Biocontrol of root and crown rot in tomatoes under greenhouse conditions using *Trichoderma harzianum* and *Paenibacillus lentimorbus*. Additional effect of solarization

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Abbreviations: Th650: Trichoderma harzianum 650
Pl629: Paenebacillus lentimorbus 629
MeBr: methyl bromide

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***Trichoderma harzianum* 650 (Th650) and *Paenebacillus lentimorbus* 629 (Pl629) selected earlier for their ability to control *Rhizoctonia solani, Fusarium solani* and *F. oxysporum in vitro*, were applied alone or combined with solarization (summer assay) and/or with methyl bromide (MeBr) (summer and winter assays) to a soil with a high inoculum level, for the control of tomato root rot caused by the complex *F. oxysporum* f. sp. *lycopersici* - *Pyrenochaeta lycopersici* - *Rhizoctonia solani.* Evaluations were also performed independently for root damage caused by *P. lycopersici*, and also for *R. solani* in the summer assay. MeBr decreased tomato root damage caused by the complex from 88.7% to 21.2% and from 78.4% to 35.7% in the summer and in the winter assay, respectively. None of the bio-controllers could replace MeBr in the winter assay, but Th650 and Pl629 reduced root damage caused by this complex in the summer assay. Treatments with bio-controllers were improved by their combination with solarization in this season. Independent evaluations showed that the positive control of Th650 towards *R. solani* and the lack of effect on *P. lycopersici* correlates well with the endochitinase pattern expressed by Th650 in response to these phytopathogens. Root damage caused by *R. solani* can be controlled at a similar level as it does MeBr in summer assays, thus representing an alternative to the use of this chemical fungicide for the control of this phytopathogen.**

Root and crown rot of tomatoes can be observed after attack by several phytopathogens, including*Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani.* In addition, corky root is developed after attack by *Pyrenochaeta lycopersici* (Campbell and Shishkoff, 1990). The presence of all these three pathogens is commonly found in the rhizosphere of tomato plants cultivated under greenhouse conditions and during the same season, in V Region - Chile. Under these circumstances, the term attack by the complex will be used rather than the name of the disease caused by *F. oxysporum* f. sp. *lycopersici* – *P. lycopersici* – *R. solani*.

The natural control of several phytopathogens is based on the presence of suppressive soils where several biocontrol microorganisms are detected, such as those belonging to *Trichoderma*, *Gliocadium*, *Pseudomonas* and *Bacillus* genera, among others (Weller et al. 2002; Montealegre et al. 2003; Guo et al. 2004; Huang et al. 2005). In Chile, the control of the complex already mentioned is accomplished through fumigation with MeBr. This chemical contaminates the environment, affects the ozone layer, destroys the soil microflora, and must be applied every season because of its null residual activity and the rapid re-colonisation of soils by the phytopathogens (Ristaino and Thomas, 1997; Pinkerton et al. 2002). Considering the difficulty to generate suppressive soils similar to the natural ones, the use of selected biocontrol microorganisms could provide an alternative to the use of chemical fungicides. *Trichoderma* spp. has proved to be useful in the control of phytopathogens affecting different crops (Chet and Inbar, 1994; Benítez et al. 2004). On the other hand, solarization constitutes an additional alternative to control soil-borne phytopathogens, because it is a process that employs solar radiation to heat soil producing temperatures that are detrimental to these type of pathogens (Eshel et al. 2000). Also, it has been recently used along with *T. harzianum*, in integrated disease management (Levy et al. 2004).

Our group has been working in biocontrol of several pathogens that infect tomato roots, selecting both bacteria and fungi with biocontrol activity (Reyes et al. 2000; Lespinasse et al. 2001; Montealegre et al. 2003). We have described the biocontrol of *R. solani* and of *F. solani* by an isolate of *P. lentimorbus* (Montealegre et al. 2003), and the biocontrol of *P. lycopersici* (Pérez et al. 2002), and of *R. solani* (Santander et al. 2003) by isolates of *T. harzianum.* Also, the effect of solarization on *F. oxysporum*, *R. solani* and *P. lycopersici* has been tested (Montealegre et al. 1996; Santander et al. 2003).Nevertheless, it is unknown whether the combination of selected native fungi (*T. harzianum*) and bacteria (*P. lentimorbus*) and the use of solarization are able to replace MeBr in the control of tomato root pathogens.

