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TRICHODERMA IN ORGANIC AGRICULTURE

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SUMMARY

Biocontrol, or Biological Control, can be defined as the use of natural organisms, or genetically modified, genes or gene products, to reduce the effects of undesirable organisms to favour organisms useful to human, such as crops, trees, animals and beneficial microorganisms. This strategy of control is ecologicaly clean and compatible with different models of agriculture: organic, biological and integrated pest/pathogen management (IPM) programmes.

The main antagonist used in disease control in Agriculture is the fungus *Trichoderma harzianum* Rifai, a low cost biocontrol agent that can establish itself in different pathosystems, has moderate effects on soil balance and does not harm benefitial organims that contribute towards pathogen's control. This biocontrol agent has not harmful effects on humans, wild life and other beneficial organisms. *T. harzianum* is a safe and effective biocontrol agent in both natural and controlled environments that does not accumulate in the food chain and to which it has not been described resistance.

Trichoderma strains used as biocontrol agents can act: a) colonizing the soil and/or parts of the plant, occupying a physical space and avoiding the multiplication of the pathogens; b) producing cell wall degrading enzymes against the pathogens; c) producing antibiotics that can kill the pathogens; d) promoting the plant development and e) inducing the defensive mechanisms of the plant.

Antifungal formulations based on *Trichoderma* strains and proteins require, as in the case of chemical fungicides, a costly and sound registration process previous to their commercialization. For this reason, many of these biological products are being offered to the farmers under the category of fertilizers and other commercial products that are not tightly regulated, and, hence, they do

not offer sufficient guarantee of quality and sanitary control. This fraud must be prosecuted since most of these wrongly registered formulations have not got a *Trichoderma* inoculum, shelflife or other properties stated in their label.

We have developed *Trichoderma* formulations against the main avocado root pathogens: *Phytophthora cinnamomi* and *Dematophora necatrix*.

Key Words: *Trichoderma*, biological control.

INTRODUCTION

Trichoderma is a fungal genus that was described in 1794, including anamorphic fungi isolated primarily from soil and decomposing organic matter (Persoon 1794). Strains within this genus include a wide spectrum of evolutionary solutions that range from very effective soil colonizers with high biodegradation potential, to non-strict plant symbionts that colonize the rhizosphere. Species concepts within *Trichoderma* are very wide, which has resulted in the recognition of many infraspecific groups. Some groups of biotypes within this conglomerate are able to antagonize phytopathogenic fungi by using substrate colonization, antibiosis and/or mycoparasitism as the main mechanisms. This antagonistic potential is the base for effective applications of different *Trichoderma* strains as an alternative to the chemical control against a wide set of fungal plant pathogens (Chet 1987; Harman and Björkman 1998). As a consequence of the variety of activities displayed by the *Trichoderma* strain conglomerate, a large range of applications have been developed: the antagonistic potential is the basis for the effective control of a wide set of phytopathogenic fungi (Papavizas, 1985; Samuels, 1996) and the biodegradative capacity is a source of useful enzymes in different industrial sectors (Harman and Kubicek 1998).

BIODIVERSITY OF *TRICHODERMA*

Most of the *Trichoderma* species are morphologically very similar and were considered for many years as a single species: *T. viride* (Bisby 1939). Since new species were discovered, a consolidated taxonomical scheme was needed and Rifai (1969) proposed and defined nine morphological species aggregates. DNA methods brought additional valuable criteria to the taxonomy of *Trichoderma* which are being used today for studies that include identification (Hermosa *et al.* 2001; Lübeck *et al.* 2000) and phylogenetic classification (Kullnig-Gradinger *et al.* 2002; Lieckfeldt and Seifert 2000). Most isolates of the genus *Trichoderma* that were found to act as mycoparasites of many economically important aerial and soil-borne plant pathogens, have been classified as *T. harzianum* Rifai (Gams and Meyer, 1998). Due to the fact that the species *"harzianum"* is generally considered as a group made of mycoparasitic and biocontrol strains, and there is large morphological plasticity that results in character overlaps with other species, the identification of the species may be difficult. Several authors have reported a large genetic variability among *T. harzianum* isolates (Bowen *et al.* 1996; Gomez *et al.* 1997; Grondona *et al.* 1997; Muthumeenakshi *et al,* 1994). In fact, it has been demonstrated that at least four distinct species are present within the biocontrol *T. harzianum* aggregate: *T. harzianum s.str., T. atroviride, T. longibrachiatum* and *T. asperellum* (Hermosa *et al.* 2000). Coevolution of organisms antagonistic to pathogens results in many *Trichoderma* strains being inactive against fungi other than those against which they were originally selected. This is strongly advantageous in that they are less likely to act against non-target organisms, but it does mean that a new selection process must take place for each crop/pathogen combination (Grondona *et al.*, 1997).

