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**Control of soilborne pathogens of tomato using a commercial formulation of *Streptomyces griseoviridis* and solarization**

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## UNIVERSITÀ DEGLI STUDI DI TORINO

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24 **Improving the efficacy of biocontrol agents against soilborne pathogens<sup>1</sup>**

25

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27

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34

35 **Abstract**

36 Technical, economical and environmental factors are forcing to adopt new sustainable methods,  
37 such as the use of microbial antagonists, for the control of soilborne pathogens. Research has  
38 mainly focused on antagonistic fungi and bacteria, often not providing consistent or satisfying  
39 results. Biocontrol agents can be combined with other chemical products, with physical methods  
40 (solarization or steam sterilization) or agronomical practices, such as enhancement of suppressive  
41 soils, use of amendments or microbial optimization in the case of soilless systems. Different  
42 biocontrol strategies should be developed for different pathogens. The use of microorganisms can  
43 play an important role in a more complex vision of crop protection, as a key elements of IPM  
44 programs. Mixtures of antagonists with complementary activities could be developed, but the need  
45 for multiple registration is critical. Genetic manipulation could result in new biocontrol strains with  
46 increased production of toxic compounds or lytic enzymes, improved space or nutrient competence,  
47 wider host range or enhanced tolerance to abiotic stresses. The potential risks related to the  
48 environmental release of genetically modified microorganisms must be carefully assessed. Genes

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49 and enzymes involved in the biocontrol mechanism could be applied directly or transferred to crops.  
50 Finally, the production system, formulation and methods of application (soil and seed treatment or  
51 microbial colonization of the hydroponic nutrient solution) are crucial to maintain and improve the  
52 efficacy of microbial antagonists.

53

54 **Keywords:** antagonist, biological control, formulation, integrated control, genetically modified  
55 microorganism, mass production, method of application, risk assessment, soilborne diseases.

56

## 57 **1. Introduction**

58 Meeting the goal of improving soilborne disease control for efficient and sustainable production  
59 systems requires a reduction of the chemical inputs in agriculture. Various movements or parties see  
60 increased pesticide application as a threat to public health and the environment. Agronomists  
61 experience growing problems with the build up of resistance to the pesticides in target pathogen  
62 populations (Dekker, 1976; Brent and Hollomon, 1998) and the withdrawal of pesticides from the  
63 market (Gullino and Kuijpers, 1994). Of special relevance, in the case of many vegetable and  
64 ornamental crops, is the loss of methyl bromide as soil fumigant (Katan, 1999; Gullino et al., 2003).  
65 Furthermore, the private industry is coming across increasing costs in discovering and developing  
66 new molecules for disease control application. Consequently, there is a growing awareness that  
67 integrated pest management (IPM) strategies can provide more environmentally sound and  
68 economically feasible alternatives for soilborne disease control. Biological control with introduced  
69 microorganisms has been identified as an important component of IPM strategies (Cook, 1993).  
70 Many studies have been devoted to the identification of microorganisms able to reduce the activity  
71 of soilborne pathogens during the four past decades (Baker and Snyder, 1965).  
72 Many laboratories around the world have developed their own microorganisms and this allowed the  
73 collection of important contributions about the biology of pathogens and antagonists. However, in

74 spite of the initial great optimism and extensive later research efforts, progress in achieving  
75 commercial, large-scale usage of biological pest control has been slow.

76 Most experiences were on a laboratory scale, thus avoiding the problems related to antagonist mass  
77 production and biofungicide formulation, and disease control trials were performed in simplified  
78 environments such as growth chambers, experimental greenhouses or small field plots, thus  
79 avoiding the risk of large scale experiments. When trials move towards the farm scale, many  
80 antagonists fail to confirm their activity or, when tested in different environments, behave  
81 unpredictably, and consequently lack reliability (Mathre et al., 1999).

82 Biofungicides presently meet many constraints and it is not a straightforward task to find, develop  
83 and implement practically feasible biocontrol products against soilborne diseases. Nevertheless,  
84 there are also reasons for stating careful optimism. There are many possibilities for combining  
85 various biocontrol agents, with each other, or with agronomical, physical or chemical control  
86 methods. In particular, by combining different methods of control, the aim is to obtain a synergistic  
87 effect, rather than additive. For that reason, a complete comprehension of the mode of action and  
88 mechanism of control is needed. A good combination is obtained when both partners benefit from  
89 the combined treatment. For example, combining a biocontrol agent (BCA) with a fungicide  
90 improves the control by the BCA and, at the same time, it enables to reduce the fungicide dosage,  
91 rendering its use more acceptable. Moreover, the combination of control methods provides a wider  
92 spectrum of control, which is especially needed to replace the wide spectrum fumigants, such as  
93 methyl bromide. Finally, combining different control methods enables to make use of less effective  
94 control methods which cannot stand by themselves, but in combination they can contribute to the  
95 control.

96 Other possibilities to improve the antagonist effectiveness include the genetic manipulation of the  
97 microorganisms and the enhancement of the mass production, formulation and methods of  
98 application. The main possibilities to improve the efficacy of the biocontrol agents against soilborne  
99 pathogens are the topic of this brief review.

100

## 101 **2. Combination with chemical pesticides**

102 The combined use of BCAs (biocontrol agents) and chemical pesticides has attracted much  
103 attention in order to obtain synergistic or additive effects against the target organisms (Locke et al.,  
104 1985). Reduced amounts of fungicide can stress and weaken the pathogen and render its propagules  
105 more susceptible to subsequent attack by the antagonist (Hjeljord and Tronsmo, 1998).  
106 Replacement of some of the chemical fungicide treatments with BCAs does not only reduce the  
107 input of chemicals but can also result in improved disease control. For this reason, BCAs are  
108 required to be resistant to chemicals. Either the natural resistance of some antagonists towards  
109 specific fungicides could be exploited or resistant mutants can be produced, by selection on  
110 pesticide-containing media (Abd-El Moity et al., 1982), by UV-induced mutation (Postma and  
111 Lutikholt, 1993), or by protoplast fusion (Minucci et al., 1991).

112 The high antagonistic activity of benzimidazole resistant isolates of *Fusarium* antagonists permitted  
113 the combination of chemical and biological control measures, leading to improved control of  
114 fusarium wilt of carnation or cyclamen (Garibaldi et al., 1990a; Minuto et al., 1995a). Seed  
115 treatments with BCAs which are insensitive to pesticides used in traditional seed technology are  
116 used in commercial delivery systems based on antagonistic *Trichoderma* spp. (Harman and  
117 Björkman, 1998). Some heavy metal-resistant mutants of *Trichoderma* spp. selected on heavy  
118 metal-rich artificial media were effective antagonists of *Fusarium* spp., *Pythium* spp. and  
119 *Rhizoctonia* spp. (Kredics et al., 2001). These mutants might be of value for use with heavy metal-  
120 containing pesticides, as part of an integrated plant protection system.

121 By using chemical and biological control measures together, the duration of active disease control  
122 will be extended and the chances for the development of fungicide resistance can be reduced.  
123 Chemicals are effective under climatic conditions or level of disease pressure in which the  
124 antagonist is less effective, but a BCA can colonize roots, wounds or senescing plants tissue before  
125 the pathogen (Ahmad and Baker, 1987; Garibaldi et al. 1990b; Hjeljord and Tronsmo, 1998).

126

### 127 **3. Combination with soil disinfestation**

128 Soil disinfestation is commonly used for sanitation in low technology houses and fumigation with  
129 chemicals, heating, steaming and solarization are the main methods in use (Garibaldi and Gullino,  
130 1995). Soilborne pathogens were effectively controlled by using methyl bromide (Bell et al., 1996).  
131 However, concern regarding the potential of methyl bromide to deplete ozone led to its inclusion  
132 among the substances controlled by the Montreal protocol which use must be eliminated by the end  
133 of 2004 from industrialized countries (Gullino et al., 2003). Although the targets are soilborne  
134 pathogens, soil disinfestation can also have deleterious effects on beneficial soil microorganisms,  
135 but some genera, such as *Trichoderma* spp. and *Gliocladium* spp., are often less sensitive to  
136 fumigants and other chemicals used in disinfestation, resulting in recolonization of the soil by these  
137 organisms (Garibaldi and Gullino, 1995). There is, however, a high risk of recolonization of the  
138 biological vacuum, with pathogens, leading to more serious disease problems (Jarvis, 1989).  
139 Incorporation of antagonists following disinfestation of the soil has been shown to be effective in  
140 controlling *Rhizoctonia solani* on carrots (Strashnow et al., 1985), *Rh. solani* and *Sclerotium rolfsii*  
141 on tomato and peanuts (Elad et al., 1982). Resting propagules of pathogens such as microsclerotia  
142 can be predisposed to infection by *Gliocladium* spp. as a result of the treatment (Tjamos, 1992).  
143 Among available alternatives, biological control and soil solarization, alone or in combination, are  
144 promising methods for soilborne disease control (Minuto et al., 1995b; Katan, 1996). Solarization,  
145 carried out by covering the soil with transparent film during the hot season, has been widely  
146 exploited in a number of warm countries as well as in climatically marginal ones (Katan and  
147 DeVay, 1991). In Northern Italy, its efficacy is improved when applied in the greenhouse (Garibaldi  
148 and Gullino, 1991). Solarization proved to be a potential control strategy both in combination with  
149 *Trichoderma harzianum* against *Rhizoctonia solani* (Chet et al., 1982), as in combination with  
150 *Gliocladium virens* against southern blight of tomatoes (Ristaino et al., 1991). Sivan and Chet  
151 (1993) combined the application of *Trichoderma harzianum* with soil solarization under field

152 conditions, obtaining a significant disease control of fusarium crown and root rot of tomato and a  
153 significant yield increase. Minuto et al. (2004) demonstrated that the combination of soil  
154 solarization and *S. griseoviridis* was effective against fusarium and verticillium wilts and corky  
155 root, increasing the range of pathogens controlled with respect to the single treatments. A  
156 significant increase in the weight of the single fruit was noticed and a higher yield per square meter,  
157 when solarization and biocontrol agents were applied together, confirming the potential additive  
158 effect caused by the commercial biofungicide and solarization in terms of yield increase.  
159 Combining BCA with solarization may also enable the shortening of the solarization process and  
160 the use under marginal conditions.  
161 BCAs can also be applied after steam disinfestation of soils and substrates, an approach largely  
162 applicable in the case of ornamental and vegetable crops (Hoitink et al., 1991; Gullino, 1992).

