spp. (Harman et al., 1981). Also, pea (*Pisum sativum* L.) seedlings treated...
with *T. harzianum* strains Th1 and N47 exhibited an increase in the number of nodules per root system (Naseby et al., 2000).

BCAs can elicit a reaction in plants similar to a defence response to invading pathogens. When exposed to a xylanase enzyme produced by *T. viride*, tobacco (*Nicotiana tabaccum*) had a hypersensitive response as indicated through discoloration, nucleus condensation and plasmolysis in cells surrounding the infection site (Yano et al., 1998). A hypersensitive response occurs when a plant detects the presence of an invading pathogen and induces rapid cell death near the infection site, to prevent the spread of the pathogen (Mechdy, 1994).

Strain T-203 of *T. harzianum* was reported to penetrate plant roots and induce host defences, including chemical and structural changes. In cells adjacent to invaded cells, the plant responded by strengthening cell walls and depositing appositions that prevented further invasion. Penetration by *T. harzianum* also elevated the production of peroxidases and chitinases that had an inhibitory effect on invading fungi, both in the roots and leaves. However, the elevated enzyme production began to decrease 120 h after inoculation, indicating through discolouration, nucleus condensation and plasmolysis in cells surrounding the infection site, to prevent the spread of the pathogen (Mechdy, 1994).

Strain T-39 (Trichodex Bioworks Inc.) for 30 days showed no difference in lifespan, brood size or hive weight compared to unexposed bees (Brownold et al., 1998). Spores of *Trichoderma* spp. have been identified in the air in sawmills (Simeray et al., 1997) and were associated with allergic alveolitis in sawmill employees (Halpin et al., 1994). Those working in BCA production or application of formulations containing spores may be at risk for health effects. *Trichoderma* spp. have been associated with the formation of toxins which cause intestinal haemorrhaging, diarrhoea, cellular destruction of intestinal mucus membranes and oedema in exposed mammals (Ueno, 1984; Coulombe, 1993). Also, a specific strain of *T. koningii* has been associated with shellfish contamination causing immediate immobilisation of blue mussels (*Mytilis edulis* L.). Examination of exposed mussels revealed that the main area of contamination was the digestive gland with some diffusion into other parts of the animal (Sallenave et al., 1999). Exposure to the toxin may indirectly influence animals, including humans that use the mussels as a food source.

### 3.2. Gliocladium spp.

The genus *Gliocladium* consists of several species of fungi that are antagonistic to plant pathogens. *G. virens* has been shown to control soil-borne plant disorders, such as cotton seedling disease caused by *P. ultimum* (Paulitz and Linderman, 1991) through the production of several antibiotics (Howell, 1991; Avent et al., 1993; Di Pietro et al., 1993). *G. virens* also produces an endochitinase that inhibited spore germination and damaged cell walls of exposed *Botrytis cinerea* Pers.: Fr. This endochitinase had a synergetic effect with gliotoxin, a toxin produced by many species in the genus *Gliocladium*, which may be necessary for effective biological control. The weakening of the cell wall by this enzyme facilitated entry by the antibiotic gliotoxin, resulting in control of the pathogenic fungus (Di Pietro et al., 1993). Likely all fungi, including mycorrhizal fungi, that have cell walls composed of chitin, would be at risk for attack from *G. virens*. However, *G. virens* did not negatively impact the ability of the mycorrhizal fungus,
Glomeromycota Becker and Gerdemann, to colonise plant roots (Paulitz and Linderman, 1991). Gluovirin, a toxin produced by some Gliocladium spp., also had no impact on several species of bacteria including Bacillus thuringiensis Berliner, Pseudomonas fluorescens Migula and Xanthomonas malvacearum (Smith) Dowson (Howell and Stipanovic, 1983) but more studies are needed to determine if there is a threat to mycorrhizal fungi and soil bacteria. Gliocladium spp. produce metabolites that can directly affect growth and development of the host plant. G. virens produces the compound viridiol when incubated on certain high-nutrient substrates that reduces germination and growth of several plant species including lettuce (Lactuca sativa L.), mustard (Brassica juncea L.), curly dock (Rumex crispus L.), and eastern black nightshade (Solanum ptycanthium Dun.). Some plants, including soybean, safflower (Carthamus tinctorius L.), and sugar beet (Beta vulgaris L.), were not affected, whereas shoot and root dry weights were reduced in 30 of 32 species treated with G. virens (Jones et al., 1988). Viridiol was toxic to germinating pigweed (Amaranthus retroflexus L.) (Howell and Stipanovic, 1984) but its production is dependent on substrate (Jones et al., 1988) and synthesis is unlikely to pose a significant threat to host plants. The compound, gliotoxin, produced by many members of Gliocladium (Howell, 1991; Arent et al., 1993; Di Pietro et al., 1993; Howell and Stipanovic, 1995; Haraguchi et al., 1996), may inhibit acetolactate synthase, which catalyses the production of branched chain amino acids, in tobacco. Growth reduction in gliotoxin-treated plants was overcome when plants were supplied with the amino acids valine, leucine, and isoleucine (Haraguchi et al., 1996). G. virens also induced host defences in cotton (Gossypium hirsutum L.) grown from seeds treated with the fungus. These defences included increased terpenoid production, the BCA (Howell et al., 2000). This toxin can reduce the formation of ATP in rabbit muscle cells due to the inhibition of creatine kinase (Hurne et al., 2000), and interferes with blood clotting as it inhibits the ADP-induced aggregation of platelets in mammals (Sakai and Watanuki, 1987). The inhibition of alcohol dehydrogenase in liver cells is associated with the covalent bonding of gliotoxin to enzyme structure or through production of reactive oxygen species in the redox cycling of gliotoxin (Waring et al., 1995).