The use of *Trichoderma* species as biological control agents has been investigated for over 70 years but it is only relatively recently that strains have become available commercially. Many *Trichoderma* strains, mainly *T. harzianum*, *T. viride* and *T. virens* (formerly *Gliocladium virens*), have been identified as having potential applications in biological control and a partial list of genera of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria, Botrytis, Chondrostereum, Colletotrichum, Dematophora, Diaporthe, Endothia, Fulvia, Fusarium, Fusicladium*, *Helminthosporium, Macrophomina*, *Monilia, Nectria, Phoma, Phytophthora, Plasmopara, Pseudoperonospora*, *Pythium, Rhizoctonia, Rhizopus, Sclerotinia, Sclerotium, Venturia, Verticillium,* and wood rot fungi (Lumsden *et al*., 1993; Monte, 2001).

Biocontrol agents are widely regarded by the general public as "natural" and therefore non-threatening products, although risk assessments must clearly be carried out on their effects on non-target organisms. Moreover, knowledge concerning the behaviour of such antagonists is essential for their effective use.

MECHANISMS OF ACTION

The choice of active *Trichoderma* strains is important in designing effective and safe biocontrol strategies. Many species of *Trichoderma* have multiple strategies for fungal antagonism, and indirect effects on plant health (such as plant growth promotion effects and fertility improvements) also vary. Some strains are potent antibiotic producers, and their suitability for use in biocontrol systems must be carefully assessed. However, many other active strains have no antibiotic capacity, and these are likely to be more useful in food production systems. *Trichoderma* biocontrol strains have evolved numerous mechanisms for both attacking other fungi and enhancing plant and root growth (Harman 2000). The colonization of the root system by rhizosphere competent strains of *Trichoderma* results in increased development of root and/or aerial systems and crop yields (Harman and Kubicek 1998). Other activities, like the induction of plant systemic resistance and antagonistic effects on plant pathogenic nematodes (Sharon *et al*. 2001), have also been described. These facts strongly suggest that during the plant-*Trichoderma* interactions, the fungus participates actively in protecting and improving its ecological niche. The dual roles of antagonistic activity against plant pathogens and promotion of soil fertility make *Trichoderma* strains appealing alternatives to soil fumigation technologies such as methyl bromide.

Strains of *Trichoderma* may also be aggressive biodegraders (Wardle *et al*. 1993) and act as competitors to fungal pathogens in their saprofitic phases, especially when nutrients are a limiting factor (Simon and Sivasithamparam 1989). Strains have been reported as promoting activities of non-pathogenic bacteria (Vrany *et al*. 1990) and mycorrhizal fungi (Calvet *et al*. 1993). In the 1990s, the ability of *Trichoderma* strains to synthesize substances inducing SAR-like responses in plants was shown (Elad 1996; Enkerli *et al*. 1999). Molecules produced by *Trichoderma* and/or its metabolic activity also have potential for promoting plant growth (Yedidia *et al.* 1999). Application of the species *T. harzianum* to plants resulted in improved seed germination, increased plant size, and augment of leaf area and weight (Altomare *et al.* 1999; Inbar *et al*. 1994). The scenario of combined systemic biofungicides and plant growth promoters has great market potential if the molecular basis of the activities can be identified.

The strong biodegradation and substrate colonization performances of *Trichoderma* strains is the result of an amazing metabolic versatility and a high secretory potential which leads to the production of a complex set of hydrolytic enzymes. Similarly, the mycoparasitic process is based on the secretion of a rich cocktail of cell wall degrading enzymes (CWDEs) able to hydrolize the cell wall of various hosts (Kubicek *et al.* 2001). Among others, chitinases (de la Cruz *et al.* 1992), b-1,3- glucanases (de la Cruz *et al*. 1995b; Lorito *et al.* 1994; Noronha and Ulhoa 1996), b-1,6-glucanases (de la Cruz *et al*. 1995a; de la Cruz and Llobell 1999), a-1,3-glucanases (Ait-Lahsen *et al.*

2001) and proteases (Geremia *et al.* 1993; Suárez 2001) have been described as important components of the multi-enzymatic system of *Trichoderma* strains. Some of these proteins display strong antifungal activities when are applied *in vitro*, alone and/or combined, against plant pathogens (Harman 2000). Some lytic enzymes can be involved in both antagonistic and saprophytic processes providing an evolutionary advantage to strains with both biodegrading and antagonistic potential, for the efficient colonization of different ecological niches in soil. A principal role in mycoparasitism has been attributed to chitinases (Lorito 1998) and glucanases (Benítez *et al.* 1998). However, fungal proteases may also be significantly involved in cell wall degradation, since fungal cell walls contain chitin and glucan polymers embedded in and covalently linked to a protein matrix (Kapteyn *et al.* 1996).