163

#### 164 **4. Application of agronomical practices for biocontrol**

165 Several agronomical methods permit to promote biocontrol agents. These include advantageous  
166 regimes for choosing crop plants, soil types, crop rotations, soil amendments, and the application of  
167 suitable ploughing, planting and sowing regimes.

168 Natural disease suppressive soils are good examples in which the indigenous microflora effectively  
169 protects plants against soilborne pathogens. Suppressiveness has been described for many  
170 soilborne pathogens, including *Fusarium oxysporum* (Scher and Baker, 1982; Alabouvette, 1986;  
171 Garibaldi et al., 1990b), *Gaeumannomyces graminis* var. *tritici* (Shipton, 1975), *Pythium ultimum*  
172 (Garibaldi et al., 1993), and *Rhizoctonia solani* (Lucas et al., 1993). Suppressiveness can be  
173 induced, transferring the characteristics to conducive soils. Isolation of microorganisms responsible  
174 for suppressiveness has permitted to inoculate soils and substrates, seeds and multiplicative material  
175 with the antagonists (Garibaldi et al. 1987). Some biofungicides have been developed studying  
176 suppressive soils. A biofungicide (White et al., 1990) based on the K61 strain of *Streptomyces*  
177 *griseoviridis* isolated from *Sphagnum* peat (Tahvonen, 1982) can control or suppress some root rot



178 and wilt diseases, caused by *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp.,  
179 colonizing the rhizosphere prior to pathogens. A strain of *Gliocladium virens* isolated in the late  
180 1980s from a soil in Maryland, was developed to control *Pythium ultimum* and *Rhizoctonia solani*  
181 in soilless mixes (Lumsden and Locke, 1989). The nonpathogenic *Fusarium oxysporum* strain  
182 Fo47, originally isolated from a fusarium suppressive soil in France is marketed as liquid  
183 formulation for soilless culture such as tomato in rockwool (Alabouvette et al., 1998). Another  
184 strain of *F. oxysporum* isolated from soils suppressive to fusarium wilt of carnation is the active  
185 ingredient of a biological product against different *formae speciales* of *F. oxysporum* (Aloi et al.,  
186 1992).

187 Organic amendments such as green manures, stable manures, and composts have long been  
188 recognized to facilitate biological control if applied before planting (Lumsden et al., 1983). A great  
189 diversity of microorganisms contribute to biocontrol provided by composts (Kwok et al., 1987;  
190 Alvarez et al., 1995). Fluorescent pseudomonads *Pantoea* and *Bacillus* spp. were the most effective  
191 species against *Pythium* damping-off on cucumber (Boehm et al., 1997) present in a composted  
192 pine bark-amended potting mix. Moreover, composts can serve as an ideal source of nutrients for  
193 antagonists into soils and offer an opportunity to introduce and establish specific BCAs into soils,  
194 which in turn leads to a sustainable disease control based on the activities of microbial communities  
195 (Hoitink and Bohem, 1999). At least two separate mechanisms of biocontrol exist in suppressive  
196 composts. The first, typical of properly prepared composts, is general suppressiveness that operates  
197 primarily against *Pythium* and *Phytophthora*, based on the competition of nutrients. If composts  
198 lack readily-available nutrients, these pathogens may be suppressed (Hoitink et al., 1991). The  
199 second mechanism of suppression is more specific, it is required for pathogens such as *Rhizoctonia*  
200 *solani* and it appears to be related primarily to mycoparasitism (Hoitink et al., 1997). Strains of  
201 *Trichoderma hamatum* and *Flavobacterium balustinum* are among the most effective strains (Kwok  
202 et al., 1987).

203 Greenhouse conditions offer good opportunities for the application of BCAs, mostly due to their  
204 more uniform and controlled environment (Albajes et al., 1999). Antagonist to be used in  
205 greenhouses should be selected in order to fit the set of conditions, such as pH, temperature and  
206 matrix potential, of the cultivation environment (Paulitz and Bélanger, 2001). Moreover, cultural  
207 practices and amendments inducing disease suppressiveness in soil or soilless media are more  
208 effective in greenhouses than in the open field.

209 In closed recirculating soilless systems, zoosporic pathogens can easily spread in the water systems,  
210 in absence of the microbial buffer produced in soil by the activity of the saprophytic microflora  
211 (Gullino and Garibaldi, 1994). Fortunately, under such conditions it is quite easy to introduce a  
212 BCA at the beginning of the cropping period to prevent colonization of the substrate by the  
213 pathogenic fungi. *Trichoderma* spp. are naturally found as a part of the indigenous microflora in  
214 soilless systems (Jensen and Lumsden, 1999). Also bacteria seem to be well adapted to soilless  
215 cultivation due to the high water content of such systems (Paulitz, 1997). Some strains of  
216 *Pseudomonas fluorescens* and *P. corrugata* reduced zoospore germination and motility of *Pythium*  
217 sp. rot in hydroponically cultivated cucumber (Rankin and Paulitz, 1994). A good example of  
218 exploitation of biocontrol in hydroponic cropping is represented by the microbial optimization of  
219 soilless systems through the application of microorganisms able to colonize the rooting system of  
220 plants grown under a strictly controlled environment (Van Os and Postma, 2000). A slow sand  
221 filtration technique was tested both alone and in combination with different antagonistic strains  
222 belonging to *Fusarium* spp. and *Trichoderma* spp., isolated from gerbera rhizosphere and applied  
223 into the soilless systems (Grasso et al., 2003). This disinfection technique can be successfully  
224 combined with the application of antagonistic microorganisms (Garibaldi et al., 2003).

225

## 226 **5. Mixtures of antagonists**

227 Association of several microorganisms is needed to control different diseases that affect the same  
228 crop. Most of the BCAs are specific only for a given type of target pathogen. Although this property

229 represents an advantage from the environmental point of view, it creates great difficulties to the  
230 growers who need to control several plant pathogens in the same crop. Moreover, the combination  
231 of two or more antagonists means also multiple registration processes, with increased costs and  
232 difficulties in matching all the studies required by the strict legislation. However this option could  
233 be feasible with products already registered: biofungicides based on different antagonistic strains  
234 may be labeled as compatible with each other and proposed for joint use.

235 Alabouvette et al. (1996) demonstrated that a synergistic effect can be obtained in controlling  
236 *Fusarium oxysporum* f.sp. *radicis-lycopersici* by combining a fluorescent *Pseudomonas* sp. with a  
237 non-pathogenic *F.oxysporum*. The non-pathogenic *F.oxysporum* competes for carbon sources while  
238 the bacterial antagonist produces a siderophore competing for iron (Lemenceau et al., 1993).  
239 Moreover it was noted that the antagonistic strain Fo47 was less sensitive to pseudobactin-mediated  
240 iron competition than the pathogenic *F. oxysporum* f.sp. *dianthi*. Park et al. (1988) also showed that  
241 interaction between the bacterium *P. putida* and saprophytic strains of *F.oxysporum* could achieve  
242 effective control of *Fusarium oxysporum* f. sp. *cucumerinum*.

243 A positive, possibly synergistic, interaction between *Trichoderma* spp. strains and bacterial  
244 antagonists, such as *Pseudomonas syringae* has been reported for combined applications in the  
245 control of plant pathogens (Whipps et al., 1997). Part of the mechanism could be explained with the  
246 positive interaction among the lipodepsipeptides of the bacterial antagonist and the fungal cell wall-  
247 degrading enzymes of the fungal biocontrol agent (Fogliano et al., 2002). An effective control was  
248 also demonstrated for the combination of *Trichoderma harzianum* protecting against infection by  
249 *Pythium ultimum* in the rhizosphere and *Pythium nuun* reducing inoculum density of the same  
250 pathogen in the soil mass (Paulitz et al., 1990).