The risk of gliotoxin depends on the rate of exposure and it has been shown that the production of gliotoxin by G. virens is transient. The toxin is only produced during a 16 h period corresponding to the stage of fastest mycelial dry weight increase (Wilhite and Straney, 1996) and may not pose a significant threat to humans or animals.

3.3. Pythium oligandrum

The oomycete, P. oligandrum Dreschs., is antagonistic to several species of fungi including B. cinerea, Acrocnemium strictum Gams., Acremonium apiti (Smith and Ramsey) Gams., Paeochyomyces spp., Penicillium albidum Sopp., Phialophora malorum Kidd and Beaumont, Scopulariopsis brevicaulis (Sacc.) Bainier and Humicola fusco-atra Traaen (Vesely and Heydinek, 1984), P. ultimum and R. solani (McQuilken et al., 1990). The ability of P. oligandrum to control damping off diseases is believed to be due to competition for resources between the BCA and the pathogens (Fossey and Deacon, 1986). However, P. oligandrum can also control diseases through direct attack. Ultrastructural evidence of the interactions between P. oligandrum, P. ultimum, Pythium aphanidermatum (Edison) Fitzpatrick, F. oxysporum Schlectend.: Fr. L. sp. radicis-lacopersici Jarvis and Shoemaker, Verticillium albo-atrum Kleb. and R. solani showed that P. oligandrum attacks fungi through mycoparasitism.
and the production of antibiotics (Benhamou et al., 1999).

The antagonistic behaviour of *P. oligandrum* towards both oomycete and true fungi may pose a problem in mushroom cultivation as *P. oligandrum* has been associated with a cropping disorder in the commercial fungus, *A. bisporus*. Eventually, crops of *A. bisporus* recovered indicating that the effect of *P. oligandrum* was transient (Fletcher et al., 1990). Precautions must be taken to determine the risk associated with any agent accidentally transferred to a non-target system.

*P. oligandrum* has been shown to affect plant growth. For example, the rate of growth of cucumber seedlings inoculated with *P. oligandrum* was initially reduced but, after 4 days, no difference was found between untreated and treated plants. In contrast, after 8 days, *P. oligandrum* actually stimulated root growth of the treated seedlings (Wulff et al., 1998).

Picard et al. (2000) reported that *P. oligandrum* produced a substance known as oligandrin, a proteinaceous molecule translocated via the vascular system. When tomato (*Lycopersicum esculentum* Mill.) was treated with oligandrin, a plant defence response was initiated and plants exhibited a faster response to invasion by the parasite, *Phytophthora parasitica* Dast., compared to untreated ones. Tomato plants exposed to oligandrin showed limited hyphal colonisation by the parasite, while untreated plants showed fungal colonisation and proliferation in nearly all tissues. This reduced invasion of treated plants was likely due to the deposition of amorphous substances along the penetration peg of the pathogen. In plants treated with oligandrin, hyphae showed nuclear densification and cytoplasmic distortion and contraction. Exposure to compounds produced by *P. oligandrum* may be beneficial to the plant in prevention of disease. However, if plant defence mechanisms are relatively non-specific, colonisation by beneficial fungi and formation of mycorrhizae may be hindered or inhibited.

