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Biological Control—Microbial Pesticides

Biological control using microbial pesticides has become important in recent years. Introduced microbial biocontrol agents (as opposed to a natural population of microbes) are called "microbial pesticides" according to the Environmental Protection Agency (EPA) (Harman, 2000). Several microbial pesticides are now commercially available. Fungal, bacterial, and viral biocontrol agents are available. They effectively control fungal, bacterial, and viral diseases. Modes of action of these biocontrol agents are described. Conditions favorable for effective action of them in controlling diseases are also discussed.

TRICHODERMA

Trichoderma species are the most important biocontrol agents. Many have been developed as commercial products. In 1999, retail sales of a product (Topshield and Rootshield) based on a single strain of *T. harzianum,* strain T-22, totaled around 3 million dollars in the United States (Harman, 2000). Commercial production of *Trichoderma* has been reported from France, New Zealand, Sweden, Poland, Denmark, Russia, Israel, Bulgaria, China, and India. There are many constraints in developing *Trichoderma* as biocontrol agents. *Trichoderma* colonizes in the spermosphere effectively, but they normally do not survive well in the rhizosphere. *Trichoderma* spp. achieve only transitory localized dominance of the rhizosphere, and these are active in only some soils and seasons (Deacon, 1994). Hence, *Trichoderma* species are likely to be effective for seed and seedling diseases, but not against diseases of a mature crop. However, crop losses will be greater when mature crops are affected, and seed and seedling diseases can be effectively controlled by seed treatment with chemicals at a very low cost. Under such conditions, the use of *Trichoderma* spp. will be limited. Another important constraint is that *Trichoderma* spores are quiescent and inactive in soil. Hence, *Trichoderma* strains cannot be added as spores. It may be easier to mass multiply fungi in the form of spores.

Several technologies were recently developed to make use of *Trichoderma* in the control of soil-borne diseases of crops at different maturity stages. *Trichoderma harzianum* strain T-22 with rhizosphere competence was developed by protoplast fusion technology (Harman, 2000). *Rhizosphere competence* is defined as the ability of a microorganism to grow and function in the developing rhizosphere. Strains that were fused were T-95 of *T. harzianum,* a rhizosphere competent mutant produced from strain T-12. T-12 was more capable of competing with spermosphere bacteria than T-95 under iron-limiting conditions; both were strong biocontrol agents. Some strains of *Trichoderma,* such as the strain T39, can induce systemic resistance, and such strains can induce resistance against diseases at any stage of the crop (De Meyer et al., 1998). Technology to apply *Trichoderma* in the form of actively growing germlings instead of spores was developed to obtain an active population of *Trichoderma* in soil to control soil-borne pathogens.

Formulations of Trichoderma

Liquid media based on molasses and molasses yeast have been used widely for the production of *Trichoderma*. The addition of complex organic materials, such as V8 juice, yeast extract, or protease peptone, increased conidial production in *T. harzianum*. The addition of osmotica such as polyethylene glycol improved conidial production of *T. harzianum* and resistance of conidia to desiccation (Whipps, 1996). *Trichoderma harzianum* has been produced in diatomaceous earth granules impregnated with 10 percent molasses. Spores, cells, or biomass are concentrated directly from liquid media by centrifugation and filtration. Biomass may be dried, milled, and incorporated into a range of dusts, alginate granules, pellets or prills, wettable powders, emulsifiable liquids or gels. Talc formulations, kaolin-based microgranules, and alginate pellet, prill, or granule formulations are available.

Conidia of *Trichoderma* are added to a bran-sand mixture, and after one to three days of incubation, this germling preparation is added to soil where colony-forming units of the antagonists continue to increase. This method provides a means of achieving an active population of antagonists in the soil. A medium supplemented with ground corn cobs was developed for applying *T. koningi* in the field (Latunde-Dada, 1993). Alternatively, a fermenter biomass of *Gliocladium* and *Trichoderma* spp. was added to a vermiculite-bran mixture moistened with 0.05 M HCl. After drying, the preparation can be remoistened with 0.05 M HCl and germlings produced as before (Lewis et al., 1991).

Seed-coating formulations have also been developed. A liquid coating formulation comprises a suspension of aqueous binder (pelgel or polyox—N-10), finely ground solid particulate matter (Agro-Lig or muck soil), and the biocontrol agent (Taylor et al., 1991). This is sprayed onto seeds in a tumbling drum. Agro-Lig has chemical and physical characteristics favorable for the growth of *Trichoderma*. This type of formulation was very effective in the control of damping-off of cucumber caused by *Pythium* (Taylor et al., 1991). Adding compounds to the seed coating that specifically enhance growth of *Trichoderma* is highly beneficial. Inclusion of specific polysaccharides and polyhydroxy alcohols improves biocontrol activity of *Trichoderma* (Nelson et al., 1988).

Method of Application of Trichoderma

Mathre et al. (1999) stated that nearly all commercialized microorganisms rely upon application of the antagonist "directly and precisely to the infection court" when and where needed. Seed treatment is the most effective method (Mathre et al., 1999). Seed-coating formulations will be useful. Seed priming is also recommended. Seed priming is the process in which hydration of the seed is controlled to a level that permits pregerminative metabolic activity to take place without emergence of the radicle. Two priming systems are available. Osmopriming utilizes aerated aqueous solutions of salts or polyethylene glycol, generating osmotic potential in the primary solution. Solid matrix priming (SMP) involves the use of moist, porous solid materials, such as powdered coal or peat, generating matric potential. Combining SMP with *Trichoderma* spp. for control of seedling diseases has been used successfully on a wide range of plants (Harman et al., 1989).

Actively growing germling populations can be applied to soil. *Trichoderma* can be applied as granules or as a drench. A single application of *T. harzianum* T-22 as RootShield granules in a greenhouse provided protection of a tomato crop against *Fusarium* crown and root rot of the mature crop. An in-furrow drench was more effective in both root colonization and disease control than a seed treatment (Harman, 2000).

In crops that are transplanted, the granules can be applied in the nursery. Tomatoes were grown in a potting mix containing the granular formulation of *T. harzianum* T-22, which permitted roots to become colonized, and then transplanted to the field. This treatment reduced *Fusarium* crown and root rot at the harvest of mature fruit (Datnoff et al., 1995). *Trichoderma* can also be applied as a spray. *Trichoderma harzianum* T-22 is effective in the control of fruit and foliar diseases when applied as a spray to these plant parts. T-22 should be applied at least once every 10 days when disease pressure is

high, because it cannot extensively grow on and colonize newly formed leaf tissues. The fungus colonizes grape or strawberry flowers and immature fruits (Harman, 2000). Diseases controlled by foliar spray include powdery mildews of *Catharanthus* and pumpkins, *Botrytis cinerea* on strawberry, downy mildew of snapdragons, and turf-grass pathogens such as *Rhizoctonia solani* and *Pythium* spp. (Harman, 2000).

Bumble bees *(Bombus impatiens)* and honey bees *(Apis mellifera)* have been used to deliver *Trichoderma* to the flowers of crop plants. Bees exiting the hive pass through a device that requires them to come into contact with *Trichoderma* products containing these spores. They subsequently deliver substantial amounts of *Trichoderma harzianum* T-22 or similar fungi to the strawberry or other flowers. This method of delivery was more effective than spray applications for control of *B. cinerea* and has proven effective over several years and trials as standard chemical applications (Kovach et al., 2000; Harman, 2000).