251 By combining specific strains of microorganisms, multiple traits antagonizing the pathogen can be  
252 combined and this may result in a higher level of protection. When *P. putida* strain WCS358,  
253 competing for iron through the production of its pseudobactin siderophore, was combined with *P.*  
254 *putida* strain RE8, inducing systemic resistance against *F. oxysporum* f.sp. *raphani*, fusarium wilt

255 suppression was significantly enhanced (de Boer et al., 2003). Previously, a mixture of three  
256 different plant growth-promoting rhizobacteria (PGRP), applied as a seed treatment, showed  
257 intensive plant growth promotion and reduction of multiple cucumber diseases (Raupach and  
258 Kloepper, 1998). Another innovative approach for improving soilborne disease control could be the  
259 development of cocktails containing strains that communicate with each other to maximize  
260 antibiotic production and disease control (Becker et al., 1997; Davelos et al., 2004).  
261 Hoitink et al. (1991) incorporated several antagonists in combination in peat substrates rendering  
262 them disease suppressive. A broad spectrum biological control of *Pythium*, *Phytophthora*, and  
263 *Rhizoctonia solani* requires the introduction into or presence of organic nutrients in the soil to  
264 maintain several taxa of biocontrol agents (Hoitink and Boehm, 1999). The composition of the  
265 microflora active in control changes as the organic matter decomposes, while the microbial carrying  
266 capacity of the amendment declines.

267

## 268 **6. Genetic manipulation**

269 Despite years of research and development, significant questions regarding the physiological and  
270 ecological constraints that limit biological control remain unanswered. Molecular and genomic tools  
271 offer new possibilities for improving the selection, characterization, and management of biological  
272 control. Development in proteomics and functional genomics will give us the possibility to  
273 determine and follow expression of crucial genes of BCA's during mass production, formulation,  
274 storage and application. Moreover, molecular techniques allow modification of wild type strains to  
275 improve their ability to control soilborne diseases. Unwanted genes might be deleted, or one or  
276 more genes, coming from different antagonists, could be added.

277 For fungi without functional or easily induced sexual stages, four methods are usually employed for  
278 genetic manipulation: conventional mutagenesis induced by chemicals (Howell and Stipanovic,  
279 1983) or by ultraviolet light (Baker, 1989); protoplast fusion (Harman et al., 1989; Minucci et al.,  
280 1991); transposon mutagenesis (Brown and Holden, 1998); and transformation. Most frequently,

281 protoplasts are used for introduction of exogenous DNA in presence of polyethylene glycol (PEG)  
282 and calcium chloride (Penttilä et al., 1987). Whole fungal cells can be transformed using the lithium  
283 acetate method (Dickman, 1988), by electroporation (Goldman et al., 1990), by particle  
284 bombardment (Lorito et al., 1993) or by *Agrobacterium*-mediated transformation (Zeilinger, 2004).  
285 Bacteria transformation can be performed by using electroporation, osmotic shock, or *Escherichia*  
286 *coli*-mediated conjugation.

287 One of the modes of action of antagonists is mycoparasitism, due to the production of lytic  
288 enzymes, such as chitinases, proteases and glucanases, able to degrade the pathogen cell walls.  
289 Genes encoding chitinases have been cloned from a wide variety of microorganisms, including  
290 *Serratia marcescens* (Sundheim et al., 1988) and *Trichoderma harzianum* (de la Cruz et al., 1992:  
291 cloning of *chit33*; Garcia et al., 1994: cloning of *ech42*). Baek et al. (1999) reported an increased  
292 and decreased antagonistic activity against *Rhizoctonia solani* on cotton strains of *T. virens*  
293 containing two *ech42* copies and a disrupted *ech42* gene copy. With constitutive expression of  
294 *chit33* in *T. harzianum*, the recombinant strains provided a superior biocontrol activity against *Rh.*  
295 *solani* on agar plates (Limon et al., 1999). An endophytic strain of *Pseudomonas fluorescens* has  
296 been transformed with the *chiA* gene encoding the major chitinase of the *S. marcescens*, showing an  
297 effective control of *Rh. solani* on bean seedlings under plant growth chamber conditions (Downing  
298 and Thomson, 2000).

299 Chitinase genes may also be used to improve resistance of plants against fungal pathogens. Genes  
300 from biocontrol fungi encoding endochitinases or exochitinases were inserted into potato and  
301 tobacco (Lorito et al., 1998), apple (Bolar et al., 2001), broccoli (Mora and Earle, 2001), or  
302 *Brassica juncea* (Mondal et al., 2003).

303 Many bacterial or fungal strains used in biocontrol produce antibiotics that inhibit the growth of  
304 other fungi. Introduction of the gene encoding the housekeeping sigma factor into a strain of  
305 *Pseudomonas fluorescens* increased the production of pyoluteorin and 2,4-diacetylphloroglucinol  
306 (DAPG) (Schnider et al., 1995). Another strain of *P. fluorescens*, antagonist of *Rh. solani* in

307 seedlings, has been genetically modified for increased production of the antibiotic pyrrolnitrin  
308 (Ligon et al., 1996), by modifying or introducing an extra copy of the wild type global regulator  
309 gene *gacA*. The GacS/GacA two-component system globally exerts a positive effect at a  
310 posttranscriptional level on the production of extracellular metabolites required for the control of  
311 plant diseases in many pseudomonads (Haas and Keel, 2003). A strain of *Pseudomonas putida* has  
312 been genetically modified to have improved activity against soil-borne pathogens, carrying the *phz*  
313 or the *phl* biosynthetic gene loci and constitutively producing either phenazine-1-carboxylic acid  
314 (PCA) or DAPG (Bakker et al., 2002). Genes for the biosynthesis of antibiotics can be transferred  
315 to antagonistic strains extending the range of pathogens controlled. Transfer of the *phzH* gene of *P.*  
316 *chloraphis*, necessary for the biosynthesis of phenazine-1-carboxamide (PCN) and the control of  
317 *Fusarium oxysporum* f. sp. *radicis-lycopersici*, to the PCA-producing biocontrol strains *P.*  
318 *fluorescens* and *P. aureofaciens* enabled these strains to produce PCN instead of PCA and suppress  
319 tomato foot and root rot (Chin-A-Woeng et al., 2001).

320 Soil bacteria, called plant growth promoting-rhizobacteria (PGPR), are found to be beneficial to the  
321 plant. Indole-3-acetic acid (IAA) production by PGPR may play a role in the construction of short  
322 root systems and giving advantage of root colonization (Suzuki et al., 2003). The molecular  
323 mechanisms involved in the root colonization are under study nowadays. The *rpoS* gene in  
324 *Pseudomonas putida* was essential for plant root colonization under competitive conditions from  
325 other microbes (Miller et al., 2001). Camacho Carvajal et al. (2002) have characterized a NADH  
326 dehydrogenases of *Pseudomonas fluorescens* WCS365 and their role in competitive root  
327 colonization. These PGPR could be genetically modified to improve root colonization and,  
328 consequently, biocontrol of phytopathogens. The introduction of the *sss* colonization gene of the  
329 tomato-*Fusarium oxysporum* f. sp. *radicis-lycopersici* biocontrol strain *Pseudomonas fluorescens*  
330 into poor and good colonizer strains of *P. fluorescens* increased the competitive tomato root tip  
331 colonization ability several times, showing that improvement of the colonization ability by genetic  
332 engineering is a realistic goal (Dekkers et al., 2000). Moreover, avirulent mutants of *Ralstonia*

333 *solanacearum* have been used in the biocontrol of bacterial wilt in potato (Smith and Sadler, 2001).  
334 These strains containing transposon-induced insertions in the *hrp* gene (Frey et al., 1994) were able  
335 to invade the plant, survive and multiply within the plant excluding pathogenic strains.  
336 The role of siderophore production by biocontrol agents in the rhizosphere has been studied with  
337 molecular methods for many years (Haas, 2003). A strain of *Pseudomonas fluorescens* was  
338 genetically modified to utilize additional ferric siderophores (Moenne Loccoz et al., 1996).  
339 A new and challenging branch of genetic transformation of biocontrol agents could be the insertion  
340 of genes that improves tolerance of antagonist to abiotic stresses, such as genes for increased  
341 tolerance or resistance to cold, heat, drought, high salinity, heavy metal rich soils, or acid soils.  
342 Although dependent on political decisions, if genetically modified antagonists could be used  
343 unrestrictedly, various efficient combinations of plant pest resistance and biocontrol regimes could  
344 then be designed. A possible crop protection method could then be the creation of plant cultivars  
345 promoting survival and activity of specific BCAs.  
346 In order to release GM-biocontrol agents into the environment – at this stage there are great  
347 difficulties in registering GM-BCA – a careful evaluation of the potential risks associated should be  
348 carried out. Safety issues involve displacement of nontarget microorganisms, allergenicity or  
349 toxicity to humans and other animals, toxicity and pathogenicity of nontarget organisms, genetic  
350 and transgene stability (Cook et al., 1996; van Elsas and Migheli, 1999). In particular, genetic  
351 stability of both manipulated and wild-type bacteria and fungi is a key factor in their safe  
352 application. Care should be taken to ensure maximum stability of the transforming DNA within the  
353 host genome and to avoid horizontal gene transfer. The strain K1026 of *Agrobacterium radiobacter*,  
354 derived from the strain K84 producer of agrocin 84 and effective antagonist of *A. tumefaciens*, was  
355 designed with a deletion in the transfer region of the plasmid (Jones et al., 1988). The resulting  
356 strain is unable to transfer its genes giving immunity to agrocin 84. If new transgenic hypervirulent  
357 biocontrol agents are created, the prevention, containment, or mitigation of uncontrollable spread of  
358 such microorganisms becomes a urgent need. Gressel (2001) proposes a series of potential means,

359 such as asporogenic deletion mutants, transgenic suppression of melanization, terminator strategy or  
360 hypervirulence genes flanked with transgenetic mitigators.