### 3.4. *Taloromyces flavus*

*T. flavus* (Klöcher) Stolk and Sasmon has been shown to be effective against sunflower (*Helianthus annuus* L.) wilt (McLaren et al., 1994) caused by *S. sclerotiorum* and for controlling stem rot in beans (Madi et al., 1997), caused by *Sclerotinia rolfsii* Sacc.

The action may be due to direct mycoparasitism of the sclerotia but an extra-cellular chitinase secreted by *T. flavus* may also play a role (Madi et al., 1997). The release of antibiotics and the production of glucose oxidase may be another mechanism by which *T. flavus* controls pathogens. Glucose oxidase catalyses conversion of glucose to gluconate and hydrogen peroxide, which may be partially responsible for biological control of pathogens, such as *Verticillium dahliae* Kleb (Murray et al., 1997). Hydrogen peroxide and antibiotics produced by *T. flavus* inhibited melanisation of sclerotia of *S. rolfsii* and *V. dahliae* (Madi et al., 1997). Prevention of melanin synthesis in the sclerotium may make the resting structure more susceptible to damage by ultraviolet light or parasitism by other organisms (Hawke and Lazarovits, 1994). The possible non-target effects of *T. flavus* should be further examined, especially with regards to soil fungi.

### 3.5. *Coniothyrium minitans*

*C. minitans* Campbell has been used to control *Sclerotinia* spp. in dry beans (Gerlagh et al., 1999; Huang et al., 2000), potato (*Solanum tuberosum* L.), carrot (*Daucus carota* L.) and chicory (*Cichorium intybus* L.) (Gerlagh et al., 1999). *C. minitans* exhibited a mycoparasitic behaviour as its mycelia surrounded the sclerotium and produced compounds that dissolved the walls of the overwintering structure. The BCA then grew within and around cells of sclerotial tissue, resulting in plasmolysis, aggregation of cytoplasm and vacuolisation in cells of *S. sclerotiorum* (Huang and Kokko, 1987). *C. minitans* has been shown to be highly specialised to *Sclerotinia* spp. (Whipps and Gerlagh, 1992; McLaren et al., 1996) and may not pose a significant threat to other economically and ecologically important fungal species.

### 3.6. *Ampelomyces quisqualis*

*A. quisqualis* Ces. is an aggressive parasite of several species of powdery mildew, including *Uncinula necator* (Schwein) Burrill, *Sphaerotheca macularis* (Wallr.: Fr.) (Falk et al., 1995) and *Sphaerotheca fuliginea* (Schlechtend.: Fr.) (Sundheim and Krekling, 1982; Falk et al., 1995). The mycoparasite invades conidia and hyphae of *S. fuliginea*, through
a combination of mechanical and enzymatic forces, and hyphae grow within hyphae of the host resulting in death of the cells (Sundheim and Krekling, 1982). Once again, it may be possible for this BCA to attack non-target fungal species and until its host range is identified, it is difficult to determine the risk to beneficial fungi and other soil organisms.

4. Summary and conclusions

The release of BCAs can be positive in terms of the production of food and fibre since they are considered less toxic to humans and environment than synthetic chemical pesticides. BCAs may also represent an acceptable and effective means of disease management since microbial organisms may control resistant pests and reduce the possibility of development of further resistance.

BCAs, however, may also pose risks to non-target organisms. An organism that appears able to control a plant disease without harming the host plant may still pose a risk to other organisms in the same target environment. It is difficult to test the interaction of a BCA with all organisms in an environment and unknown pathogenic relationships with non-target fungi, bacteria, plants or animals could be discovered after release. Mycorrhizal and non-target saprophytic fungi are particularly at risk since most BCAs are parasitic on at least one fungal species. Toxins produced by a BCA may not only harm fungal species but also plants, marine and terrestrial animals, while workers who manufacture and apply these agents are at particular risk for respiratory diseases and allergies.