The present work reports the biocontrol activity of *T. harzianum* 650 and of *P. lentimorbus* 629 and solarization for the control of the complex. Selection of the biocontrol agents was done based on previous results on *R. solani* and *F. solani* (Escobar et al. 2004) and taking into account that each *Trichoderma* isolate behaves differentially when confronted to the same pathogen (Pérez et al. 2002).

MATERIALS AND METHODS

Microorganisms and culture conditions: Th650 and Pl629

The fungus was isolated from tomato monoculture suppressive soils. It was cultured on potato-dextrose-agar, PDA (DIFCO), for the obtention of conidia (Lu et al. 2004). These were used as inoculum for liquid cultures of the fungus for: a) its characterization in terms of the secretion of biocontrol enzymes; b) for obtention of the inoculum to be applied to tomato seeds and c) for formulations. The innoquity of this *Trichoderma* isolate on tomato seedlings was tested as follows: a) tomato seeds of variety 593 were covered with a sodium alginate based formulation containing Th650 (Montealegre and Larenas, 1997) and placed in speedlings containing a previously sterilized mixture of perlite: vermiculite = 1:1 (w/w); and b) tomato seedlings were transplanted to speedlings containing the mixture already mentioned plus 0.2 g of alginate pellets (1.1×10^5 cfu) per seedling placed in a single hole. The corresponding controls were run with seeds without treatment, and with seedlings placed in speedlings in the absence of alginate pellets. Seedlings were maintained

Table 1. Winter assay. Effect of Th650, Pl629 and MeBr on damage level caused by *P. lycopersici*, on % total damage of roots caused by the complex *F. oxysporum* f. sp. l*ycopersici – P. lycopersici - R. solani*, and in production of tomato plants.

Treatments	*Damage level P. lycopersici* [1]	% of total damage of roots caused by the complex *Rhizoctonia solani, F. oxysporum* f. sp. *lycopersici and P. lycopersici* [2]	Total Production (kg/plant)	1st quality fruits[3] (kg/plant)	% of 1st quality fruits in total plants of the assay
Control	3.8 a	78.4 a	1.3 b	0.9 a	61.0 a
Th650[4]	3.7 a	71.7 ab	1.9 a	1.2 ab	65.4 a
Pl629[5] + Th650	3.2 ab	65.5 abc	1.7 ab	1.1 ab	65.9 a
Pl629	3.2 ab	60.4 abc	1.7 ab	1.2 ab	72.7 a
CH_3Br[6] + Th650	2.7 bc	49.2 bcd	2.1 a	1.3 b	62.6 a
CH_3Br + Pl629	2.3 bc	45.1 cd	2.1 a	1.5 b	72.7 a
CH_3Br	2.2 c	35.7 d	1.9 a	1.2 ab	67.0 a

Different letters within the same column mean significant differences at $p \leq 0.05$

(1) Damage 0-5 (Campbell and Shishkoff, 1990).
(2) Percentage of total root damage caused by the complex *Rhizoctonia solani*, *F. oxysporum* f. sp. *lycopersici* and *P. lycopersici.*
(3) Harvest up to 3rd bunch in December 6, 2001.
(4) *Trichoderma harzianum* was applied to the soil 7 days before transplanting (1 g pellet (4.2×10^6 cfu/g pellet)/L soil). When used along with *P. lentimorbus*, it was applied to the soil at the time of transplanting.
(5) *Paenebacillus lentimorbus* application: 2 ml (5×10^9 cfu/ml)/seedling in the speedling, 10 days before transplanting.
(6) Applied as Metabromide 980 (98 MeBr : 2 chloropicrine) in a dosage of 75.5 g/m^2 before transplanting (July 15, 2001). Application: June 28, 2001 – July 3, 2001. Aeration until July 5, 2001.

under glasshouse at 15 - 25ºC with daily watering with the addition of fertilizers. Evaluations were done as described by Montealegre et al. (2003), further considering % emergence and rate of emergence for seed treatment; seedling mortality and dry weight (aerial and root portions) both for seed and seedling treatments. Parameters were checked after plants reached a development corresponding to four – five true leaves.

The bacterium was stored in tubes containing B King medium at 5ºC, or in flasks containing TSB (tryptone soy broth) plus glycerol at -21ºC (Raupach and Kloepper, 1998). It was cultured in B King medium for the obtention of the inoculum: a) to be used directly on the tomato plants or b) for formulation. The innoquity of this bacterial isolate towards tomato seedlings was established as described (Montealegre et al. 2003).