The production of secondary metabolites by *Trichoderma* strains also shows great variety and application potential. *Trichoderma* strains seem to be an inexhaustible source of antibiotics, from the acetaldehydes gliotoxin and viridin (Dennis and Webster 1971), to alpha-pyrones (Keszler *et al.* 2000), terpenes, polyketides, isocyanide derivatives, piperacines, and complex families of peptaibols (Sivasithamparam and Ghisalberti 1998). All these compounds produce synergistic effects in combination with CWDEs, with strong inhibitory activity on many fungal plant pathogens (Lorito *et al.* 1996; Schirmböck *et al.* 1994). The potential of genes involved in biosynthetic pathways of antibiotics [e.g. polyketides, Sherman (2002) and peptaibols (Wiest *et al.* 2002)] with applications in human and veterinary medicine is not been explored yet.

Trichoderma is not only a good biocontrol agent, but also a general fertility promoter. In the absence of pathogens, application of appropriate *Trichoderna* formulations (following solarization and/or preceding fumigation with authorized and environmentally-friendly chemicals) can also serve to promote plant growth and crop precocity, increase fruit production and reduce chemical treatments.

SELECTION OF *TRICHODERMA* STRAINS

Once active strains have been identified with the *in vitro* assays, a further selection must be done by studying other factors such as: 1) activity *in vivo* using experimentally induced diseases on plants, 2) tolerance of high or low temperatures (necessary to survive other IPM treatments), 3) suitability for formulation as foliar sprays and/or soil enhancements (e.g. high sporulation levels, rapid growth in bulk conditions), 4) specificity (strains should be inactive against beneficial organisms and plant crops), 5) long-term survival in field conditions, 6) interactions with other *Trichoderma* strains already present in the cropping systems, 7) compatibility with agrochemicals used in the crop, or 8) shelflife and inoculum efficacy under commercial conditions.

TRICHODERMA IN AVOCADO PROTECTION

We have developed a biocontrol formulation, based on *Trichoderma* conidia, that was tested with satisfactory results.against the main avocado root pathogens: *Phytophthora cinnamomi* and *Dematophora necatrix* in plantations maintained in the ecological conditions of Motril (Granada, Spain). *D. necatrix* is more resistant than *P. cinnamomi* to the action of *Trichoderma* biocontrol strains. However, being more difficult, the control by *Trichoderma* of root diseases caused by *D. necatrix* is effective (Freeman *et al.*, 1986).

TRICHODERMA PROTEIN FORMULATIONS

Trichoderma protein extracts with high glucanase and chitinase activities, directly obtained from wild type strains (Lorito, 1998), have been demonstrated to be effective as biofungicides. They can also be combined with chemicals (carbendazim, iprodione) with synergistic effects, and are stable enough to be considered for commercial application. We have investigated the antifungal properties of the proteins produced by *Trichoderma* species in laboratory and field conditions, defining the concentration of protein necessary to produce fungicide effects. It is recommended that any protein formulations contains at least one enzyme from each of the following classes: endochitinase, exochitinase, endoglucanase, exoglucanase (ß-1,3 plus ß-1,6), proteases and cellulalase (endocellulase). More than two enzymes from each class did not provide additional antifungal effect. In the field trials carried out with *Trichoderma* protein extracts, increased average weight of both roots and fruit per plant was detected in plots treated with *Trichoderma* proteins. The protein filtrates increased the total useful fruit weight by increasing the number of fruits of commercial size. These tests showed that *Trichoderma* chitinases and glucanases have no effect on the plant even if relatively large quantities are injected into plant tissues. CWDEs are not harmful to humans and animals, as indicated by eco-toxicological tests for registration of strains of *Trichoderma* for use as biocontrol agents in USA and the EU, and degrade into environmentally friendly residues. CWDEs can be effectively combined with whole-organism *Trichoderma* control, with considerable opportunities for synergism. CWDEs are particularly suited to post-harvest control.

The genes coding for protein production can be introduced into suitable organisms to be used as cell factories for large-scale production of CWDEs.

TRICHODERMA GENES

Several methods for applying both biocontrol and plant growth promotion exerted by *Trichoderma* strains have recently been demonstrated and it is now clear that hundreds of separate genes and gene products are involved in the processes of mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of enzymes produced by pathogens (Monte 2001). Some of these genes have been identified, cloned from *Trichoderma* spp. (that offer great promise as transgenes to produce crops that are resistant to plant diseases since transgenic expression of high levels of chitinolytic and glucanolytic *Trichoderma* enzymes do not affect plant morphology, development or yield, or infection by arbuscular mycorrhizal fungi), patented and used to transgenically increase plant disease resistance (Lorito *et al.* 1998), but most of them are still unexploited for developing new biotechnologies.

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