361 Finally, the members of the APS Biological Control Committee asserted that significant investment  
362 in genomic analyses of biological control agents will lead to increased efficacy and application of  
363 biological control of plant pathogens and recently proposed a list of recommended BCAs for  
364 genomic sequencing.

365

## 366 **7. Mass production**

367 A critical factor that must be considered when selecting a BCA for commercial development is the  
368 availability of a cost-effective production and stabilization technology that yields an optimally  
369 effective form of the antagonist. More studies on the practical aspects of mass-production and  
370 formulation need to be undertaken to make new biocontrol products stable, effective, safer and  
371 more cost-effective (Fravel et al., 1999).

372 Major characteristics to market a biofungicide are the following (Agosin and Aguilera, 1998):  
373 abundant and cost-effective production of microbial propagules; ability to survive downstream  
374 process; stability and adequate shelf life of the final product upon storage, preferably without  
375 refrigeration; tolerance to environmental variations in temperature, desiccation, irradiation and  
376 relative humidity in order to survive and establish active populations in the soil; and consistent  
377 efficacy under varying field conditions at commercially feasible rates.

378 One of the most important reasons for the limited commercial diffusion of biofungicides is the high  
379 cost of production, due to the high cost of substrate, low biomass productivity, and limited  
380 economies of scale (Rhodes, 1996). The practical efficacy of a BCA greatly depends on the quality  
381 of the inoculant, itself a function of the production and formulation processes (Whipps, 1997).

382 Two methods are commonly used for producing inoculum of BCA's: liquid and solid fermentation.  
383 In submerged fermentation, specific parameters for aeration, temperature and pH control, carbon  
384 and nitrogen sources must be developed for each organism. Extra-cellular chitinase production by



385 the chitinolytic fungus *Trichoderma harzianum* using submerged fermentation was studied  
386 (Sandhya et al., 2004). Supplementation of additional carbon sources showed no further  
387 enhancement in chitinase production while supplementation of nitrogen sources such as peptone  
388 and tryptone in the fermentation medium showed a marked increase in production. Many BCAs are  
389 easily produced in liquid culture in lab scale, but when produced in large scale, they do not produce  
390 the expected quantity or quality of propagules, essentially for the low oxygen availability in  
391 fermenters. Some filamentous fungi need high oxygen transfer for growth and especially for  
392 sporulation.

393 The choice of the adequate propagule of the microorganism is important to provide the desired shelf  
394 life. Some bacteria are easily dried and can be provided as dry cells or formulated further. Survival  
395 stage structures of the organism, such as chlamydo spores, microsclerotia, ascospores, or endospores  
396 are generally preferred. Sporulating Gram-positive bacteria, such as *Bacillus* spp. and *Streptomyces*  
397 spp., offer endospores resistant to desiccation and heat that can be formulated readily into stable  
398 products, such as dry powder. Instead, Gram-negative bacteria, such as *Pseudomonas* spp., are  
399 generally formulated as frozen cell pellets that must be kept at low temperature until application,  
400 which remain a major obstacle for their large-scale use (Slininger et al., 1996).

401 If the microorganism is produced by liquid fermentation, it is necessary to reduce the volume of  
402 liquid or obtain a final dry formulation. Before drying, the microorganism propagules are separated  
403 by filtration or centrifugation. The strain Fo47 of *Fusarium oxysporum* has been produced in  
404 submerged fermentation, removing the growth medium by filtration and the propagules were mixed  
405 with talcum powder used as an inert carrier and then dried at 18-20°C for two days (Durand et al.,  
406 1989). Drying can also be accomplished by freeze-drying, atomization, or bed-fluid drying,  
407 preserving the inoculum for a long time with high viability (Beudeker et al., 1989). Rapid drying  
408 can cause cell membrane damage, particularly if heating is used to speed drying. For this reason, a  
409 glycerol-enriched medium was developed to produce high levels of desiccation-tolerant conidia of  
410 *Trichoderma harzianum* (Jin et al., 1996).

411 A biofungicide based on the K61 strain of *Streptomyces griseoviridis* is produced by fermentation  
412 followed by lyophilization. The commercial product contains at least  $10^8$  dormant spores/g and is  
413 stable for 12 months at temperatures inferior to  $8^\circ\text{C}$  (Tahvonen and Avikainen, 1987). Sarabatnam  
414 and Traquair (2002) demonstrated that vegetative propagules from actively growing filaments are  
415 acceptable inoculants for *Streptomyces* sp. formulations with better shelf life at  $4^\circ\text{C}$ . The final  
416 product is easy to apply by mixing it with nutrient solution delivered to plants in soilless cultures or  
417 by mixing with potting mixtures.

418 Solid-state fermentation mimics the natural environment conditions and habitat for some  
419 microorganisms (Kim et al., 1985). The moisture needed is found in the solid matrix in an adsorbed  
420 or complex form, with moisture ranging between 12 and 80%. Solid fermentation scaling, necessary  
421 for use on an industrial scale, raises engineering problems due to the build-up of temperature, pH,  
422  $\text{O}_2$ , substrate and moisture gradients. However solid fermentation possess several biotechnological  
423 advantages, among the other, higher fermentation productivity, higher end-concentration of  
424 products, higher product stability, and lower demand on sterility due to the low water activity  
425 (Holker et al., 2004). Larena et al. (2002) produced 250-fold more conidia of *Penicillium oxalicum*,  
426 antagonist of *F.oxysporum* f.sp. *lycopersici*, in solid than in liquid fermentation. Conidia produced  
427 in solid fermentation had a longer shelf life if stored at  $-20^\circ\text{C}$ .

428 Moreover, solid-state fermentation permits to save the labor and technical difficulties and generally  
429 does not need further formulation. This type of formulation is adapted for horticultural usage where  
430 it is mixed with potting mixture, but it does not enable application of the inoculant as a suspension  
431 in water. The strain Fo47 of *F. oxysporum* can be produced by solid-state fermentation either in  
432 sterilized peat or in calcinated clay, and be stored at  $4^\circ\text{C}$  or at room temperature without loss of  
433 density or activity (Olivain et al., 1999). Conidia of *Trichoderma harzianum* produced in aerial  
434 mycelium with solid-substrate fermentation persist longer under harsher environmental conditions  
435 than those produced under submerged culture conditions and wall thickness of aerial conidia is  
436 nearly twice that of submerged ones (Muños et al., 1995).

437 A new solid-state fermenter patented by Lüth and Eiben (2003) was used for *Coniothyrium minitans*  
438 spore production. A big advantage was the culture of the fungus under absolute axenic conditions.  
439 After 14 days of sterile fermentation - including *in situ* drying - the cereal grains used as a substrate  
440 were covered and filled with fungal spores. When the fermentation process is completed the conidia  
441 are separated from the culture medium using a micro-screen machine, that permits to have only the  
442 conidia of the processed fungus in the end product.

443

#### 444 **8. Formulation and methods of application**

445 Biological control has often been idealized as method for controlling plant diseases, as has  
446 happened for organic farming compared to the traditional cropping techniques. In organic farming  
447 the bottleneck is represented by keeping remunerative yield, lowering the selling price of the  
448 product. For biological control the major difficulty to reach the market and to be competitive with  
449 the chemical fungicides is represented by a consistent and reliable effectiveness and by the length of  
450 shelf life. Both problems can be faced with a scientific development of formulation of biocontrol  
451 agents. Until now, except for rare cases, formulation has been faced with an empirical approach  
452 without a methodology.

453 Obvious advantages of formulation include greater efficacy, increased shelf life, ease of handling,  
454 increased safety, lower production costs and compatibility with agricultural practices. Minuto et al.  
455 (1997) have compared different strains of antagonistic *Fusarium oxysporum* and different  
456 commercial formulations of these strains to control fusarium wilt of basil, concluding that the  
457 efficacy was strongly dependent on the formulation.

458 Formulation is dependent on the type of fermentation used. In the case of solid-state fermentation  
459 providing the carrier for the inoculum, it is not necessary to develop a sophisticated formulation  
460 process (Lewis, 1991).

461 The type of formulation desired depends on the intended use. For application to soilless cultures  
462 where the easiest way is to apply the inoculant through the drip irrigation system, a liquid

463 formulation would be preferred. A granular material would be more appropriate for combining with  
464 potting mix, while a wettable powder would be more appropriate for root dips or sprays. The  
465 application of *Coniothyrium minitans* follows one of two ways: either soil application to reduce the  
466 sclerotial inoculum-potential, or spore-sprays onto diseased plants or crop debris to sanitize the crop  
467 (de Vrije et al., 2001).

468 Often a biofungicide comprises many ingredients, such as carriers, diluents, bulking additives,  
469 membrane stabilizers, growth and contaminant suppressants, buffering systems, binders,  
470 dispersants, lubricants, activators, food sources and coating compounds, added for various  
471 purposes (Paau, 1998). These include keeping viability of antagonists, manipulating bulk for  
472 handling and delivery, promoting the activity of the BCAs, and arresting growth of potential  
473 contaminants.

474 More attention should also be devoted to the special requirements of BCAs in terms of application  
475 and delivery technology. Delivery must be easy, effective, timely, to the appropriate site of action,  
476 and compatible with available agricultural equipment. In contrast to chemical pesticides, they are  
477 living organisms, they range in size and are more susceptible to the actual conditions (temperature,  
478 humidity, pH) than chemical pesticides (Matthews, 2000). In general, most biocontrol agents are  
479 applied with the same equipment currently used to apply chemical pesticides (Mathre et al., 1999).