Time of application of *Trichoderma* is also important. *Trichoderma* can be overwhelmed by heavy disease pressure. Therefore, *T. harzianum* may be used strictly as a preventative measure; it cannot cure infections. *Trichoderma* is less effective against systemic diseases than against more superficial ones. It cannot control existing diseases, and so a good systemic fungicide must be used if diseases already exist. In conditions of high or very high disease pressure, T-22 should be used as part of an integrated chemical-biological system. A combination of chemical treatment with *Trichoderma* will be highly effective in the control of diseases. A tank mix with chemical fungicides or an alternating spray with chemical fungicides is the ideal method of application of *Trichoderma* (Harman, 2000). A combination of ozone fumigation and *T. harzianum* treatment was on par with the standard methyl bromide treatment, and the combination was significantly better than either *T. harzianum* alone or ozone fumigation alone in control of strawberry root diseases (Harman, 2000).

A single strain of *Trichoderma* may not be sufficient to be effective under all conditions and against all diseases. Mathre et al. (1999) suggested that almost invariably, a different agent might be needed for each disease. Cook (1993) stated that biological control is widely recognized as being highly disease-specific. He advocated an approach to biological control that uses mixtures of numerous agents for each disease. A mixture of *Trichoderma* spp. has been developed as commercial formulations. *T. harzianum* + *T. polysporum* (Binab—T) and *T. harzianum* + *T. viride* (Trichodowels) are the important complex products (Whipps, 1996). *Trichoderma* has been combined also with other biocontrol agents. The combination of *T. harzianum* T-22 and the mycorrhizal fungus *Glomus intradices* was more effective than either organism alone (Datnoff et al., 1995). There are also reports

that a single strain of *Trichoderma* may be capable of controlling diverse pathogens under diverse conditions (Chet, 1987; Harman, 2000).

Diseases Controlled by Trichoderma

Trichoderma has been reported to control *Rhizoctonia, Fusarium, Phytophthora,* and *Pythium* diseases in many crops, tomato root and crown rot, pumpkin and *Catharanthus* powdery mildews, gray mold *(Botrytis cinerea)* of strawberry, root rots of several crops caused by *Macrophomina phaseolina,* wheat take-all caused by *Gaeumannomyces graminis* var. *tritici, Sclerotinia* and *Verticillium* diseases of fruit trees, *Armillaria mellea* infection in trees, Dutch elm disease *(Ceratocystis ulmi),* Chestnut blight *(Endothia parasitica),* silver leaf disease of trees *(Chondrostereum purpureum),* and stem and root rot of pine *(Heterobasidion annosum)* (Harman et al., 1989; Nelson et al., 1988; Maplestone et al., 1991; Whipps, 1992, 1996; Datnoff et al., 1995; Nemec et al., 1996; De Meyer et al., 1998; Elad et al., 1999; Howell et al., 1999; Burns and Benson, 2000; Harman, 2000).

Mycoparasitism of Trichoderma

Trichoderma spp. may control diseases caused by various fungal pathogens by their various types of actions. Their modes of action include mycoparasitism, antibiosis, induced resistance, competition for nutrients or space, and inactivation of the pathogen's enzymes. Mycoparasitism involves tropic growth of the biocontrol agent toward the target fungi, lectin-mediated coiling of attachment of *Trichoderma* hyphae to the pathogen, and finally attack and dissolution of the target fungal cell wall by activity of enzymes, which may be associated with physical penetration of the cell wall. More than 20 separate genes may be involved in mycoparasitism. *Trichoderma* produces ten different chitinases and several β-1,3-glucanases and proteases. The importance of chitinases produced by the antagonist has been demonstrated in several ways. A 42-kDa endochitinase is induced before *T. harzianum* comes into contact with *Botrytis cinerea* (Zeilinger et al., 1999). A strain of *T. harzianum* deficient in the ability to produce endochitinase had reduced ability to control *B. cinerea* (Woo et al., 1999). Endochitinase was disrupted or overproduced in *T. virens,* and the resulting strains were found to have decreased or increased biocontrol activity, respectively (Back et al., 1999). Expression of endochitinase from *T. harzianum* in transgenic apple increases resistance to apple scab (Bolar et al., 2000). The endochitinase gene from *Trichoderma* confers resistance in many other transgenic plants

(Lorito et al., 1998). Contradicting reports state that chitinase may not be involved in an antagonistic action of *Trichoderma*. The activity of endochitinase was disrupted or overproduced in *T. harzianum*, but these changes had no effect on its biocontrol ability against *Rhizoctonia solani* or *Sclerotium rolfsii* (Carsolio et al., 1999). A strain of *T. harzianum* deficient in the ability to produce endochitinase had increased ability to control *R. solani* (Woo et al., 1999). These results suggest that other gene products may also be involved in the action of *Trichoderma*.

Antibiotic Production by Trichoderma

Forty-three antibiotic substances were reported to be produced by *Trichoderma* spp. (Sivasithamparam and Ghisalberti, 1998). Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes, and steroids are important and found to be associated with biocontrol activity of *Trichoderma* spp. Mutation to eliminate production of specific antibiotics is associated, in some strains, with a loss of activity against particular pathogens (Howell, 1998).

Induced Resistance by Trichoderma

Some *Trichoderma* spp. induce systemic resistance against pathogens. *Trichoderma harzianum* induces systemic resistance against powdery mildews (Elad et al., 1999), *B. cinerea* infections (De Meyer et al., 1998), and root rot of cotton (Howell et al., 1999). *Trichoderma* products (BINABTF-WP and BINABT vector) induced systemic acquired resistance in strawberry against *Botrytis cinerea* (Ricard and Jorgensen, 2000). Different elicitors, including xylanase, have been isolated from *Trichoderma* and they trigger the synthesis of various defense compounds, including phytoalexins (Calderon et al., 1993). *Trichoderma* induces the synthesis of phytoalexins involved in disease resistance in cotton (Howell et al., 1999), modifies and strengthens plant cell walls in cucumber (Yedidia et al., 1999), and increases activities of chitinase and peroxidase in cucumber tissues (Yedidia et al., 1999). Cucumber roots treated with *T. harzianum* exhibited higher activities of chitinase, β-1,3-glucanase, and peroxidase (Yedidia et al., 2000). For more information on the role of peroxidases, β-1,3-glucanases and chitinases in conferring disease resistance, see Chapter 34.

Competition for Space or Nutrients by Trichoderma

Trichoderma may compete for space or nutrients with pathogens and suppress their development. *Botrytis cinerea* conidia require external nutrients for germination and infection. When conidia of *T. harzianum* T39 were applied to leaves, germination of conidia of the pathogen was slowed, an effect attributed in part to competition (Elad et al., 1999).

Inactivation of the Pathogen's Enzymes by Trichoderma

B. cinerea depends upon production of pectolytic, cutinolytic, and cellulolytic enzymes to infect living plants. However, conidia of two strains of *T. harzianum* (T39 and NCIM 1185), when applied to the leaves, produce a serine protease that is capable of degrading the pathogen's plant cell wall degrading enzymes and thereby reducing the ability of the pathogen to infect the plant (Elad and Kapat, 1999). The biocontrol activity of T39 could be enhanced by adding additional quantities of its protease. Several protease inhibitors reduced the biocontrol activity of T39 (Elad and Kapat, 1999).

PSEUDOMONAS SPECIES

Pseudomonas spp. are the important group of biocontrol agents that have been developed as commercial products. Fluorescent pseudomonads form the major bacterial group surviving in the rhizosphere of crop plants. They are also known as plant growth promoting rhizobacteria (PGPR) because they promote plant growth by secreting auxins, gibberellins, and cytokinins (Vidhyasekaran, 1998). These pseudomonads survive in soil, rhizoplane, phylloplane, pistils, nectarines, and fruits of the plants. *Pseudomonas fluorescens, P. putida,* and *P. cepacia (Burkholderia cepacia)* are important *Pseudomonas* species and are known as highly effective biocontrol agents. They have been reported to control soil-borne, seed-borne, and air-borne fungal, bacterial, and viral pathogens.