Ecosystems are complex environments that exhibit intricate interactions between biotic and abiotic factors. Even if it is difficult to completely understand the functioning of a biological system, it is important for researchers to attempt to identify as many possible non-target effects associated with the release of any BCA. Since these agents are alive, their behaviour, development and spread may be unpredictable and humans must attempt to foresee possible complications and mitigate any undesired effects. BCAs, being perceived as 'natural' and 'low risk', are often exempted from the rigorous testing required for chemical pesticides (Lumsden and Walter, 1995; Anonymous, 2001). Due to the fact that release of an agent may have unforeseen ecological repercussions, it is important for the non-target effects of BCAs to be fully analysed. Research must determine the host range of BCAs before they are released into the environment. In weed control, BCAs are often subjected to a centrifugal phylogenetic scheme (Wapshere, 1974) to determine if they will have detrimental effects on non-target plants. This scheme involves identification of plant species closely related to the target species, which are then tested for susceptibility to the agent. Species closely related to susceptible plants are then tested to ultimately determine host range. A similar method may be used to determine the host range of plants for BCAs for plant diseases. However, since host factors, such as variable susceptibility within a single species influence disease incidence, phylogenetic testing may not fully identify vulnerable plant species and further studies of genetic relationships between hosts and pathogens are needed to produce a reliable method of determining host range (Weidemann and Tebeest, 1990).

The ability of a BCA to spread into non-target environments is also of concern. Tracking microbial agents is now possible in field situations through molecular techniques that allow determination of strain stability, integrity and monitoring (Hermosa et al., 2000, 2001; Avis et al., 2001). Molecular markers and techniques should hence be applied in field studies to unequivocally determine the spread and activity of specific BCA strains (Hintz et al., 2001).

An analysis of risks and impacts associated with release of a BCA may be performed through a biological impact assessment as proposed by Teng and Yang (1993). An assessment involves several steps including risk determination, data and information generation, synthesising knowledge on the system, prediction of impact, risk and benefit evaluation. It will obviously remain impossible to predict all impacts or interactions with non-target organisms. Unexpected effects of parasitism, predation and competition may be difficult to assess due to insufficient monitoring (Simberloff and Stiling, 1996b). It may be therefore necessary to implement the 'precautionary principle' when creating policies associated with BCAs. The precautionary principle involves use of careful measures when approaching an activity that may pose a risk to human health or the environment, even if that risk has not been clearly identified using scientific analysis (Santillo et al., 1998). Although this
principle has been criticised for delaying the imple-
m entation of technologically advanced products, it is
important to collect sufficient scientific information to
show that an agent does not pose a significant dan-
ger to non-target organisms (Saunders, 2000). Thus, a
BCA would be regarded as a possible risk until scien-
tific evidence has proven that it is safe. This will allow
use of microbial agents while maintaining adequate
monitoring and research to allow early detection and
mitigation of detrimental biological and environmen-
tal impacts.

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References

on Kentucky bluegrass grown for seed in northeastern Oregon.
Plant Dis. 80, 853–855.
Standards Board, Ottawa, Ont., Canada.
Pest Control Agents and Products. Pest Management Regulatory
Agency, Ottawa, Ont., Canada.
Arent, A.G., Hanson, J.R., Kovach, J., 1993. Metabolites of
molecular characterization of fungal biocontrol agents: some
Bailey, B.A., Lumsden, R.D., 1998. Direct effects of
Trichoderma and Gliocladium on plant growth and resistance to pathogens.
In: Harman, G.E., Kubbuck, C.P. (Eds.), Trichoderma and
Gliocladium: Enzymes, Biological Control and Commercial
Applications. Taylor & Francis, Bristol, PA, pp. 185–204.
Bano, M., Chemett, J.M., 1999. Leaf-cutting ants (Formicidae,
Attine) prune their fungus to increase and direct its productivity.
Bennamou, N., Rey, P., Picard, K., Trifilie, Y., 1999. Ultra-
structure and cytochemical aspects of the interaction between the
mycoparasite Pythium oligandrum and soilborne plant pathogens.
Phytopathology 89, 506–517.
enhancement of alfalfa-2 (2a) sweet corn by Trichoderma
Bolan, N.S., Rolston, A.D., Barrow, N.J., 1987. Effects of
vesicular-arbuscular mycorrhiza on the availability of iron
Trichoderma harzianum on honey bee survival. 1997 NYS Fruit
Project Reports. Cornell Cooperative Extension, Ithaca, NY.
spp. with Glomus mosseae and two with pathogenic fungi.
Agric. Ecosyst. Environ. 29, 39–45.
the vesicular-arbuscular mycorrhizal fungus Glomus mosseae
and some saprophytic fungi isolated from organic substrates.
characteristics, and long-term storage of fungi cultivated by
Cheeke, P.R., 1995. Endogenous toxins and mycotoxins as forage
grasses and their effects on livestock. J. Anim. Sci. 73, 909–
918.
Cook, R.J., 1988. Biological control and holistic plant-health care
Cook, R.J., Brackett, W.L., Coulson, J.R., Goettel, M.S., Humber,
R.A., Lumsden, R.D., Maddox, J.V., McManus, M.L., Moore,
Safety of microorganisms intended for pest and plant disease
control: a framework for scientific evaluation. Biol. Control 7,
333–351.
Dairy Sci. 76, 880–891.
Plant Soil 71, 455–462.
Di Pietro, A., Lorito, M., Hayes, C.K., Broadbent, R.M.,
Harman, G.E., 1993. Endochitinase from Gliocladium virens:
isoenzyme, characterization, and synergistic antifungal activity in
combination with gliotoxin. Phytopathology 83, 308–313.
antibiotic from Trichoderma koningii active against soilborne
Eichner, R.D., Warin, P., Geuze, A.M., Breathwaite, A.W.,
Millbrather, A., 1988. Gliotoxin causes oxidative damage to
plasmid and cellular DNA. J. Biol. Chem. 263, 3772–3777.
Ekern, F., Wustenfeld, K., Sée, D., 2000. The toxicity of the
fungicide procymidone to soil flagellates. Biol. Fertil. Soil 31,
76–77.
Partial control of grape powdery mildew by the mycoparasite
oligandrum associated with a cropping disorder of Agaricus
Foley, M.F., Deacon, J.W., 1986. Susceptibility of
Pythium oligandrum to antibiotic from
Trichoderma harzianum. Plant Dis. 70, 70–77.
Fracchia, S., Mujica, M.T., García-Romera, I., García-Garrido,