480

## 481 **9. Conclusions**

482

483 The history of biological control of plant pathogens is not so recent. Sandford in 1926 started  
484 biocontrol against potato scab, but research on this topic received a renewed impetus and attracted  
485 many scientists after the 1963 International Symposium held at Berkeley on Ecology of soilborne  
486 plant pathogens: prelude to biological control (Baker and Snyder, 1965; Baker and Cook, 1974).  
487 Despite many decades of research in biological control, biopesticides represent about 1% of the  
488 global pesticide market (Butt et al., 2001). BCAs still represent a specialized niche market, in spite

489 of a consensus view among the public, growers and regulators. BCAs are generally characterized by  
490 a narrow spectrum of activity, which is desirable from an environmental point of view, but not from  
491 a commercialization perspective. International agrochemical companies, characterized by large  
492 sales forces and substantial fixed costs, are not generally interested in scarcely remunerative niche  
493 products. Small enterprises, the portion of the production system normally interested in developing  
494 such a products, should have long term planning, because return from the initial costs is quite long.  
495 Biofungicides have to break into the existing agro-industrial complex: they might act differently  
496 than chemical pesticides in traditional testing situations largely built for chemical fungicides and  
497 they might need certain special arrangements for their application (Gerhardson, 2002).

498 Regulatory guidelines for biocontrol agents (BCAs) in many parts of the world are incompletely  
499 formulated or subject to frequent changes. Moreover, not all countries adopt a favorable and fast  
500 registration process for BCAs: in Europe as in the United States the small enterprises, which would  
501 develop products based on BCAs, should be prepared to deal with the complex procedure and high  
502 costs for registration of microbials (Hofstein et al., 1996; Harman, 2000).

503 Despite the difficulties encountered by the development of biofungicides, there are many reasons  
504 for being optimistic. Different strategies can be established to speed up the production,  
505 commercialization and acceptance of biocontrol agents and effectiveness of control. The use of  
506 microorganisms can play an important role in a more complex vision of crop protection, as key  
507 elements of IPM programs. Mixtures of antagonists, genetically modified antagonists, enhancement  
508 of production, formulation, and application technologies and procedures will be useful tools to  
509 accomplish this difficult and exciting task.

510

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515

516 **References**

517 Abd-El Moity, T.H., Papavizas, G.C., Shatla, N.N., 1982. Induction of new isolates of  
518 *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot on  
519 onion. *Phytopathology* 72, 396-400.

520 Agosin, E., Aguilera, J.M., 1998. Industrial production of active propagules of *Trichoderma* for  
521 agricultural uses. In: Harman, G.E., Kubicek, C.P. (Eds.), *Trichoderma & Gliocladium – Enzymes,*  
522 *biological control and commercial applications.* Taylor & Francis Ltd, London, Great Britain, pp.  
523 205-227.

524 Ahmad, J.S., Baker, R., 1987. Rhizosphere competence of *Trichoderma harzianum*.  
525 *Phytopathology* 77, 182-189.

526 Alabouvette, C., 1986. Fusarium wilt-suppressive soils from the Chateaufrenard region : review  
527 of a 10-year study. *Agronomie* 6, 273-284.

528 Alabouvette, C., Lemanceau, P., Steinberg, C., 1996. Biological control of fusarium wilts:  
529 opportunities for developing a commercial product. In: Hall, R. (Ed.), *Principles and Practice of*  
530 *managing soilborne plant pathogens,* APS Press, St Paul, United States, pp. 192-212.

531 Alabouvette, C., Schippers, B., Lemanceau, P., Bakker, P.A.H.M., 1998. Biological control of  
532 Fusarium wilts : toward development of commercial products. In: Boland, G.J., Kuykendall, L.D.  
533 (Eds.), *Plant-microbe interactions and biological control,* Marcel Dekker, New York, United States,  
534 pp.15-36.

535 Albajes, R., Gullino, M.L., Van Lenteren, J.C., Elad, Y., 1999. *Integrated Pest and Diseases*  
536 *Management in Greenhouse Crops.* Kluwer Academic Publishers, Dordrecht, Netherlands.

537 Aloï, C., Bergonzoni, P., Arteconi, M., Mallegni, C., Gullino, M.L., 1992. Biofox C: un  
538 prodotto biologico ad azione antagonistica di *formae speciales* di *Fusarium oxysporum*. *Atti*  
539 *Giornate Fitopatologiche* 1, 73-78.

540 Alvarez, B., Gagné, S., Antoun, H., 1995. Effect of compost on rhizosphere microflora of the

541 tomato and on the incidence of plant growth-promoting rhizobacteria. *Appl. Environ. Microbiol.* 61,  
542 194-199.

543 Baek, J.M., Howell, C.R., Kenerley, C.M., 1999. the role of an extracellular chitinase from  
544 *Trichoderma virens* Gv29-8 in the biocontrol of *Rhizoctonia solani*. *Curr. Genet.* 35, 41-50.

545 Baker, R., 1989. Some perspectives on the application of molecular approaches to biocontrol  
546 problems. In: Whipps, M., Lumsden, R.D. (Eds.), *Biotechnology of fungi for improving plant*  
547 *growth*. Cambridge Univ. Press, Cambridge, Great Britain, pp. 220-223.

548 Baker, K.F., Cook, R.J., 1974. *Biological control of plant pathogens*. Freeman W.H. and Co.,  
549 San Francisco, United States.

550 Baker, K.F., Snyder, W.C., 1965. *Ecology of soil-borne plant pathogens, prelude to biological*  
551 *control*. University of California Press, Berkeley, United States.

552 Bakker, P.A., Glandorf, D.C., Viebahn, M., Ouwens, T.W., Smit, E., Leeflang, P., Wernars, K.,  
553 Thomashow, L.S., Thomas-Oates, J.E., van Loon, L.C., 2002. Effects of *Pseudomonas putida*  
554 modified to produce phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol on the microflora  
555 of field grown wheat. *Antonie Van Leeuwenhoek* 81, 617-624.

556 Becker, D.M., Kinkel, L.L., Schottel, J.L., 1997. Evidence for interspecies communication and  
557 its potential role in pathogen suppression in a naturally occurring disease suppressive soil. *Can.J.*  
558 *Microbiol.* 43, 985-990.

559 Bell, C.H., Price, N., Chakrabarti, B., 1996. *The Methyl Bromide Issue*. John Wiley & Sons,  
560 New York, United States.

561 Beudeker, R.F., Van Dam, H.M., Van der Plaats, J.B., Vallenga, K., 1989. Development in  
562 baker's yeast production. In: Verachtert, H., De Mot, R. (Eds.), *A yeast: biotechnology and*  
563 *biocatalysis*, Marcel Dekker, New York, United States, pp. 103-147.

564 Boehm, M.J., Wu, T., Stone, A.G., Kraakman, B., Iannotti, D.A., et al., 1997. Cross-polarized  
565 magic-angle spinning <sup>13</sup>C nuclear magnetic resonance spectroscopic characterization of soil  
566 organic matter relative to culturable bacterial species composition and sustained biological control

567 of Pythium root rot. Appl. Environ. Microbiol. 63, 162-168.

568 Bolar, J.P., Norelli, J.L., Harman, G.E., Brown, S.K., Aldwinckle, H.S., 2001. Synergistic  
569 activity of endochitinase and exochitinase from *Trichoderma atroviride* (*T. harzianum*) against the  
570 pathogenic fungus (*Venturia inaequalis*) in transgenic apple plants. Transgenic Res. 10, 533-543.

571 Brent, K.J., Hollomon, D.W., 1998. Fungicide resistance: the assessment of risk. FRAC  
572 Monograph no. 2. CGPF, Brussels, Belgium.

573 Brown, J.S., Holden, D.W., 1998. Insertional mutagenesis of pathogenic fungi. Curr. Opin.  
574 Microbiol. 1, 390-394.

575 Butt, T.M., Jackson, C.W., Magan, N., 2001. Fungi as biocontrol agents – Progress, problems  
576 and potential. CABI Publishing, Wallingford, Great Britain.

577 Camacho Carvajal, M.M., Wijfjes, A.H., Mulders, I.H., Lugtenberg, B.J., Bloemberg, G.V.,  
578 2002. Characterization of NADH dehydrogenases of *Pseudomonas fluorescens* WCS365 and their  
579 role in competitive root colonization. Mol. Plant-Microbe Interact. 15, 662-671.

580 Chet, I., Elad, Y., Kalfon, A., Hadar, Y., Katan, J., 1982. Integrated control of soilborne and  
581 bulbborne pathogens in iris. Phytoparasitica 10, 229-236.

582 Chin-A-Woeng, T.F., Thomas-Oates, J.E., Lugtenberg, B.J., Bloemberg, G.V., 2001.  
583 Introduction of the *phzH* gene of *Pseudomonas chlororaphis* PCL1391 extends the range of  
584 biocontrol ability of phenazine-1-carboxylic acid-producing *Pseudomonas* spp. strains. Mol. Plant-  
585 Microbe Interact. 14, 1006-1015.

586 Cook, R.J., 1993. Making greater use of introduced microorganisms for biological control of  
587 plant pathogens. Annu. Rev. Phytopathol. 31, 53-80.