Diseases Controlled by Pseudomonas

The following is a list of diseases that have been reported to be controlled by saprophytic pseudomonads (Levy et al., 1998; Alstrom, 1991; Wei et al., 1991; Liu et al., 1992, 1995a,b; Wilson and Lindow, 1993; Maurhofer, Scherer, et al., 1994; Tosi and Zazzerini, 1994; Hoffland et al., 1995; Leeman et al., 1995b; Raaijmakers et al., 1995; Vidhyasekaran and Muthamilan, 1995, 1999; Benhamou et al., 1996; M'Piga et al., 1997; Vidhyase-

karan, 1998, 2001; Vidhyasekaran, Rabindran, et al., 1997; Vidhyasekaran, Sethuraman, et al., 1997; Vidhyasekaran et al., 2000):

Apple—gray mold *(Botrytis cinerea)*
Bean—halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*), root rot *(Sclerotium rolfsii)*
Chickpea—wilt (*Fusarium oxysporum* f. sp. *ciceris*)
Cotton—bacterial blight (*Xanthomonas axonopodis* pv. *malvacearum*), Fusarium wilt (*Fusarium oxysporum* Schlechtend.: Fr. f. sp. *vasinfectum*), seedling blight *(Rhizoctonia solani),* and seedling rot *(Pythium ultimum)*
Cucumber—wilt (*Fusarium oxysporum* f. sp. *cucumerinum*), damping-off *(Pythium aphanidermatum),* anthracnose *(Colletotrichum orbiculare),* angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), and mosaic *(Cucumber mosaic virus)*
Pea—wilt (*Fusarium oxysporum* f. sp. *pisi*), root rot *(Aphanomyces euteiches),* and damping-off *(Pythium ultimum)*
Peanut—late leaf spot *(Phaeoisariopsis personata),* stem rot (southern blight) *(Sclerotium rolfsii),* and rust *(Puccinia arachidis)*
Pear—fire blight *(Erwinia amylovora)*
Pigeonpea—wilt (*Fusarium oxysporum* f. sp. *udum*)
Potato—bacterial wilt and brown rot *(Ralstonia solanacearum),* black leg and soft rot *(Erwinia carotovora)*
Radish—wilt (*Fusarium oxysporum* f. sp. *raphani*)
Rice—blast *(Pyricularia oryzae),* sheath blight *(Rhizoctonia solani),* bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), sheath rot *(Sarocladium oryzae),* and tungro *(Rice tungro virus)* diseases
Safflower—rust *(Puccinia carthami)*
Sugar beet—leaf spot *(Phoma betae),* damping-off *(Pythium ultimum),* and root rot *(Aphanomyces euteiches)*
Tobacco—black root rot *(Thielaviopsis basicola),* necrosis *(Tobacco necrosis virus)*
Tomato—wilt (*Fusarium oxysporum* f. sp. *lycopersici*)
Wheat—take-all *(Gaeumannomyces graminis* var. *tritici),* leaf (brown) rust *(Puccinia triticina=Puccinia recondita),* and Septoria speckled leaf blotch *(Septoria tritici)*

Induction of Systemic Resistance by Pseudomonas Spp.

Several rhizobacterial strains were shown to elevate plant resistance against several pathogens. Strains of *Pseudomonas fluorescens, P. putida,*

and *P. aeruginosa* induced systemic resistance (ISR) in rice (Ohno et al., 1992; Vidhyasekaran, Rabindran, et al., 1997; Vidhyasekaran et al., 2000), cucumber (Liu et al., 1995a,b; Meyer et al., 1992; Raupach, 1996), radish (Leeman et al., 1995b), tobacco (Maurhofer et al., 1994a), tomato (Van Wees et al., 1997; M'Piga et al., 1997), bean (Meier et al., 1993), pea (Benhamou et al., 1996), carnation (Van Peer and Schippers, 1992), and *Arabidopsis* (Van Wees et al., 1997).

Rhizobacterial strains have been shown to induce different defense genes in plants. Maurhofer, Hase, et al. (1994) showed that ISR by *P. fluorescens* strain CHA0 in tobacco was associated with accumulation of various pathogenesis-related (PR) proteins (PR-1, PR-2, and PR-3 proteins). Inoculation of bean leaves with cells of *P. fluorescens* induced the accumulation of transcripts for chalcone synthase (CHS), chitinase, and lipoxygenase (Meier et al., 1993). Increase in peroxidase activity as well as an increase in the level of mRNAs encoding for phenylalanine ammonia-lyase (PAL) and CHS could be recorded in the early stages of the interaction between roots and various bacterial endophytes (Zdor and Anderson, 1992). Treatment with *P. fluorescens* causes increases in activities of peroxidase, lysozyme, and PAL in tobacco (Schneider and Ullrich, 1994). Van Peer et al. (1991) showed massive accumulation of phytoalexins in carnation roots colonized by the rhizobacterial strains after pathogen challenge. M'Piga et al. (1997) reported that *P. fluorescens* strain 63-28 induced accumulation of an electron-dense material in epidermal and outer cortical cells and coating of most intercellular spaces with similar substances in tomato. This aggregated material appeared to be mainly composed of phenolic compounds, especially phenols containing *O*-hydroxy groups. The deposition of β-1,3-glucans (callose) was also observed in host cell walls. Chitinases were also induced (M'Piga et al., 1997). Benhamou et al. (1996) demonstrated that pea root bacterization with *P. fluorescens* triggered a set of defense reactions that resulted in the elaboration of permeability barriers. Increases in plant lignification, phytoalexins, various lytic enzymes, and other PR proteins have been observed upon treatment of plants with different specific strains of rhizobacteria (Albert and Anderson, 1987; Frommel et al., 1991; Kloepper et al., 1993; Sayler et al., 1994; M'Piga et al., 1997; Vidhyasekaran et al., 2000).

Studies on the mode of induction of defense genes by the rhizobacterial strains reveal that different bacterial cell wall components may act as elicitors of induction of the signal transduction system. Lipopolysaccharides (LPS) of *P. fluorescens* strain WCS417r act as elicitors and induce resistance against different diseases (Duijff et al., 1997). LPS and LPS-containing cell wall preparations of *P. fluorescens* WCS417r are as effective as living WCS417r bacteria in inducing ISR in radish (Leeman et al., 1995a).

The O-antigenic side chain of the outer membrane LPS of the strain WCS417r appears to be the main determinant for induction of ISR in radish and carnation (Van Peer and Schippers, 1992; Leeman et al., 1995a; Duijff et al., 1997).

Leeman et al. (1996) demonstrated that the siderophore of WCS374r can act as an elicitor of ISR in radish. *Pseudomonas fluorescens* strain CHA0 induced systemic resistance in tobacco. A siderophore (pyoverdine)-deficient derivative of this strain no longer induced ISR (Maurhofer, Hase, et al., 1994). Leeman et al. (1996) reported that the purified siderophore, pseudobactin, from *P. fluorescens* strain WCS374 induced ISR in radish. However, a pseudobactin-deficient, *P. fluorescens* 374PSB, retained ISR-inducing activity. These results suggest that siderophore production by this strain was only partially responsible for the induction of systemic resistance in radish.