Characterization of Th650

Conidia from Th650 (1×10^6) were used to inoculate 200 mL of liquid Mandels medium using cell walls of *R. solani* or *F. oxysporum* or *P. lycopersici* as the sole carbon source, as described (Pérez et al. 2002). Supernatants from these cultures were used to characterize the isoenzymic pattern of hydrolytic enzymes involved in biocontrol (endochitinases, β-1,3-glucanases and proteases), secreted by this fungal isolate in response to the presence of cell walls of the different phytopathogens (Pérez et al. 2002). Briefly, native PAGE at pH 4.4 separated proteins (50 µg per lane) from supernatants of culture media. Endochitinase activity was visualised after incubating the polyacrylamide gel with an auxiliary 2% (w:v) agarose gel containing glycol chitin, and further incubation with 0.01% (w:v) fluorescent brightener 28. β-1,3-glucanase activity was visualised after incubating the gel with 1% (w:v) laminarin and developing bands of activity with 0.15% (w:v) triphenyltetrazolium. Protease activity was visualised after incubating an haemoglobin containing gel with Coomassie blue.

Treatments and evaluations: Treatments, solarization, evaluations of the assays, determination of the inoculum of phytopathogens found in soils

Treatments were done under commercial greenhouse conditions. They were done applying Th650 and Pl629 alone or combined with solarization and/or with methyl bromide in a soil previously selected on the basis of its high inoculum level content of *P. lycopersici*, of *R. solani* and of the complex *F. oxysporum* f. sp. *lycopersici* - *P. lycopersici* - *R. solani* in Quillota, V Region of Chile. Two assays were done during the seasons 2001 (winter assay) and 2001-2002 (summer assay) using commercial crops of the tomato variety 593, in four replicas each one containing 10 tomato plants.The biocontrol assay in winter included the following treatments: Control (no soil treatment); MeBr (Methylbromide plus chloropicrine (98:2 in %) using a dosage of 75.5 g/m^2); Th650 (1.0 g pellets containing *T. harzianum* isolate Th650 (4.2×10^6 cfu/g pellet) per litre of soil placed 5-10 cm depth in the row seven days before transplanting); Pl629 (2 mL of a *P. lentimorbus 629* suspension (5×10^9 cfu/mL) per seedling ten days before transplanting); MeBr + Th650; MeBr + Pl629; Th650 + Pl629. All treatments were applied in parallel.

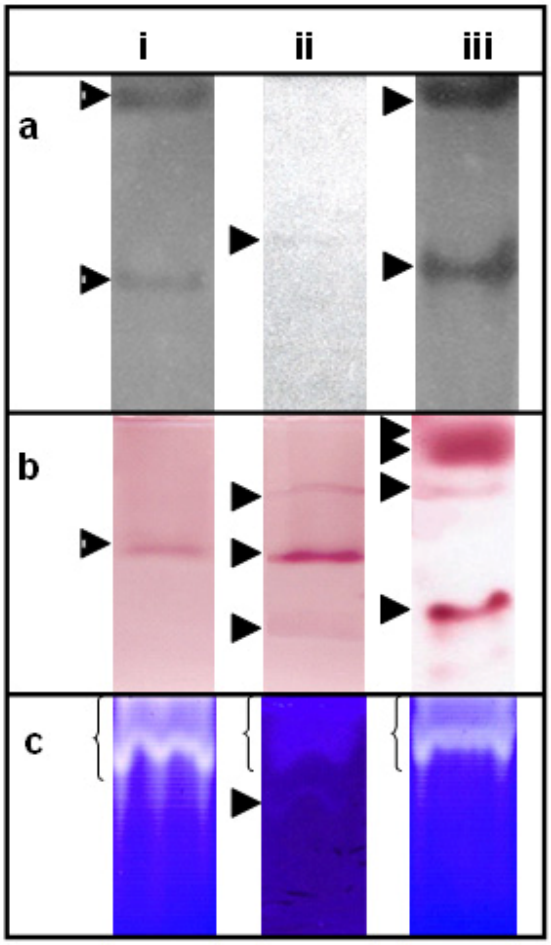

Figure 1. Native PAGE of extracellular enzymes secreted by *Trichoderma harzianum* isolate Th650 cultured in the presence of cell walls of: i) *F. oxysporum* f. sp. *lycopersici*, ii) *Pyrenochaeta lycopersici* or iii) *Rhizoctonia solani* as the sole carbon source.