588 Cook, R.J., Bruckart, W.L., Coulson, J.R., Goettel, M.S., Humber, R.A., Lumsden, R.D.,  
589 Maddox, J.V., McManus, M.L., Moore, L., Meyer, S.F., Quimby, P.C., Stack, J.P., Vaugh, J.L.,  
590 1996. Safety of microorganisms intended for pest and plant disease control: a framework for  
591 scientific evaluation. Biol. Control 7, 333-351.

592 Davelos, A.L., Kinkel, L.L., Samac, D.A., 2004. Spatial variation in frequency and intensity of



593 antibiotic interactions among Streptomyces from prairie soil. Appl. Environ. Microbiol. 70, 1051-  
594 1058.

595 De Boer, M., Bom, P., Kindt, F., Keurentjes, J.J.B., van der Sluis, I., van Loon, L.C., Bajer,  
596 P.A.H.M., 2003. Control of Fusarium wilt of radish by combining *Pseudomonas putida* strains that  
597 have different disease-suppressive mechanisms. Phytopathology 93, 626-632.

598 Dekker, J., 1976. Acquired resistance to fungicides. Annu. Rev. Phytopathol. 14, 405-428.

599 Dekkers, L.C., Mulders, I.H., Phoelich, C.C., Chin-A-Woeng, T.F., Wijfjes, A.H., Lugtenberg,  
600 B.J., 2000. The sss colonization gene of the tomato-*Fusarium oxysporum* f. sp. *radicis-lycopersici*  
601 biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild-  
602 type *Pseudomonas* spp. bacteria. Mol. Plant-Microbe Interact. 13, 1177-1183.

603 De La Cruz, J., Hidalgo-Gallego, A., Lora, J.M., Benitez, T., Pintor-Toro, J.A., Llobell, A.,  
604 1992. Isolation and characterization of three chitinases from *Trichoderma harzianum*. Eur. J.  
605 Biochem. 206, 859-867.

606 De Vrije, T., Antoine, N., Buitelaar, R.M., Bruckner, S., Dissevelt, M., Durand, A., Gerlagh,  
607 M., Jones, E.E., Lüth, P., Oostra, J., Ravensberg, W.J., Renaud, R., Rinzema, A., Weber, F.J.,  
608 Whipps, J.M., 2001. The fungal biocontrol agent *Coniothyrium minitans*: production by solid-state  
609 fermentation, application and marketing. Appl. Microbiol. Biotechnol. 56, 58-68.

610 Dickman, M.B., 1988. Whole cell transformation of the alfalfa fungal pathogen *Colletotrichum*  
611 *trifolii*. Curr. Genet. 14, 241-246.

612 Downing, K.J., Thomson, J.A., 2000. Introduction of the *Serratia marcescens* chiA gene into an  
613 endophytic *Pseudomonas fluorescens* for the biocontrol of phytopathogenic fungi. Can. J.  
614 Microbiol. 46, 363-369.

615 Durand, A., Vergoignan, C., Almanza, 1989. Studies of the survival of *Fusarium oxysporum*  
616 Fo47. Eur. J. Plant Pathol. 104, 903-910.

617 Elad, Y., Hadar, Y., Chet, I., Henis, Y., 1982. Prevention, with *Trichoderma harzianum* Rifai  
618 aggr., of reinfestation by *Sclerotium rolfsii* Sacc, and *Rhizoctonia solani* Kuhn of soil fumigated

619 with methyl bromide, and improvement of disease control in tomatoes and peanuts. *Crop Prot.* 1,  
620 199-211.

621 Fogliano, V., Ballio, A., Gallo, M., Scala, F., Lorito, M., 2002. *Pseudomonas* lipodepsipeptides  
622 and fungal cell wall-degrading enzymes act synergistically in biological control. *Mol. Plant-  
623 Microbe Interact.* 15, 323-333.

624 Fravel, D.R., Rhodes, D.J., Larkin, R.P., 1999. Production and commercialization of biocontrol  
625 products. In: Albajes, R., Gullino, M.L., van Lenteren, J.C., Elad, Y. (Eds.), *Integrated Pest and  
626 Disease Management in Greenhouse Crops*. Kluwer Academic Publishers, Dordrecht, Netherlands,  
627 pp. 365-376.

628 Frey, P., Smith, J.J., Albar, L., Prior, P., Saddler, G.S., Trigalet-Demery, D., Trigalet, A., 1994.  
629 Bacteriocin typing of *Burkholderia (Pseudomonas) solanacearum* race 1 of the West French Indies  
630 and correlation of genomic variation of the pathogen. *Appl. Environ. Microbiol.* 62, 473-479.

631 Garcia, I., Lora, J.M., de la Cruz, J., Benitez, T., Llobell, A., Pintor-Toro, J.A., 1994. Cloning  
632 and characterization of a chitinase (CHIT42) cDNA from the mycoparasitic fungus *Trichoderma  
633 harzianum*. *Curr. Genet.* 27, 83-89.

634 Garibaldi, A., Brunatti, F., Gullino, M.L., 1987. Evaluation of several antagonists and different  
635 methods of application against *Fusarium* wilt of carnation. *EPPO Bull.* 17, 625-629.

636 Garibaldi, A., Guglielmone, L., Gullino, M.L., 1990b. Rhizosphere competence of antagonistic  
637 *Fusaria* isolated from suppressive soils. *Symbiosis* 9, 401-404.

638 Garibaldi, A., Gullino, M.L., 1991. Soil solarization in southern European countries, with  
639 emphasis on soilborne disease control of protected crops. In: Katan, J., DeVay, J.E. (Eds.), *Soil  
640 solarization*. CRC Press, Boca Raton, United States, pp. 227-235.

641 Garibaldi, A., Gullino, M.L., 1995. Focus on critical issues in soil and substrate disinfestation  
642 towards the year 2000. *Acta Hortic.* 382, 21-36.

643 Garibaldi, A., Gullino, M.L., Aloi, C., 1990a. Biological control of *Fusarium* wilt of carnation.  
644 *Proc. Brighton Crop Protection Conference – Pests and Diseases*, 19-22 November 1990, Brighton,

645 Great Britain, 3A-1, 89-95.

646 Garibaldi, A., Gullino, M.L., Mocioni, M., 1993. Researches on soils suppressive to root rot  
647 caused by *Pythium ultimum*. Meded. Fac. Landbouww. Rijksuniv. Gent 58, 1307-1314.

648 Garibaldi, A., Minuto, A., Grasso, V., Gullino, M.L., 2003. Application of selected antagonistic  
649 strains against *Phytophthora cryptogea* on gerbera in closed soilless systems with disinfection by  
650 slow sand filtration. Crop Prot. 22, 1053-1061.

651 Gerhardson B., 2002. Biological substitutes for pesticides. Trends in Biotechnology 20, 338-  
652 343.

653 Goldman, G.H., van Montagu, M., Herrera-Estrella, A., 1990. Transformation of *Trichoderma*  
654 *harzianum* by high-voltage electric pulse. Curr. Genet. 17, 169-174.

655 Grasso, V., Minuto, A., Garibaldi, A., 2003. Selected microbial strains suppress *Phytophthora*  
656 *cryptogea* in gerbera crops produced in open and closed soilless systems. Phytopathol. Mediterr. 42,  
657 55-64.

658 Gressel, J., 2001. Potential failsafe mechanisms against the spread and introgression of  
659 transgenic hypervirulent biocontrol fungi. Trends Biotechnol. 19, 149-154.

660 Gullino, M.L., 1992. Integrated control of diseases in closed systems in the sub-tropics. Pestic.  
661 Sci. 36, 335-340.

662 Gullino, M.L., Camponogara, A., Gasparrini, G., Rizzo, V., Clini, C., Garibaldi, A., 2003.  
663 Replacing Methyl Bromide for Soil Disinfestation: The Italian Experience and Implications for  
664 Other Countries. Plant Dis. 87,1012-1021.

665 Gullino, M.L., Garibaldi, A., 1994. influence of soilless cultivation on soil-borne diseases. Acta  
666 Hortic. 361, 341-354.

667 Gullino, M.L., Kuijpers, L.A.M., 1994. Social and political implications of managing plant  
668 diseases with restricted fungicides in Europe. Annu. Rev. Phytopathol. 32, 559-579.

669 Haas, D., Keel, C., 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas*  
670 spp. and relevance for biological control of plant disease. Annu. Rev. Phytopathol. 41, 117-153.

671 Haas, H, 2003. Molecular genetics of fungal siderophore biosynthesis and uptake: the role of  
672 siderophores in iron uptake and storage. *Appl. Microbiol. Biotechnol.* 62, 316-330.

673 Harman, G.E., 2000. Myths and dogmas of biocontrol: changes in perceptions derived from  
674 research on *Trichoderma harzianum* T-22. *Plant Dis.* 84, 377-393.

675 Harman, G.E., Björkman, T., 1998. Potential and existing uses of *Trichoderma* and *Gliocladium*  
676 for plant disease control and plant growth enhancement. In: Harman, G.E., Kubicek, C.P. (Eds.),  
677 *Trichoderma & Gliocladium – Enzymes, biological control and commercial applications.* Taylor &  
678 Francis Ltd, London, Great Britain, pp. 229-265.

679 Harman, G.E., Taylor, A.G., Stasz, T.E., 1989. Combining effective strains of *Trichoderma*  
680 *harzianum* and solid matrix priming to provide improved biological seed treatment systems. *Plant*  
681 *Dis.* 73, 631-637.