Pyochelin, a siderophore, is produced by several rhizobacteria. Salicylic acid is a precursor of pyochelin synthesis (Leeman et al., 1996). Several genera of bacteria, including fluorescent pseudomonads, are known to synthesize salicylic acid (Dowling and O'Garra, 1994). *Pseudomonas fluorescens* strain CHA0 produces salicylic acid (Meyer et al., 1992). Leeman et al. (1996) reported that *P. fluorescens* strain WCS374 produced salicylic acid in quantities that were iron dose-dependent. *Pseudomonas fluorescens* strain WCS417r has the capacity to produce salicylic acid (Leeman et al., 1996). Salicylic acid was responsible for the induction of resistance in radish (Leeman et al., 1996). Meyer et al. (1992) reported that salicylic acid itself might function as an endogenous siderophore. Leeman et al. (1996) reported that rhizobacteria-mediated ISR is affected by iron concentration. Salicylic acid production is iron (Fe^{3+}) regulated (Leeman et al., 1996). Salicylic acid production is promoted by low iron concentrations. Increasing ferric iron concentrations in vitro reduced salicylic acid production below detectable limits by bacteria (Meyer et al., 1992). When ferric iron was applied as a soil drench, ferric iron concentration increased in planta; but it significantly reduced the level of ISR observed in cucumber. This suggests that salicylic acid may not be involved in ISR; but some other siderophores mediated by iron may be involved in induction of ISR in cucumber.

Massive accumulation of phytoalexins could be detected in roots of carnation plants treated with *P. fluorescens* only after challenge inoculation with pathogen (Van Peer et al., 1991). The induction of phenolics and callose in tomato by *P. fluorescens* strain 63-28 was substantially amplified upon infection with the pathogen (M'Piga et al., 1997). These results suggest that the rhizobacterial strains may be capable of evoking transcriptional activation of plant defense genes, the expression of which may be subsequently latent until the plant perceives signals originating from contact with the pathogen. It is also possible that besides the rhizobacterial signal molecules, pathogen's signal molecules may also be involved in the induction of ISR. Induction of H_2O_2 production due to *P. fluorescens* treatment in plants has also been reported (Jakobek and Lindgren, 1993), and H_2O_2 is known as a second messenger that triggers the synthesis of defense chemicals.

Production of Antibiotics by Pseudomonas

Pseudomonas spp. produce antibiotics that have been assigned a role in disease control. *Pseudomonas fluorescens* CHA0 produces 2,4-diacetylphloroglucinol, pyoluteorin, and pyrrolnitrin (Voisard, 1994; Maurhofer, Sacherer, et al., 1994). *Pseudomonas fluorescens* 2-79 produces phenazine-carboxylic acid, pyoverdine, and anthranilic acid, with biocontrol activity mainly due to phenazine production (Hamdan et al., 1991). *Pseudomonas fluorescens* Pf-5 produces 2,4-diacetylphloroglucinol, pyrrolnitrin, and pyoluteorin, with pyoluteorin being the most effective against *Pythium ultimum* (Loper et al., 1994). *Pseudomonas fluorescens* DR54 produces the antibiotic viscosinamide (Thrane et al., 1999).

The importance of antibiotics in the biocontrol activity of *Pseudomonas* spp. was demonstrated by several studies. Biocontrol activity could be increased by increasing the production of antibiotics. Phenazine production could be increased by introducing extra copies of biosynthetic or activator genes. This increased the biocontrol activity of the *Pseudomonas* strain (Thomashow and Pierson, 1991). Additional evidence for a role of antibiotics in biocontrol by *Pseudomonas* spp. was obtained by heterologous expression of complementary genes in strains that naturally do not produce the antibiotic, thus increasing their biocontrol activity (Hara et al., 1994) or by use of Tn5 mutagenesis to inactivate specific genes and affect pathways of antibiotic production (Schnider, Keel, Voisard, et al., 1995). In situ detection of antibiotics in the rhizosphere of plants treated with antibiotic-producing strains was demonstrated for phenazine-carboxylic acid (Thomashow et al., 1990), 2,4-diacetylphloroglucinol (Maurhofer et al., 1995), pyrrolnitrin (Kempf et al., 1994), and pyoluteorin (Maurhofer et al., 1995). In situ expression of the genes required for the synthesis of pyoluteorin, phenazines, and Oomycin A could be demonstrated by fusing reporter genes such as those encoding β-galactosidae or ice-nucleating activity to promoters of genes encoding for antibiotics (Loper and Lindow, 1994; Kraus and Loper, 1995).

A two-component regulatory system for antibiotic production in *Pseudomonas* spp. was reported. The system is based on two protein components, an environmental sensor and a cytoplasmic regulator or global activator (GacA) that mediates changes in response to sensor signals. A response reg-

ulator gene, *gacA,* was identified in *P. fluorescens* CHA0. Strains with a mutation of this gene lost the ability to produce pyoluteorin and 2,4-diacetylphloroglucinol (Laville et al., 1992). A *lemA*-like *apd* gene was found in *P. fluorescens* Pf-5. Strains with a mutation in this region failed to produce pyoluteorin and pyrrolnitrin, and lost the ability to inhibit *Rhizoctonia solani* in culture (Corbell and Loper, 1995). Antibiotic production in *Pseudomonas* spp. may be further controlled by the action of housekeeping sigma factors encoded by the *rpoS* or *rpoD* genes (Schnider, Keel, Blumer, et al., 1995).

Role of HCN, Siderophore, and β-1,3-Glucanase Produced by Pseudomonas

Pseudomonas spp. produce hydrocyanic acid (HCN), which was shown to be important in biocontrol activity (Laville et al., 1992; Voisard et al., 1994; Loper et al., 1994; Corbell and Loper, 1995). The importance of a *Pseudomonas*-produced pyoverdine siderophore in biocontrol activity was demonstrated (Hamdan et al., 1991; Maurhofer, Hase, et al., 1994; Voisard et al., 1994; Loper et al., 1994; Kraus and Loper, 1995). β-1,3-Glucanase may be involved in biocontrol activity of *Pseudomonas (Burkholderia) cepacia* (Fridlender et al., 1993).

Factors Involved in Biocontrol Efficacy of Pseudomonas

Several factors determine the efficacy of *Pseudomonas* in controlling crop diseases in the field. Effective strain selection is important (Vidhyasekaran, 1998). The antagonist inoculum dose determines the efficacy of the antagonist in controlling diseases. A threshold population density of the fluorescent pseudomonad strains of approximately 10^5 cfu per g of root is required for significant suppression of fusarium wilt of radish. When rhizosphere population densities of the strains dropped below this threshold level, the efficacy of these strains to suppress the fusarium wilt was almost lost (Raaijmakers et al., 1995). Similar results were obtained in the control of rice sheath blight by *P. fluorescens* strain Pf1 (Vidhyasekaran and Muthamilan, 1999) and *P. fluorescens* PFALR2 (Rabindran and Vidhyasekaran, 1996). The efficacy of fluorescent pseudomonads is affected drastically by increasing disease pressure. Significant wilt disease suppression in radish by fluorescent pseudomonads was observed when the disease incidence in the control (untreated) field was less than 80 percent and the antagonists did not suppress fusarium wilt when disease incidence exceeded 80 percent in the control field (Raaijmakers et al., 1995). The antagonists may be highly

useful in moderately resistant varieties rather than highly susceptible varieties (Leeman et al., 1995b). It was suggested that the antagonists should be integrated with chemical fungicides, with the two being applied alternatively (Vidhyasekaran, 1998).