T.A. Brimner, G.J. Boland / Agriculture, Ecosystems and Environment 100 (2003) 3–16
between Glomeris mosseae and arbuscular mycorrhizal open
Gerlagh, M., Goossen-van de Grinj, H.M., Fokkema, N.J.,
Vrieseman, P.F.G., 1997. Inoculation by application of Coniothyrium minitans on Sclerotinia sclerotiorum-infected
wheat. Phytopathology 89, 141–147.
Ghielmi, E.L., Sonnemann, K., 1999. Antifungal antibi-
otics produced by Trichoderma spp. Soil Biol. Biochem. 23,
1011–1020.
Gossen, B.D., Rimmer, S.R., 2000. Myths and dogmas of biocontrol: changes in
perceptions derived from research on Trichoderma harzianum for
the detection of Trichoderma atrovirens 11, a biological control
agent against scabiform fungal plant pathogens. Crit. Genet. 38,
343–350.
Harman, G.E., Björkman, T., 1998. Potential and existing uses of
Application of Fungi for Plant Disease Control and Plant
Trichoderma harzianum: Enzymes, Biological Control and
Commercial Applications. Taylor & Francis, Bristol, PA,
pp. 131–151.
Harmer, G.E., Bjerkan, T., 1998. Potential and existing uses of
Trichoderma harzianum and Gliocladium for plant disease control and plant
growth enhancement. In: Harman, G.E., Kubicek, C.P. (Eds.),
Trichoderma and Gliocladium: Enzymes, Biological Control and
Commercial Applications. Taylor & Francis, Bristol, PA,
pp. 131–151.
Trichoderma hamatum applied to seeds as a biocontrol agent.
Phytopathology 71, 569–572.
Harmer, G.E., Hayes, C.K., Lentz, M., Bradshaw, R.M., Di Pietro,
A., Petersen, C., Thomas, A., 1993. Chitinolytic enzymes of
Trichoderma harzianum: purification of chitosanase and
Hawke, M.A., Lazarovits, G., 1994. Production and manipulation of
individual microsclerotia of Verticillium dahliae for use in
studies of survival. Phytopathology 84, 883–880.
Hermosa, M.R., Gromona, I., Iturriaga, E.A., Diaz-Minguez,
classification and identification of biocontrol isolates of
Hermosa, M.R., Gromona, I., Diaz-Minguez, J.M., Iturriaga, E.A.,
for the detection of Trichoderma atrovirens 11, a biological control
agent against scabiform fungal plant pathogens. Crit. Genet. 38,
343–350.
of Arbuscular Mycorrhiza on Sustainable Agriculture and Natural Ecosystems. Birkhauser Verlag, Basel, Switzerland.


with methyl bromide or soil solarization. Crop Prot. 12, 380–386.  