(a) Endochitinases. Native PAGE at pH 4.4 separated proteins (50 µg per lane) from supernatants of culture media. Endochitinase activity was visualised after incubating the polyacrylamide gel with an auxiliary 2% (w:v) agarose gel containing glycol chitin, and further incubation with 0.01% (w:v) fluorescent brightener 28. Arrows show endochitinase activity.

(b) β-1,3-glucanases. Native PAGE at pH 4.4 separated proteins (50 µg per lane) from supernatants of culture media. β-1,3-glucanase activity was visualised after incubating the gel with 1% (w:v) laminarin and developing bands of activity with 0.15% (w:v) triphenyltetrazolium. Arrows show β-1,3-glucanase activity.

(c) Proteases. Native PAGE at pH 4.4 in haemoglobin containing gels, separated proteins (50 µg per lane) from supernatants of culture media. Protease activity was visualised after incubating the gel with Coomassie blue. Clear zones on the gel indicate proteolytic activity.

The biocontrol assay in summer included the following treatments: Control (no soil treatment); MeBr (Methylbromide plus chloropicrine (98:2 in %) using a dosage of 75.5 g/m^2); Th650 (1.0 g pellets containing *T. harzianum* isolate Th650 per litre of soil placed 5-10 cm depth in the row seven days before transplanting); Pl629 (2 mL of a *P. lentimorbus 629* suspension (5 x 10^9 cfu/mL) per seedling added 10 days before transplanting); Solarization; Solarization + Th650; Solarization + Pl629. All treatments were applied in parallel at the same time period, immediately after finishing solarization of soil.

MeBr was applied on June 28 - July 3, followed by aeration up to July 5, in the winter assay run on 2001; and on November 28, followed by aeration up to January 10, in the summer assay run on 2001-2002. Transplantation of tomato seedlings from speedlings to soil were done 7 - 10 days after aeration.

Solarization was performed between November 28, 2001 and January 10, 2002, which corresponds to late Spring – beginning Summer in Chile. Well prepared soil, moistened close to field capacity, was covered with a 40 micron thickness transparent polyethylene film. Temperature up to 10 cm depth was monitored during solarization. Inoculum was estimated before and after the procedure as described (Santander et al. 2003).

The evaluation of the assays (depending on the soil used for them) considered:

a) Root damage (corky root) caused by *P. lycopersici* (Campbell and Shishkoff, 1990);

b) Crown damage in % caused by *R. solani* (perimeter affected);

c) % total damage of roots caused by the complex *F. oxysporum* f. sp. *lycopersici* - *R. solani* - *P. lycopersici*;

d) Total yield;

e) First quality fruits.

Results were analysed using ANOVA and Duncan's tests at $p<0.05$. Percent values were transformed into Bliss degree as described (Rustom et al. 1989) for the statistic analysis.

The determination of the inoculum of phytopathogens found in soils was done as follows: soil samples were taken up to 10 cm depth. 1 g of soil was suspended in 99 mL sterile water or saline solution. After stirring, 0.1 mL of the supernatant was diluted to 100 mL, and 0.1 mL of the dilution was plated on selected media according to Singleton et al. (1992).

Formulations

Th650 was formulated as alginate pellets, as described (Montealegre and Larenas, 1997), reaching a concentration of 4.2 x 10^6 cfu/g pellets in assay 1 (winter) and of 570.000 cfu/g pellets in assay 2 (summer). Pl629 was formulated as described (Raupach and Kloepper, 1998).