The method of application also determines the efficacy of pseudomonads. Seed treatment with these antagonists appears to be very effective. These bacteria establish well in the rhizosphere when introduced through seed treatment (Vidhyasekaran, Sethuraman, et al., 1997; Vidhyasekaran and Muthamilan, 1999). Seed treatment of peas with *Pseudomonas aureofaciens (P. chlororaphis)* protects pea plants against *R. solani* (Koch et al., 1998). Biopriming of treated seeds increases the population of the antagonists in seed and effectively controls diseases (Callan et al., 1990; Vidhyasekaran and Muthamilan, 1995). Soil application was shown to be effective in control of soilborne diseases (Hagedorn et al., 1993). Root dip treatment for transplanted crops can be highly useful. A root dip into suspensions of *P. aureofaciens* protects strawberry against *Phytophthora fragariae* var. *fragariae* (Koch et al., 1998). Several workers have successfully used foliar spray of these antagonists to control foliar diseases (Mew and Rosales, 1986; Vidhyasekaran, Rabindran, et al., 1997).

Pseudomonads should be used as powder formulations only. Ten-day-old bacterial cultures in a liquid nutrient medium are ineffective in controlling diseases. Several formulations with different carrier materials were developed for application in the field. Peat- and talc-based formulations are commonly used (Hagedorn et al., 1993; Hofte et al., 1991; Vidhyasekaran, Sethuraman, et al., 1997). Granular preparations of *P. aureofaciens* UKM B-111 based on clay minerals show high survival, preservation of antagonistic activity, and stability of composition during long-term storage (Kurdish et al., 1999). Vermiculite-based formulation of *P. putida* is effective in controlling *Fusarium oxysporum* f. sp. *cucurbitacearum* infection in cucumber (Amer and Utkhede, 2000).

OTHER ORGANISMS

Gliocladium

The fungus *Gliocladium catenulatum* (Primastop) was developed as a biofungicide. It was registered in the United States by Kemira Agro OY. The same product will be registered as PreStop in Europe (Niemi and Lahdenpera, 2000). This product has proven effective in the control of damping-off of vegetables, herb, and ornamental seedlings, root and stem rot diseases in vegetables and ornamentals, *Didymella* in cucumber and tomato, and gray

mold in ornamentals. It is effective against various soilborne diseases caused by *Pythium, Phytophthora,* and *Rhizoctonia,* foliar diseases caused by *Didymella, Alternaria,* and *Botrytis,* and storage diseases caused by *Helminthosporium* and *Rhizoctonia* (Niemi and Lahdenpera, 2000).

Pythium Oligandrum

Pythium oligandrum was developed as a commercial product named Polygandron. The addition of *P. oligandrum* zoospores to soil reduced the ability of sclerotia of *Sclerotinia sclerotiorum* to germinate. *Pythium oligandrum* reduces the survival of *S. sclerotiorum* present naturally in soils through mycoparasitic activity (Madsen and Neergaard, 1999). The cell-wall-degrading enzymes *N*-acetyl-β-D-glucosaminidase, endo-chitinase, β-glucanase, β-glucosidase, cellobiohydrolase, and protease were detected in culture filtrates of *P. oligandrum* cultivated with *S. sclerotiorum* (Madsen and Neergaard, 1999). *P. oligandrum* may also act by inducing resistance in plants. A low molecular weight protein termed *oligandrin* was obtained from culture filtrates of *P. oligandrum* (Picard et al., 2000). This protein induces plant defense reactions that help restrict stem cell invasion by *Phytophthora nicotianae* var. *parasitica.* Oligandrin is similar to elicitins. Oligandrin-treated plants show reduced disease incidence in tomato caused by *P. nicotianae* var. *parasitica* (Picard et al., 2000).

Verticillium Lecanii

Verticillium lecanii is a promising biocontrol agent of rusts and powdery mildews that effectively controls cucumber powdery mildew caused by *Spherotheca fuliginea* (Askary et al., 1998). *Verticillium lecanii* grows over a wide temperature range, and a water film, or at least high humidity, is required for conidial germination. Thus, water is an important environmental factor in the control of powdery mildews on cucumber and rose by *V. lecanii* (Verhaar et al., 1996). The development of *V. lecanii* in pustules of *Puccinia striiformis* was best at 95 to 100 percent relative humidity (RH), whereas no development was observed at 80 percent RH (Mendgen, 1981). This suggests that high humidity is required for effective control of the pathogen by *V. lecanii. Verticillium lecanii* produces antibiotics and hydrolytic enzymes and they may be involved in the parasitism of *V. lecanii* on *Penicillium digitatum* (Benhamou and Brodeur, 2000).

Yeasts

The yeast *Torulopsis candida* (= *Candida famata*) effectively controls *Penicillium digitatum* infection on citrus fruits (Arras et al., 1999). Another yeast, *Debaryomyces hansenii,* reduces *Penicillium digitatum* decay on orange fruits. The yeast elicited production of phytoalexins, scopoletin, and scoparone, and did not produce toxic substances against the pathogens, *Penicillium digitatum* and *Botrytis cinerea.* This suggests that the yeast may reduce the fungal infection by activating host's defense mechanisms (Arras and Arru, 1999). The yeasts *Candida saitoana* and *C. oleophila* control postharvest diseases of apple and citrus fruits (El-Ghaouth et al., 2000). The yeast *(Candida guilliermondii)* suspension, when sprayed two to five times at 7 to 10 day intervals, reduces decay caused by *B. cinerea* in both table grapes (cultivars Thomson Seedless and Superior Seedless) and wine grapes (cultivar Sauvignon blane), and rots caused by *Aspergillus niger* in wine grapes (Zhavi et al., 2000). Another yeast, *Pichia membranefaciens,* controls storage rot of nectarine fruits caused by *Rhizopus stolonifer* (Fan and Tian, 2000).

Aureobasidium

A cosmopolitan yeastlike fungus, *Aureobasidium pullulans,* colonizes leaf surfaces and is a potential biocontrol agent for plant pathogens. It controls *Botrytis cinerea* on apples, *Penicillium digitatum* on grapefruits, *B. cinerea, Rhizopus stolonifer,* and *Aspergillus niger* on table grapes and *B. cinerea* and *R. stolonifer* on cherry tomatoes (Schena et al., 1999). Preharvest application of *Aureobasidium pullulans* isolate L47 on table grapes results in a significant reduction of postharvest rot caused by *B. cinerea* (Schena et al., 1999). *Aureobasidium pullulans* controlled apple decay caused by *B. cinerea* and *Penicillium expansum.* The yeastlike fungus induced a transient increase in β-1,3-glucanase, chitinase, and peroxidase activities in apple tissues, and all three enzymes are involved in host defense mechanisms (Ippolito et al., 2000; Castoria et al., 2001). The biocontrol agent also has capacity to out-compete pathogens for nutrients and space (Ippolito et al., 2000; Castoria et al., 2001).

Penicillium

Talaromyces flavus (anamorph *Penicillium dangeardii*) is known to control *Verticillium* wilt of potato, artichoke, and olive. *Talaromyces flavus* isolate Tf-1 suppressed *Verticillium* wilt incidence in eggplant (Fravel and

Roberts, 1991). Purified glucose oxidase from *T. flavus* significantly reduced the growth rate of *V. dahliae* in the presence, but not in the absence, of eggplant roots. This suggests that glucose from the roots is metabolized by glucose oxidase to form hydrogen peroxide, which is toxic to *V. dahliae* (Fravel and Roberts, 1991).

Coniothyrium

The fungal mycoparasite *Coniothyrium minitans* applied as a spray reduces *Sclerotinia sclerotiorum* (white mold) infection in bean, potato, carrot, and chicory (Gerlagh et al., 1999). *C. minitans*-based formulations have been developed as Coniothyrin and Contans in Russia and Germany, respectively.