682 Hjeljord, L., Tronsmo, A., 1998. *Trichoderma* and *Gliocladium* in biological control: an  
683 overview. In: Harman, G.E., Kubicek, C.P. (Eds.), *Trichoderma & Gliocladium – Enzymes,*  
684 *biological control and commercial applications.* Taylor & Francis Ltd, London, Great Britain, pp.  
685 131-151.

686 Hofstein, R., Daoust, R.A., Aeschlimann, J.P., 1996. Constraints to the development of  
687 biofungicides: the example of „AQ-10”, a new product for controlling powdery mildews.  
688 *Entomophagia* 41, 455-460.

689 Hoitink, H.A.J., Boehm, M.J., 1999. Biocontrol within the context of soil microbial communities:  
690 a substrate dependent phenomenon. *Annu. Rev. Phytopathol.* 37, 427-446.

691 Hoitink, H.A.J., Inbar, Y., Boehm, M.J., 1991. Status of compost-amended potting mixes  
692 naturally suppressive to soilborne diseases of floricultural crops. *Plant Dis.* 75, 869-873.

693 Hoitink, H.A.J., Stone, A.G., Han, D.Y., 1997. Suppression of plant diseases by composts.  
694 *HortSci.* 32, 184-187.

695 Holker, U., Hofer, M., Lenz, J., 2004. Biotechnological advantages of laboratory-scale solid-  
696 state fermentation with fungi. *Appl. Microbiol. Biotechnol.* 64, 175-186.

697 Howell, C.R., Stipanovic, R.D., 1983. Gliovirin, a new antibiotic from *Gliocladium virens* and  
698 its role in the biological control of *Pythium ultimum*. *Can. J. Microbiol.* 29, 321-324.

699 Jarvis, W.R., 1989. Epidemiology of *Fusarium oxysporum* f.sp. *radicis-lycopersici*. In: Tjamos,  
700 E.C., Beckman, C. (Eds.). *Vascular wilt diseases of plants*, NATO ASI Series H28,  
701 Springer-Verlag, Berlin, Germany, pp. 397-411.

702 Jensen, D.F., Lumsden, R.D., 1999. Biological control of soilborne pathogens. In: Albajes, R.,  
703 Gullino, M.L., Van Lenteren, J.C., Elad, Y. (Eds.), *Integrated Pest and Diseases Management in*  
704 *Greenhouse Crops*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 319-337.

705 Jin, X., Taylor, A.G., Harman, G.E., 1996. Development of media and automated liquid  
706 fermentation methods to produce desiccation-tolerant propagules of *Trichoderma harzianum*. *Biol.*  
707 *Control* 7, 267-274.

708 Jones, D.A., Ryder, M.H., Clare, B.G., Farrand, S.K., Kerr, A., 1988. Construction of a Tra-  
709 deletion mutant of pAgK84 to safeguard the biological control of crown gall. *Mol. Gen. Genet.* 212,  
710 207-214.

711 Katan, J., 1996. Soil solarization: integrated control aspects. In: Hall, R. (Ed.), *Principles and*  
712 *practice of managing soilborne plant pathogens*, APS Press, St. Paul, United States, pp. 250-278.

713 Katan, J., 1999. The methyl bromide issue. Problems and potential solutions. *J. Plant Pathol.* 81,  
714 153-159.

715 Katan, J., DeVay, J.E., 1991. *Soil solarization*. CRC Press, Boca Raton, United States.

716 Kim, J.H., Hosobuchi, M., Kishimoto, M., Seki, T., Yoshida, T., Taguchi, H., Ryu, D.D.Y.,  
717 1985. Cellulase production by a solid state culture system. *Biotechnol. Bioeng.* 27, 1445-1450.

718 Kredics, L., Antal, Z., Manczinger, L., Nagy, E., 2001. Breeding of mycoparasitic *Trichoderma*  
719 strains for heavy metal resistance. *Lett Appl Microbiol.* 33, 112-116.

720 Kwok, O.C.H., Fahy, P.C., Hoitink, H.A.Y., Kuter, G.A., 1987. Interactions between bacteria  
721 and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media.  
722 *Phytopathology* 77, 1206-1212.

723 Larena, I., Melgarejo, P., De Cal, A., 2002. Production, survival, and evaluation of solid-  
724 substrate inocula of *Penicillium oxalicum*, a biocontrol agent against fusarium wilt of tomato.  
725 *Phytopathology* 92, 863-869.

726 Lemenceau, P., Bakker, P.A.H.M., De Kogel, W.J., Alabouvette, C., Schippers, B., 1993.  
727 Antagonistic effect on nonpathogenic *Fusarium oxysporum* strain Fo47 and pseudobactin 358 upon  
728 pathogenic *Fusarium oxysporum* f.sp. *dianthi*. *Appl. Environ. Microbiol.* 59, 74-82.

729 Lewis, J.A., 1991. Formulation and delivery systems of biocontrol agents with emphasis on  
730 fungi. In: Keister, D.L., Cregan, P.B. (Eds.), *The rhizosphere and plant growth*. Kluwer Academic  
731 Publishers, Dordrecht, Netherlands, pp. 279-287.

732 Ligon, J.M., Lam, S.T., Gaffney, T.D., Hill, D.S., Hammer, P.E., Torkewitz, N., 1996.  
733 Biocontrol: genetic modifications for enhanced antifungal activity. In: Stacey, G., Mullin, B.,  
734 Gresshoff, P.M. (Eds.), *Biology of plant-microbe interactions*. Int. Soc. Plant-Microbe Interact., St.  
735 Paul, United States, pp. 457-462.

736 Limon, M.C., Pintor-Toro, J.A., Benitez, T., 1999. Increased antifungal activity of *Trichoderma*  
737 *harzianum* transformants that overexpress a 33-kDa chitinase. *Phytopathology* 89, 254-261.

738 Locke, J.C., Marois, J.J., Papavizas, G.C., 1985. Biological control of Fusarium wilt of  
739 greenhouse-grown chrysanthemums. *Plant Dis.* 69, 167-169.

740 Lorito, M., Hayes, C.K., Di Pietro, A., Barman, G.E., 1993. Biolistic transformation of  
741 *Trichoderma harzianum* and *Gliocladium virens* using plasmid and genomic DNA. *Curr. Genet.* 24,  
742 349-356.

743 Lorito, M., Woo, S.L., Garcia, I., Colucci, G., Harman, G.E., Pintor-Toro, J.A., Filippone, E.,  
744 Muccifora, S., Lawrence, C.B., Zoina, A., Tuzun, S., Scala, F., Fernandez, I.G., 1998. Genes from  
745 mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. *Proc. Natl.*  
746 *Acad. Sci. U. S. A.* 95, 7860-7865.

747 Lucas, P., Smiley, R.W., Collins, H.P., 1993. Decline of *Rhizoctonia* root rot on wheat in soils  
748 infested with *Rhizoctonia solani* AG-8. *Phytopathology* 83, 260-265.

749 Lumsden, R.D., Lewis, J.A., Millner, P.D., 1983. Effect of composted sewage sludge on several  
750 soilborne plant pathogens and diseases. *Phytopathology* 73, 1543-1548.

751 Lumsden, R.D., Locke, J.C., 1989. Biological control of damping-off caused by *Pythium*  
752 *ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. *Phytopathology* 79, 361-  
753 366.

754 Lüth, P., Eiben, U., 2003. Solid-state fermenter and method for solid-state fermentation. United  
755 States Patent US 6,620,614 B1

756 Mathre, D.E., Cook, R.J., Callan, N.W. 1999. From discovery to use – Traversing the world of  
757 commercializing biocontrol agents for plant disease control. *Plant Dis.* 83, 972-983.

758 Matthews, G.A., 2000. Pesticide application methods. Blackwell Science Ltd., Oxford, Great  
759 Britain.

760 Miller, C.D., Kim, Y.C., Anderson, A.J., 2001. Competitiveness in root colonization by  
761 *Pseudomonas putida* requires the *rpoS* gene. *Can. J. Microbiol.* 47, 41-48.

762 Minucci, C., Migheli, Q., Gullino, M.L., 1991. Protoplast fusion for the production of superior  
763 strains of antagonistic *Fusarium* spp. In: Beemster, A.B.R., Bollen, G.J., Gerlagh, M., Ruissen,  
764 M.A., Schippers B., Tempel, A. (Eds.), *Biotic Interactions and soil-borne diseases*, Elsevier,  
765 Amsterdam, Netherlands, pp. 316-321.

766 Minuto, A., Migheli, Q., Garibaldi, A., 1995a. Evaluation of antagonistic strains of *Fusarium*  
767 spp. in the biological and integrated control of Fusarium wilt of cyclamen. *Crop Prot.* 14, 221-226.

768 Minuto, A., Migheli, Q., Garibaldi, A., 1995b. Integrated control of soil-borne plant pathogens  
769 by solar heating and antagonistic microorganisms. *Acta Hort.* 382, 173-182.

770 Minuto, A., Minuto, G., Migheli Q., Mocioni, M., Gullino, M.L., 1997. Effect of antagonistic  
771 *Fusarium* spp. and different commercial biofungicide formulations on Fusarium wilt of basil  
772 (*Ocimum basilicum* L.) *Crop Prot.* 16, 765-769.