RESULTS AND DISCUSSION

Characterisation of biocontrollers

Th650 grown in the presence of cell walls from the different phytopathogens of the complex showed: a) secretion of two chitinases, one β-1,3-glucanase and a wide band of protease activity in response to cell walls of *F. oxysporum* sp. *lycopersici*, b) secretion of one chitinase, three β-1,3-glucanases and high protease activity in response to cell walls of *P. lycopersici*, and c) two chitinases, four β-1,3-glucanases and high protease activity in response to cell walls of *R. solani* (Figure 1). Therefore, Th650 has the ability to secrete three different types of hydrolytic enzymes involved in biocontrol against the three phytopathogens tested. The differences observed both in isoenzymic patterns and levels of enzyme activity could reflect the ability to induce the expression of specific genes for the degradation of the polysaccharides found in the cell walls from the different phytopathogens tested, in response to the presence of their cell walls (Pérez et al. 2002). In fact, Th650 expressed and secreted two endochitinases towards *R. solani* and *F. oxysporum* one endochitinase towards *P. lycopersici*; four β-1,3-glucanase isoenzymes in response to the presence of cell wallsfrom *R. solani*, three in response to *P. lycopersici* and only one in to *F. oxysporum* (Figure 1).Also, the highest endochitinase and β-1,3-glucanase activities were observed in supernatants of Th650 cultures with *R. solani* cell walls as the sole carbon source. The secretion of chitinolytic enzymes and β-1,3-glucanases has been detected in the rhizosphere of soybean seedlings inoculated with *T. harzianum* and planted in a soil infested with *R. solani* demonstrating that *T. harzianum* was the source of these enzymes in response to the presence of *R. solani* (dal Soglio et al. 1998). These enzyme activities have been also detected in supernatants from other *T. harzianum* isolates in response to *R. solani* or *F. oxysporum* or *P. lycopersici* cell walls (Pérez et al. 2002), although isoenzymic patterns differ among isolates. The secretion of difusible and volatile antibiotics, previously demonstrated for Th650 (Escobar et al. 2004), along with the secretion of biocontrol enzymes, indicate that Th650 has the ability to use different biocontrol mechanisms (Benítez et al. 2004) against the phytopathogens already mentioned.

On the contrary, the secretion of antibiotics appears to be the most probable biocontrol mechanism of Pl629, similar to other antagonistic bacteria (Guo et al. 2004), because it is unable to secrete any of the biocontrol enzymes mentioned above (Montealegre et al. 2003). As opposed to Pl629, *Serratia* or *Bacillus cereus* include the secretion of chitinases within their biocontrol mechanisms (Ordentlich et al. 1988; Frankowski et al. 2001; Huang et al. 2005). From this point of view, the combined use of Th650 plus Pl629 would cover a wide spectrum of antagonistic mechanisms.

Innoquity test of Th650 on tomato plants

No lesions were observed in roots or in crowns of tomato plants, neither seed germination was affected by the presence of Th650, suggesting that this *Trichoderma* isolate is not pathogenic for tomato seeds or seedlings. A 100% emergence at day 7 was observed for all seeds, and no significant differences were observed related to mortality, dry weight of roots or aerial portion, crown diameter and seedling height. Based on these results, any negative effect of Th650 was discarded.

Biocontrol assay in winter

Control of root damage. The ability of Th650 and of Pl629 to decrease root damage caused either by *P. lycopersici* or by the complex of pathogens in the winter assay, is shown in Table 1. Analysis of soils showed that they contained 1.6 to 2.0 x 10^4 cfu/g soil of *P. lycopersici*, 5.3-6.3 x 10^4 cfu/g soil of *F. oxysporum* and 1.72-2.27 x 10^5 cfu/g soil of *R. solani*, which is considered a high inoculum. Treatments which included MeBr alone or MeBr plus either Th650 or Pl629 showed significant differences in reducing % root damage caused by *P. lycopersici* when compared to control; although no differences were observed among these three treatments suggesting that the effect is mainly due to MeBr. It was expected no root lesion in treatment with MeBr, but a 2.2 damage index was observed suggesting that re-colonisation of soil by *P. lycopersici* could have been produced. This agrees with previous studies that showed that re-colonisation occurred because of the biological void produced as a consequence of MeBr treatment (Montealegre et al. 1996). As the decrease in % root damage showed no significant differences when compared to MeBr alone, it is possible that none of the bioantagonists could prevent the re-colonisation by *P. lycopersici*. These results agree with the fact that BL629 does not secrete any enzyme system involved in bio-control, and that Th650 express only one chitinase with very low activity in response to the presence of this phytopathogen, which could be insufficient to control the development of *P. lycopersici* (Figure 1). Therefore, it appears that chitinases secreted by bio-controllers are important in preventing *P. lycopersici* development as well as tomato root damage, which agrees with the fact that a different *T. harzianum* isolate (isolate 11) accomplished an effective control of *P. lycopersici*, that correlates with a high production of chitinase activity expressed as multiple isoforms (Pérez et al. 2002). It is also important to mention that the % root damage observed in the presence of Th650 or of Pl629 alone or in combination among them, did not show significant differences from the control, suggesting that none of the antagonists could prevent the damage caused by *P. lycopersici*, as opposite to the findings of *in vitro* experiments (Pérez et al. 2002). In addition, it must be taken into account that according to optimal temperature development, *P. lycopersici* is the main pathogen found during winter season (Jones et al. 1991). In this season its development is higher than the one observed for *F. oxysporum* f. sp. *lycopersici* but similar to that of *R. solani*. On the other hand, optimal development

Table 2. Summer assay. Effect of Th650, Pl629, solarization and of MeBr on damage level caused by *P. lycopersici*, on % total damage of roots caused by *R. solani* and by the complex *F. oxysporum* f. sp. l*ycopersici* – *P. lycopersici* - *R. solani*, and in production of tomato plants.