Nonpathogenic Isolates of Pathogens

Nonpathogenic isolates may induce resistance against pathogenic isolates of the same pathogen and other pathogens. Nonpathogenic isolates of *Rhizoctonia* (np-R) protect seedlings against damping-off caused by virulent isolates of *Rhizoctonia* species of different anastomosis groups (Sneh, 1999). Some np-R isolates induced plant resistance against *R. solani, Pythium aphanidermatum,* and *Pseudomonas syringae* pv. *lachrymans* in cucumber (Sneh, 1999). Binucleate *Rhizoctonia* fungi are another group of biocontrol agents that effectively control diseases of potato, bean, sugar beet, cucumber, pepper, *Catharanthus,* and turf grass caused by *Rhizoctonia* and *Pythium* spp. Colonization of host tissues by nonpathogenic isolates triggers production of host defense compounds such as peroxidases, glucanases, and chitinases (Burns and Benson, 2000).

Reduced-pathogenicity isolates of *Colletotrichum gloeosporioides (Glomerella cingulata)* delayed anthracnose symptom development in avocado fruits induced by virulent isolates (Yakoby et al., 2001). Preinoculation of avocado fruit with reduced-pathogenicity isolates induced resistance that was accompanied by an increase in the levels of preformed antifungal dienes (Yakoby et al., 2001). Less aggressive strains of *Ralstonia solanacearum* induced resistance against aggressive strains of *R. solanacearum* (Trigalet et al., 1998). Similarly, nonpathogenic isolates of *Fusarium oxysporum* induced resistance against pathogenic *F. oxysporum* strains in tomato, carnation, and sweet potato (Whipps, 1996). Commercial formulations of nonpathogenic strains of *F. oxysporum* and *R. solanacearum* are available in Europe.

Ulocladium

The fungus *Ulocladium atrum* competes saprophytically with *Botrytis* spp. during the colonization of necrotic plant tissues. The inoculum potential of *B. cinerea* is reduced by antagonistic interaction with *U. atrum,* leading to slower disease epidemic (Kohl et al., 1998). In cyclamen *(Cyclamen persicum),* naturally senesced leaves within the dense canopy play a crucial role in *Botrytis* epidemics. Since healthy leaves are normally resistant to conidial infections, *B. cinerea* depends on dead tissues for initial entry into the plant. Stimulated by this food base, the inoculum potential of the pathogen increases within the canopy of the single plant to such a level that healthy petioles and leaf blades can then be infected (Kohl et al., 2000). Biocontrol of *B. cinerea* by *U. atrum* could be achieved by competitively excluding the pathogen from colonizing necrotic leaves present within the cyclamen canopy. Repeated applications of conidial suspensions of *U. atrum* controlled the disease as effectively as the grower's standard fungicide program (Kohl et al., 1998, 2000).

Phialophora

Phialophora spp. are known to control wheat take-all caused by *Gaeumannomyces graminis* var. *tritici*. A *Phialophora* sp. (isolate I-52) was isolated from soil in a wheat field exhibiting suppression of take-all disease (Mathre et al., 1998). I-52 was grown on a variety of autoclaved organic substrates, including oat, millet, and canola seed. Each of these provided significant disease control when added to the seed furrow. Seed treatment was ineffective (Mathre et al., 1998). A *Phialophora* strain was commercialized in Australia (Wong et al., 1996).

Cryphonectria Hypoviruses

Some viruses may affect the virulence of crop pathogens. The hypovirulence caused by *Cryphonectria* hypoviruses (CHVs) on *Cryphonectria parasitica,* the causal agent of chestnut blight, is a typical example for this group (Robin et al., 2000). Infections by hypovirulent isolates result in superficial cankers on both European *(Castanea sativa)* and American *(C. dentata)* chestnut trees, whereas virus-free isolates cause deep, lethal cankers. CHVs are cytoplasmic double-stranded RNA viruses that move into conidia, but not into ascospores, and can be transmitted from an infected isolate to a virus-free isolate through hyphal anastomosis (Robin et al., 2000). In France, the Ministry of Agriculture has promoted an intensive re-

lease of hypovirulent isolates of *C. parasitica* for control of chestnut blight on 180,000 ha since 1974 (Robin et al., 2000). Mixtures of hypovirulent isolates, selected according to the vegetative compatibility types present in the populations of *C. parasitica,* were released in France. Every year in these orchards, all newly formed cankers are treated with hypovirulent isolates by introducing the mycelia into holes in the margin of the canker. This treatment shows a curative effect and contributes to the healing of the canker when the virus is successfully transmitted. The hypoviruses have a preventative effect depending on the spread of hypovirulent strains within the area where they are released. Hypoviruses are used to manage *C. parasitica* in Italy also (Bisiach et al., 1991). The major problem in exploiting hypoviruses as biocontrol agents is the existence of several vegetative compatibility (VC) types in the populations of *C. parasitica.* Most of the virus isolates are specific to the VC types. The rate of transmission of the virus is negatively correlated with the number of vegetative incompatibility genes that differ between mycelia that anastomose. In North America, the lack of spread of hypoviruses is characterized by high VC diversity. In Europe, low VC-type diversity favors the spread of hypovirulent isolates of *C. parasitica* and the recovery of European chestnut in many areas (Bissegger et al., 1997).

Bacillus Species

Different *Bacillus* spp. have been developed as microbial pesticides. *Bacillus subtilis* is available as a commercial formulation named FZB24. It is produced in a multi-stage liquid fermentation process from a stock culture that guarantees a uniform strain identity. The spores formed in this process are separated from the culture broth and then dried and formulated together with protective colloids, inert material, and other additives. The formulated end product has a storage stability of at least two years. This product was registered in Germany (Junge et al., 2000). The mode of action of this strain has been reported. The bacterium might act by competition by temporary colonization of the rhizosphere and rhizoplane. *Bacillus subtilis* has the ability to form antibiotics in vitro, but the in vivo production of antibiotics has not been demonstrated. *Bacillus subtilis* induces resistance by activation of defense genes. The bacterium also promotes plant and root growth, probably by producing cytokinins and auxins. The stronger root system ultimately leads to an uptake of water and nutrients. The growth promotion leads to the possibility of disease escape (Kilian et al., 2000). *Bacillus subtilis* strain BACT-0 formulations with vermiculite or kaolin as carrier material effectively control *Pythium aphanidermatum* infection in lettuce (Amer and Utkhede, 2000). *Bacillus cereus* applied as seed treatment was as

effective as the fungicide metalaxyl in the management of seedling diseases in lucerne (Kazmar et al., 2000). *Bacillus subtilis* strain EBW-1 as a root dip effectively controls *Agrobacterium tumefaciens* crown gall in apples (Utkhede, 1999). Addition of nitrate to soil increased the efficacy of *B. subtilis* in control of soil-borne fungal diseases (Knox et al., 2000). When used as a spray, *B. polymyxa* decreases the spread of rose powdery mildew and rust on leaves of *Antirrhinum* (Saniewska et al., 1998).

Pantoea

Pantoea agglomerans (Erwinia herbicola) is an effective biocontrol agent against the apple and pear fire blight pathogen *Erwinia amylovora* (Johnson et al., 2000). *Pantoea agglomerans* was sprayed on pear and apple cultivars and the effect of environmental factors on the spread of the biocontrol agent was assessed. The introduced bacteria colonized the blossoms of the inoculated trees, and temperature was found to be the important variable affecting successful spread of this biocontrol agent from blossom to blossom. The bacterial populations were positively correlated with mean degree hours per day during bloom and negatively correlated with the proportion of days with rain (Johnson et al., 2000). *Pantoea agglomerans* is spread by honey bees *(Apis mellifera)* in apple and pear orchards in New Zealand (Vanneste et al., 1999) and the United States (Pusey, 1999). *Pantoea agglomerans* strain E25 was highly effective in the control of the fire blight pathogen in the United States (Pusey, 1999). *Pantoea agglomerans* effectively controls apple storage diseases caused by *Botrytis cinerea* and *Penicillium expansum* (Sobiczewski and Bryk, 1999).