773 Minuto, A., Spadaro, D., Garibaldi, A., Gullino, M.L., 2004. Control of soilborne pathogens of  
774 tomato using a commercial formulate of *Streptomyces griseoviridis* and solarization. *Crop Prot.*,

775 submitted.

776 Moenne Looco, Y., McHugh, B., Stephens, P.M., McConnel, F.I., Glennon, J.D., Bowling,  
777 D.N., Ogara, F., 1996. Rhizosphere competence of fluorescent *Pseudomonas* sp. B24 genetically  
778 modified to utilize additional ferric siderophores. FEMS Microbiol. Ecol. 19, 215-225.

779 Mondal, K.K., Chatterjee, S.C., Viswakarma, N., Bhattacharya, R.C., Grover, A, 2003.  
780 Chitinase-mediated inhibitory activity of Brassica transgenic on growth of *Alternaria brassicae*.  
781 Curr. Microbiol. 47, 171-173.

782 Mora, A., Earle, E.D., 2001. Combination of *Trichoderma harzianum* endochitinase and a  
783 membrane-affecting fungicide on control of *Alternaria* leaf spot in transgenic broccoli plants. Appl.  
784 Microbiol. Biotechnol. 55, 306-310.

785 Muñoz, G., Agosin, E., Cotoras, M., San Martín, R., Volpe, D., 1995. Comparison of aerial and  
786 submerged spore properties of *Trichoderma harzianum*. Curr. Genet. 32, 225-230.

787 Olivain, C., Steinberg, C., Dreumont, C., Stawiecki, K., Pouhair, D., Wadoux, P., Alabouvette,  
788 C., 1999. Production of a mixed inoculum of *Fusarium oxysporum* Fo47 and *Pseudomonas*  
789 *fluorescens* C7 in peat. In: Alabouvette, C. (Ed.), Second International Fusarium Biocontrol  
790 Workshop. INRA, Dijon, France.

791 Paau, A.S., 1998. Formulation of beneficial organisms applied to soil. In: Burges, H.D. (Ed.),  
792 Formulation of microbial biopesticides. Kluwer Academic Publishers, Dordrecht, Netherlands, pp.  
793 235-254.

794 Park, C.S., Paulitz, T.C., Baker, R., 1988. Biocontrol of *Fusarium* wilt of cucumber resulting  
795 from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium*  
796 *oxysporum*. Phytopathology 78, 190-194.

797 Paulitz, T.C., 1997. Biological control of root pathogens in soilless and hydroponic systems.  
798 HortScience 32, 193-196.

799 Paulitz, T.C., Bélanger, R.R., 2001. Biological control in greenhouse systems. Annu. Rev.  
800 Phytopathol. 39, 103-133.



801 Paultiz, T.C., Ahmad, J.S., Baker, R., 1990. Integration of *Pythium nuun* and *Trichoderma*  
802 *harzianum* isolate T-95 for the biological control of Pythium damping-off of cucumber. Plant Soil  
803 121, 243-250.

804 Penttilä, M., Nevalainen, H., Rättö, M., Salminen, E., Knowles, J., 1987. A versatile  
805 transformation system for the cellulytic filamentous fungus *Trichoderma reesei*. Gene 61, 155-164.

806 Postma, J., Luttikholt, A.J.G., 1993. Benomyl-resistant *Fusarium*-isolates un ecological studies  
807 on the biological control of *Fusarium* wilt in carnation. Netherlands J. Plant Pathol. 99, 175-188.

808 Rankin, L., Paulitz, T.C., 1994. Evaulation of rhizosphere bacteria for biological control of  
809 Pythium root rot of greenhouse cucumbers in hydroponic culture. Plant Dis. 78, 447-451.

810 Raupach, G.S., Kloepper, J.W., 1998. Mixtures of plant growth-promoting rhizobacteria  
811 enhance biological control of multiple cucumber pathogens. Phytopathology 88, 1158-1164.

812 Rhodes, D.J., 1996. Economics of baculovirus-insect cell production systems, Cytotechnol. 20,  
813 291-297.

814 Ristaino, J.B., Perry, K.B., Lumsden, R.D., 1991. Effect of solarization and *Gliocladium virens*  
815 on sclerotia of *Sclerotium rolfsii*, soil microbiota, and the incidence of southern blight of tomato.  
816 Phytopathology 81, 1117-1124.

817 Sabaratnam, S., Traquair, J.A., 2002. Formulation of a *Streptomyces* biocontrol agent for the  
818 suppression of Rhizoctonia damping-off in tomato transplants. Biol. Control 23, 245-253.

819 Sandhya, C., Adapa, L.K., Nampoothiri, K.M., Binod, P., Szakacs, G., Pandey, A., 2004.  
820 Extracellular chitinase production by *Trichoderma harzianum* in submerged fermentation. J Basic  
821 Microbiol. 44, 49-58.

822 Scher, F.M., Baker, R., 1982. Effect of *Pseudomonas putida* and a syntetic iron chelator on  
823 induction of suppressiveness to fusarium wilt pathogens. Phytopathology 72, 1567-1573.

824 Schnider, U., Keel, C., Blumer, C., Troxler, J., Défago, G., Haas, D., 1995. Amplification of the  
825 housekeeping sigma factor in *Pseudomonas fluorescens* CHA0 enhances antibiotic production and  
826 improves biocontrol abilities. J. Bacteriol. 177, 5387-5392.

827       Shipton, P.J., 1975. Take-all decline during cereal monoculture. In: Bruehl, G.W. (Ed.), Biology  
828 and control of soil-borne plant pathogens, APS Press, St. Paul, United States, pp. 137-144.

829       Sivan A., Chet I., 1993. Integrated control of fusarium crown and root rot of tomato with  
830 *Trichoderma harzianum* in combination with methyl bromide or soil solarization. Crop Prot. 12,  
831 380-386.

832       Slininger, P.J., Van Cauwenberge, J.E., Bothast, R.J., Weller, D.M., Thomashow, L.S., Cook,  
833 R.J., 1996. Effect of growth culture physiological state, metabolites, and formulation on the  
834 viability, phytotoxicity, and efficacy of the take-all biocontrol agent *Pseudomonas fluorescens* 2-79  
835 stored encapsulated on wheat seeds. Appl. Microbiol. Biotechnol. 45, 391-398.

836       Smith, J.J., Saddler, G.S., 2001. The use of avirulent mutants of *Ralstonia solanacearum* to  
837 control bacterial wilt disease. In: Jeger, M.J., Spence, N.J. (Eds.), Biotic interactions in plant-  
838 pathogen-associations. CABI Publishing, New York, United States, pp. 159-176.

839       Strashnow, Y., Elad, Y., Sivan, A., Chet, I., 1985. Integrated control of *Rhizoctonia solani* by  
840 methyl bromide and *Trichoderma harzianum*. Plant Pathol. 34, 146-151.

841       Sundheim, L., Poplawsky, A., Ellingboe, H., 1988. Molecular cloning of two chitinase genes  
842 from *Serratia marcescens* and their expression in *Pseudomonas* species. Physiol. Mol. Plant Pathol.  
843 33, 483-491.

844       Suzuki, S., He, Y., Oyaizu, H., 2003. Indole-3-Acetic acid production in *Pseudomonas*  
845 *fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. Curr.  
846 Microbiol. 47, 138-143.

847       Tahvonen, R., 1982. Preliminary experiments into the use of *Streptomyces* spp. isolated from  
848 peat in the biological control of soil and seedborne diseases in peat culture. J. Sci. Agric. Soc. Finl.  
849 54, 357-369.

850       Tahvonen, R., Avikainen, H., 1987. The biological control of seed-borne *Alternaria*  
851 *brassicicola* of cruciferous plants with powdery preparation of *Streptomyces* sp. J. Agric. Sci. Finl.  
852 59, 199-208.

853 Tjamos, E.C., 1992. Selective elimination of soilborne plant pathogens and enhancement of  
854 antagonists by steaming, sublethal fumigation and soil solarization. In: Tjamos, E.C., Papavizas,  
855 G.C., Cook, R.J. (Eds.), *Biological control of plant diseases: progress and challenges for the future*,  
856 Plenum Press, New York, United States, pp. 1-15.

857 Van Elsas, J.D., Migheli, Q., 1999. Evaluation of the risks related to the release of biocontrol  
858 agents active against plant pathogens. In: Albajes, R., Gullino, M.L., van Lenteren, J.C., Elad, Y.  
859 (Eds.), *Integrated pest and disease management in greenhouse crops*, Kluwer Academic Publishers,  
860 Dordrecht, Netherlands, pp. 377-393.

861 Van Os, E.A., Postma, J., 2000. Prevention of root diseases in closet soilless growing systems  
862 by microbial optimisation and slow sand filtration. *Acta Hort.* 532, 97-102.

863 Whipps, J. M., 1997. Developments in the biological control for soil-borne plant pathogens.  
864 *Adv. Bot. Res.* 26, 1-134.

865 White, J.G., Linfield, C.A., Lahdenpera, M.-L., Uoti, J., 1990. Mycostop – A novel biofungicide  
866 based on *Streptomyces griseoviridis*. *Proc. Brighton Crop Protection Conference – Pests and*  
867 *Diseases*, 19-22 November 1990, Brighton, Vol. 1, 221-226.

868 Zeilinger, S., 2004. Gene disruption in *Trichoderma atroviride* via *Agrobacterium*-mediated  
869 transformation. *Curr. Genet.* 45, 54-60.