Treatments	*Damage level P. lycopersici* (1)	% of total damage of roots caused by *R. solani or* the complex *R. solani*, *F. oxysporum* f. sp. *lycopersici and P. lycopersici* (2)		Total Production (kg/plant)	1st quality fruits(3) (kg/plant)	% of 1st quality fruits in total plants of the assay
		R. solani	Complex			
Control	3.9 a	63.8 a	88.7 a	1.77 ab	1.20 a	67.7 a
CH_3Br (4)	1.1 c	16.0 e	21.2 d	2.12 a	1.32 a	62.6 a
Th650 (5)	3.8 a	26.8 bcd	80.4 b	1.69 ab	1.21 a	71.0 a
Solarization	3.2 b	32.0 bc	66.9 c	2.08 ab	1.45 a	69.9 a
Solarization(6) + Th650	3.3 b	21.9 cde	75.8 bc	2.09 ab	1.40 a	67.9 a
Pl629(7)	3.5 ab	34.4 b	77.1 bc	1.65 b	1.08 a	65.9 a
Solarization + Pl629	3.1 b	17.3 de	68.6 c	2.14 a	1.48 a	68.7 a

Different letters within the same column mean significant differences at $p \leq 0.05$

(1) Damage 0-5 (Campbell and Shishkoff, 1990).
(2) Percentage of total root damage caused by the complex *Rhizoctonia solani*, *F. oxysporum* f. sp. *lycopersici* and *P. lycopersici*
(3) Harvest up to 3rd bunch in June 8, 2002.
(4) Applied as Metabromide 980 (98 MeBr : 2 chloropicrine) in a dosage of 75.5 g/m^2 before transplanting (January 17, 2002). Application: November 28, 2001. Aeration until January 10, 2002.
(5) *Trichoderma harzianum* was applied to the soil 7 days before transplanting (1 g pellet (4.2 x 10^6 cfu/g pellet)/L soil)
(6) Solarization (November 28, 2001 – January 10, 2002).
(7) *Paenebacillus lentimorbus* application: 2 ml (5 x 10^9 cfu/ml)/seedling in the speedling, 10 days before transplanting.

of Th650 is at 28ºC, and although it may grow at winter temperatures its development is much slower than the one observed for *P. lycopersici* (Escobar et al. 2004). In consequence, winter low temperatures favours the development of *P. lycopersici* over Th650. A similar situation could be happening for Pl629, whose optimal growth temperature is of 28ºC (Montealegre et al. 2003). Therefore, the lack of effect of Th650 or of Pl 629 could be attributed in part to the advantage in development of *P. lycopersici* over these two antagonists in the winter season, but also to a lack of ability of the biocontrollers to prevent *P. lycopersici* development because they are not expressing enzymatic effective bio-control mechanisms, whose presence in other *Trichoderma* isolates results in the control this tomato root pathogen (Pérez et al. 2002).

The % root damage produced by the presence of the complex of pathogens was significantly different when control was compared to treatments that contained MeBr. The treatment with this latter compound was not improved by the addition of Pl629 or by Th650, suggesting that the decrease in root damage was due to the presence of MeBr rather than to the presence of the bacterial or the fungal antagonist. As in the previous case, the advantage in development of *P. lycopersici* and *R. solani* in the winter season could explain results in terms of root damage. Based on the results obtained in this winter assay, we decided to omit treatments that included the combined use of Th650 and Pl629, MeBr and Th650 and MeBr and Pl629 in the summer assay.

Fruit yield and quality. When this assay was evaluated in terms of yield and fruit quality, significant differences were observed among treatments that included MeBr, or Th650 and control in terms of total yield per plant in the winter season (Table 1). In these conditions, Th650 was as good as MeBr alone, suggesting that although it is not controlling root damage it is improving yield as compared to control. This could be due to a yield promoting activity of *Trichoderma* species, similar to that described for a growth promoting activity of these fungi (Baker et al. 1984). Combination of this fungus and MeBr did not improve statistically the effect of any of them. Replacement of Th650 by Pl 629 neither improved total yield.