Lyophilized, talc-based, and whey-based formulations of *P. agglomerans* were developed (Ozaktan et al., 1999). In these formulations, the bacteria survived up to 180 days of storage at 10°C and up to 60 days at 24°C. Talc-based formulations were more effective in reducing pear fruit and blossom blight caused by *E. amylovora* than the lyophilized and whey-based formulations (Ozaktan et al., 1999).

Agrobacterium, Serratia, Streptomyces, and Rhizobium

Agrobacterium radiobacter is an effective biocontrol agent that controls crown gall of various crops caused by *Agrobacterium tumefaciens.* Commercial formulations of *A. radiobacter* are available in the United States, Australia, and New Zealand (Whipps, 1996). *Agrobacterium radiobacter* produces the bacteriocin agrocin 84, which inhibits *A. tumefaciens. Agrobacterium radiobacter* strains, which lack agrocin 84 production, are not ef-

fective against the crown gall pathogen. Increased doses of the antagonist may reduce the disease severity only to certain extent. The amount of disease suppression per unit of antagonist dose decreased with increasing antagonist dose (Johnson and DiLeone, 1999).

Serratia marcescens controls several diseases. It controls *Sclerotinia minor* infection in lettuce (El-Tarabily et al., 2000). *Serratia plymuthica* strain RIGG4 stimulates defense reactions in cucumber seedlings inoculated with *Pythium ultimum* (Benhamou et al., 2000). The antagonist induced cell wall apposition in cucumber, and callose, pectin, and cellulose appeared in the wall appositions (Benhamou et al., 2000). *Streptomyces griseoviridis* is another important biocontrol agent. Commercial formulations of the bacterium are available and it controls *Alternaria brassicicola* in cauliflower and damping off of pepper (Whipps, 1996). Some *Rhizobium* strains also acted as biocontrol agents (Simpfendorfer et al., 1999).

COMMERCIALLY AVAILABLE MICROBIAL PESTICIDES

Commercialization of biocontrol agents as microbial pesticides requires several steps, beginning with initial discovery and then proceeding through testing of efficacy, prototyping, and then commercial production, extensive large-scale field testing, toxicology and environmental tests, registration, and marketing (Harman, 2000). Toxicological tests, such as oral, dermal, ocular, respiratory, and health hazards using test animals and fish, should show no adverse effects and the microorganism should not be a pathogen. Success in selling biocontrol products requires that potential users and distributors be educated and convinced about the value of a biological product that is probably more conceptually difficult to use than standard pesticides. It requires several years and millions of dollars to bring a single biocontrol agent to market and to become profitable. Hence, only a few biocontrol agents have been developed as commercial products. They are mainly produced by small companies in different countries. Actizyme is a commercial granulated formulation of *Bacillus subtilis* (Walker and Morey, 1999). TriD25 is a dry formulation containing *Trichoderma koningii* and *T. harzianum* (Walker and Morey, 1999). FZB24 is a product of Bayer AG, Germany, and it consists of *B. subtilis* (Kilian et al., 2000). The yeast *Candida oleophila* is commercially available under the trade name Aspire (El-Ghaouth et al., 2000). Two strains of the bacterium *Pseudomonas syringae* are available under the trade names Biosave-100 and Biosave-110 (El-Ghaouth et al., 2000). The following are the other commercially available microbial pesticides:

Agrobacterium radiobacter—Diegall (Fruit growers Chemical Co., New Zealand), Galltrol (AgBio Chem, Inc., California), Norbac 84-C (New Bioproducts Inc., California), NoGall (Root Nodule, Pty Ltd., Australia; Bio-Care Technology, Pty Ltd., Australia)

Ampelomyces quisqualis—AQ10 (Ecogen, Langhorne, Pennsylvania)

Bacillus subtilis—Kodiak, Kodiak (A-13), and Epic (MB 1600) (Gustafson, Inc., Texas), Bactophyt (NPO Vector, Novosibirsk, Russia), System 3 (GBO3) (Helena Chemical Co., Tennessee)

Coniothyrium minitans—Coniothyrin (Russian Govt.), Contans (Prophyta Biologischer Pflanzenschutz GmbH, Germany)

Fusarium oxysporum (nonpathogenic)—Fusaclean (Fo47) (Natural Plant Protection, Noguerres, France), Biofox-C (S.I.A.P.A., Bologna, Italy)

Gliocladium catenulatum—PrimaStop (Kemira OY, Finland), PreStop (Kemira OY, Finland)

Peniophora (Phlebia) gigantea—Pg suspension (Ecological Laboratory Ltd., U.K.), Rotstop (Kemira OY, Finland)

Pseudomonas (Burkholderia) cepacia—Intercept (Soil Technologies, Fairfield, Iowa), Blue Circle and Deny (CTT Corporation, Carlsbad, California)

Pseudomonas fluorescens—BioCoat (SandG Seeds, BV, the Netherlands), Conqueror (Mauri Foods, Australia), Dagger (no longer available)

Pythium oligandrum—Polygandron (Vyzkummy ustov rastlinnej, Slovak Republic)

Ralstonia solanacearum (nonpathogenic)—PSSOL (Natural Plant Protection, Noguerres, France)

Streptomyces griseoviridis –Mycostop (Kemira OY, Finland), Stimagrow (Kemira OY, Finland)

Trichoderma harzianum T-22—Topshield, aka 1295-22, KRL—AG2, ATC 20847 (Bioworks, Geneva, NY), T-22G and T-22B (TGT Inc., New York), RootShield (Bioworks, Geneva, NY)

Trichoderma harzianum—T-35 (Makhteshim-Agan Chemicals, Israel), Harzian 20 and Harzian 10 (Natural Plant Protection, Noguerres, France), F-stop (Eastman Kodak Co., United States TGT Inc., New York), Supraavit (Bonegaard and Reitzel, Denmark)

Trichoderma harzianum strain T39—Trichodex (Makhteshim-Agan Chemicals, Israel)

Trichoderma harzianum + *T. polysporum*—BINAB-T and W (Bio-Innovation AB, Toreboda, Sweden)

Trichoderma harzianum + *T. viride*—Trichodowels, Trichoject, Trichopel, and Trichoseal (Agrimm Technologies Ltd., New Zealand)

Trichoderma spp.—Trichodermin (Bulgarian and Russian Governments), Promot (JH Biotech, Inc., Ventura, California), Solsain, Hors-solsain,

Plantsain (Prestabiol, Montpellier, France), ANTI-FUNGUS (Grondontsmettingen De Ceuster, Belgium), Ty (Mycontrol, Israel)

Trichoderma virens (Gliocladium virens)—GlioGard and SoilGard (Grace-Sierra Co., Maryland)

Trichoderma viridae—Bip T (Poland)

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– 18 –

Biological Control—Mycorrhiza

Mycorrhizal fungi are symbiotic organisms that live on the roots of several plants. Among them, endomycorrhizae are important because they confer resistance against pathogens. Several mycorrhizal fungi have been developed as commercial products and they can be used to reduce losses caused by diseases.

WHAT IS A MYCORRHIZA?

Mycorrhiza is a symbiotic association of a fungus with the roots of a plant. Three distinct classes of mycorrhiza are recognized: *ectomycorrhiza, endomycorrhiza,* and *ectendomycorrhiza.* In the case of ectomycorrhiza, the fungal symbionts penetrate intercellularly and partially replace the middle lamella between the cortical cells of the roots. This hyphal arrangement around such cortical cells is called the "Hartig net." The ectomycorrhizal fungal symbionts also form a dense, usually continuous, hyphal network or fungal mantle over the feeder root surface. Ectomycorrhizal association is normally seen on tree species. The majority of ectomycorrhizal fungi belong to the division Basidiomycota and the families of Amanitaceae, Tricholomataceae, Rhizopogonaceae, and Boletaceae. In contrast, the endomycorrhizal fungus penetrates the cortical cells of the feeder roots intracellularly. Such fungal symbionts form large vesicles and arbuscules in cortical tissues, and hence, they are called *vesicular-arbuscular mycorrhiza* (VAM) or *arbuscular mycorrhiza* (AM). Such fungi, however, do not form a dense fungal mantle. Instead they develop a loose, intermittent arrangement of mycelium with large spores on the root surface. Endomycorrhizal colonization takes place in many annual crops that do not form ectomycorrhiza, and the fungal symbionts belong to the division Oomycota and the family Endogonaceae.

Ectendomycorrhiza is the third class of mycorrhiza. This type of mycorrhiza is present on the roots of certain tree species under specific ecological situations. This type of mycorrhiza resembles ectomycorrhiza, in that it forms a Hartig net and a fungal mantle, and also resembles endomycorrhiza,

because of the intracellular penetration of cortical tissue by these fungi. Mycorrhizal symbiosis plays a key role in nutrient cycling in the ecosystem and protects plants against environmental stress (Barea and Jeffries, 1995). Mycorrhizal fungi alter host physiology and induce biochemical changes in the host metabolism. An altered host metabolism may result in resistance against plant pathogens (Vidhyasekaran, 1990).

DISEASES CONTROLLED BY MYCORRHIZAL FUNGI

Since the 1970s, several researchers have demonstrated that endomycorrhizal fungi, such as *Glomus mosseae, G. fasciculatum, G. intraradices,* and *G. multiculae,* could effectively control various diseases. The following are important diseases that are controlled by mycorrhizal fungi (Caron, 1989; Jalali, 1990; Linderman, 1994; Azcon-Aguilar and Barea, 1996; Cordier, 1998; Sharma and Adholeya, 2000):

Alfalfa—root rot *(Thielaviopsis basicola),* wilt *(Verticillium dahliae)*
Citrus—root rot *(Phytophthora parasitica),* root rot *(Thielaviopsis basicola)*
Cotton—wilt *(Verticillium dahliae),* root rot *(Thielaviopsis basicola)*
Cucumber—root rot *(Rhizoctonia solani),* damping-off (*Pythium* spp.)
Onion—pink root *(Pyrenochaeta terrestris)*
Pea—root rot *(Thielaviopsis basicola)*
Peanut—root rot *(Sclerotium rolfsii)*
Poinsettia—damping-off *(Pythium ultimum)*
Soybean—root rot *(Fusarium solani),* root rot (*Phytophthora megasperma* var. *sojae*)
Tobacco—root rot *(Thielaviopsis basicola)*
Tomato—wilt (*Fusarium oxysporum* f. sp. *lycopersici*), crown and root rot (*F. oxysporum* f. sp. *radicis lycopersici*), root rot *(Thielaviopsis basicola),* damping-off *(Pythium aphanidermatum),* blight *(Phytophthora parasitica)*
Wheat—take-all *(Gaeumannomyces graminis)*

MECHANISMS INVOLVED IN BIOCONTROL BY MYCORRHIZAE

Several hypotheses have been proposed to explain the mechanisms involved in the control of diseases by mycorrhizal fungi (Larsen, 2000).

Mycorrhizae may enhance nutrient uptake by plants and may strengthen the plants against pathogens. These fungi enhance root growth, and robust root development may compensate damage caused by pathogens. Exudates of roots colonized by arbuscular mycorrhizal fungi may affect the pathogen population in the mycorrhizosphere. Experiments in vitro showed that after 48 h in the presence of exudates from strawberry roots colonized by *Glomus etunicatum* and *G. monosporum,* sporulation of *Phytophthora fragariae* was reduced by about 64 and 67 percent, respectively (Norman and Hooker, 2000). A similar trend was observed in an in vivo system, with a 68 percent reduction in sporulation of *P. fragariae* in the mycorrhizosphere of colonized plants relative to sporulation in the mycorrhizosphere of uncolonized plants (Norman and Hooker, 2000).

An altered host metabolism may contribute to disease resistance induced by mycorrhizae (Benhamou et al., 1994; Cordier, 1998). Accumulation of phenolics in roots of plants due to mycorrhizal infection has been widely reported (Krishna and Bagyaraj, 1983; Cordier, 1998), and the role of phenolics in disease resistance is known. Increased accumulation of phytoalexins due to mycorrhizal infection has also been reported (Morandi et al., 1984; Harrison and Dixon, 1993). Chitinase transcripts accumulate in bean colonized by arbuscular mycorrhizal fungus *Glomus intraradices* (Blee and Anderson, 1996). β-1,3-Glucanase transcripts accumulate in and around arbuscule containing cells (Lambias and Mehdy, 1995). Chitinases and β-1,3-glucanases are important pathogenesis-related proteins that are involved in disease resistance (Vidhyasekaran, 1997, 2002). Dehne and Schoenbeck (1978) showed increased lignification of cells in the endodermis of mycorrhizal tomato and cucumber plants. Lignification is involved in cell wall thickening. Thus, mycorrhizal fungi may induce resistance against plant pathogens by several methods.

MASS PRODUCTION OF MYCORRHIZAL FUNGI

Endomycorrhizal fungi are obligate symbionts and hence they cannot be produced in a nutrient medium. They should be produced on living roots. This method is tedious and the risk of contamination with pathogens exists. AM fungi are mass multiplied on plants growing in disinfested soil. A highly susceptible trap plant is used for the multiplication of AM fungi. Stock cultures of these fungi are maintained in the form of colonized roots. These roots (with spores) are used to produce large amounts of inoculum in soil-based media. Soil in nursery beds are sterilized with methyl bromide. The AM fungal propagules are added to the soil and seeds of the trap plant (particularly monocots) are sown. The beds are watered regularly and kept

free from weeds. After about three months, the spore production in infected roots is assessed and used for application in the field.

Soilless substrates are also used for multiplication of AM fungi. Shredded bark, calcined montmorillonite clay, expanded clay aggregates, pertile, soilrite, and vermiculite have been used as inert substrates. The plants are grown in these substrates in the presence of AM fungal propagules, with frequent addition of nutrient solutions. Colonized roots and spores can be produced in hydroponics. Precolonized plants on sterile substrate are needed for this system. AM fungi can be propagated by growing precolonized plants in a defined nutrient solution that flows over the host roots. In an aeroponic system, the roots of the host are bathed in a fine mist of defined nutrient solutions suspended in air. Root-organ culture (axenic culture) is also used for multiplication of the mycorrhizal fungi (Sharma and Adholeya, 2000).

COMMERCIALIZATION OF MYCORRHIZAE

A few companies have taken up commercial production of arbuscular mycorrhizas. AGC Microbio of Cambridge, United Kingdom, produces mycorrhizal fungi in the trade name of Vaminoc. The other companies producing mycorrhizal fungi include: Bio-Enhancement Technologies, Camarillo, California; Horticultural Alliance, Sarasota, Florida; Plants Health Care, Pittsburg, Pennsylvania; Tree Pro, West Lafyette, Illinois; Biological Crop Protection, Wye, United Kingdom; Mikko-Tek Labs, Timmons, Ontario, Canada; Premier Tech, Quebec, Canada; Biorize, Dijon, France; Central Glass Co., Tokyo, Japan; and Global Horticare, Lelystad, The Netherlands.

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