First quality fruits produced after any of the treatments were not different from controls, with the exception of MeBr $\pm$ Th650 or Pl629. These results could be explained as a result of the growth promoting activity of *Trichoderma* and *Paenebacillus* (Benítez et al. 2004; Guo et al. 2004). Finally, the presence of these bio-controllers is not affecting the % of first quality fruits.

Biocontrol assay in summer

Control of root damage. When the assay was performed in the summer season, we could include solarization in addition to some of the treatments performed in winter. Percent root damage caused by *P. lycopersici* or *R. solani* or the complex is shown in Table 2.

Root damage of tomato plants caused by *P. lycopersici* in the summer season was similar to that observed in the winter season assay. Treatment with MeBr resulted in less root damage being also the most effective treatment, suggesting that fumigation with MeBr was favoured by the soil temperature. Solarization also reduced significantly root damage caused by this pathogen. During solarization, soil temperature reached a mean of 32ºC, with maximum temperatures at 41.7ºC, as compared to controls where a mean of 28ºC was observed with a maximum value of 30.9ºC. The differences in the temperatures reached could explain the benefits of solarization, due to its deleterious effect on the soil-borne pathogens (Montealegre et al. 1996).

As in the winter season assay, Th650 did not show any biocontrol activity against *P. lycopersici* although the soil temperature during the summer assay was not limiting fungal development. Pl629 alone behaved as Th650 alone, and combination of both bio-controllers with solarization did not improve the effect of the latter treatment. Taking these results altogether, it appears that the lack of biocontrol of Th650 and of Pl629 is not due to a seasonal effect but to their lack of capacity to express effective biocontrol mechanisms on *P. lycopersici.* On the contrary, both Th650 and Pl629 prevented root damage caused by *R. solani* when applied alone or after solarization. Expression of chitinases, β-1,3-glucanases and proteases by Th650 in terms of number of isoenzymes and level of activity (Figure 1) is clearly better towards *R. solani*, fact that could account for the ability of Th650 to control root damage produced by this pathogen. On the other hand, although Pl629 does not secrete these enzyme systems, it can produce antibiotics that may be affecting *R. solani* development, or improving root development as has been described for other growth-promoting bacteria (Guo et al. 2004). Solarization alone significantly decreased root damage produced by *R. solani*, and when combined with Pl629 showed better results than the treatment alone. Therefore, it may be suggested that this bacterium might be controlling the re-colonisation of soil by *R. solani* after solarization, as opposite to what occurs for *P. lycopersici*. The addition of Th650, although decreased the % of total damage, was not significantly different from solarization alone. Finally, although fumigation with MeBr was statistically different from solarization alone, when Th650 or Pl629 were used in combination with solarization, results were comparable to those obtained with MeBr, thus constituting good alternatives to the use of MeBr for the control of *R. solani*.

The % root damage caused by the complex in the summer season was significantly reduced by MeBr (Table 2) as was observed in the winter season (Table 1). The summer assay also showed that solarization was effective in controlling root damage produced by the complex, but its effect could not be compared to the use of MeBr. The weakening and delayed mortality of *F. oxysporum* already described (Assaraf et al. 2002) could be contributing to control the root damage already mentioned. The use of Th650 or of Pl629 decreased % total damage, effect that could be attributed to an effective action of the bio-controllers on *R. solani*. Th650 was less efficient than solarization, and Pl629 showed no significant differences with solarization in reducing % root damage. The combined use of Pl629 or Th650 with solarization did not improve the effect of the biocontrollers or solarization alone.

Fruit yield and quality. The treatments used for the summer season assay did not result in significant differences either in fruit yield or in fruit quality (Table 2). Moreover, MeBr alone was not better than the other treatments when total yield or first fruit quality were evaluated.

Taken together the results of both assays, it may be concluded that Th650 and Pl629 do not express bio-control mechanisms enough to prevent *P. lycopersici* development, but appear to be good alternatives to the use of MeBr for the control of *R. solani*. Solarization prevents root damage due to the presence of *P. lycopersici* or *R. solani* or of the complex; nevertheless, this prevention did not result in improvement of fruit yield or quality. In addition, solarization could be improved by the addition of Th650 or Pl629 for the control of *R. solani*.

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