



## Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress Fusarium wilt of tomato

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Received 2 November 2000; received in revised form 24 October 2001; accepted 5 November 2001

### Abstract

It has been reported that plant growth media amended with composted bark suppress Fusarium wilts whereas media amended with composted municipal sludge aggravate this disease. However, in this study, a compost prepared from vegetable and animal market wastes, sewage sludge and yard wastes showed a high ability to suppress Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* race I. The ability of this compost to suppress Fusarium wilt of tomato was compared with that of a peat mix (peat:vermiculite, 1:1 v/v) and a naturally suppressive soil from Chateaufort, France. The compost and the soil from Chateaufort were highly suppressive, whereas the peat mix was highly conducive. Amendment with this compost significantly ( $P < 0.05$ ) increased the suppressiveness of the peat mix. Biotic and abiotic properties were compared among these substrates. The peat mix was acidic, and had a low EC, whereas the compost was basic and a high EC. The compost–peat mix had a similar pH to the compost, however EC was approximately half that of the compost. The bacterial populations and microbial activity were highest in the compost and the compost–peat mix. Compost (10%; v/v), *Trichoderma asperellum* isolates isolated from natural compost–peat mix, and the nonpathogenic biocontrol agent *F. oxysporum* Fo47 isolated from Chateaufort soil were inoculated into sterilized compost–peat mix and Chateaufort soil to assess their ability to restore suppressiveness in the sterilized substrates. Both the natural compost and the *T. asperellum* isolates significantly ( $P < 0.05$ ) increased the suppressive ability of sterilized compost–peat mix and Chateaufort soil. Fo47 was relatively the most effective biocontrol agent. The incidence of Fusarium wilt was lowest in tomato plants grown in either sterilized compost–peat mix or Chateaufort soil inoculated with this strain. Our results show that the use of some composted sewage sludge in the plant growth medium is effective for suppression of Fusarium wilt at the early stage of plant growth. In addition, the *T. asperellum* isolates isolated from the suppressive compost–peat mix appear to have the potential to be a new alternative of biocontrol of Fusarium wilt. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Biological control; Chateaufort soil; Compost; *Fusarium oxysporum*; Peat; *Trichoderma asperellum*

### 1. Introduction

Soils and potting media may have a natural ability to reduce the incidence of plant diseases. This quality of such substrates is referred to as disease suppression and may be achieved by either limiting growth or saprophytic survival of pathogen, expression of the disease, or both. In most cases, suppression is based mainly on interactions between the pathogen and some populations of the saprophytic microflora. Such interactions are influenced by the abiotic characteristics of the soil (Alabouvette, 1999). Microbial communities contribute to the suppression through all four principal mechanisms of biological

control: competition, antibiosis, parasitism/predation and induced systemic resistance (Hoitink and Boehm, 1999).

Composts prepared from heterogeneous wastes and used in container media or as soil amendments may have highly suppressive effects against diseases caused by a variety of soilborne plant pathogens such as *Pythium* spp. (Mandelbaum and Hadar, 1990; Pascual et al., 2000), *Phytophthora* spp. (Hoitink and Boehm, 1999; Widmer et al., 1999), *Rhizoctonia* spp. (Kuter et al., 1983; Tuitert et al., 1998) and *Fusarium* spp. (Chef et al., 1983; Trillas-Gay et al., 1986). The physical and chemical properties and the presence of beneficial microorganisms account for the suppressive effects of such composts (Hoitink et al., 1993). The degree of decomposition of the organic matter critically affects the composition of bacterial taxa as well as the populations and activities of biocontrol agents. Therefore it is a key factor in disease suppression (Hoitink and Boehm, 1999).

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Utilization of suppressive soils and container media also provides a potential tool for management of *Fusarium* wilts. These diseases cause severe losses on many crops, especially in high temperature regions, and are difficult to control. The ability of some soils to suppress *Fusarium* wilts has been studied extensively (Louvet et al., 1981; Alabouvette, 1986). Several microorganisms have been shown to cause or contribute to the suppressive effect of various soil systems to *Fusarium* wilts, most notably populations of nonpathogenic strains of *Fusarium oxysporum* and fluorescent *Pseudomonas* spp. (Kloepper et al., 1980; Lemanceau et al., 1992; Larkin et al., 1996; Duijff et al., 1999). Abiotic properties such as the nature of clays and pH interact with these microbial populations that support suppressiveness (Alabouvette, 1999). Among isolates of nonpathogenic *F. oxysporum*, Fo47, isolated from a suppressive soil from Chateaufort, France, has been shown to effectively suppress *Fusarium* wilt of tomato (Larkin and Fravel, 1998; Fuchs et al., 1999). Competition for nutrients seems to be a major mechanism of biological control for this strain (Larkin and Fravel, 1999) even though competition for colonization of the root surface and tissues (Eparvier and Alabouvette, 1994; Olivain and Alabouvette, 1997; Duijff et al., 1999) and induced systemic resistance (Fuchs et al., 1997; Duijff et al., 1998) may also be involved.

Biotic and abiotic factors also play a role in suppression of compost-amended substrates to *Fusarium* diseases (Chef et al., 1983; Ueda et al., 1990). Bark composts, with high C/N ratio, immobilize nitrogen and suppress *Fusarium* wilt. However, composted sewage sludge, which usually presents low C/N ratio and releases ammonia, aggravates *Fusarium* wilt diseases (Tayama, 1987). Several microorganisms isolated from compost such as strains of *Trichoderma hamatum*, have been reported to effectively suppress diseases caused by *F. oxysporum* (Trillas-Gay et al., 1986; Mousseaux et al., 1998). Isolates of several *Trichoderma* spp. are effective antagonists for control of plant pathogens in composted bark amended substrates (Hoitink and Fahy, 1986). These antagonistic interactions with other fungi typically have been classified as based on antibiosis, mycoparasitism and competition for nutrients (Hjeljord and Tronsmo, 1998). Once established, some *Trichoderma* isolates can compete and colonize potential infection courts (Hjeljord and Tronsmo, 1998). Others induce systemic resistance in plants (Zhang et al., 1997).

In this report we present a short bioassay (25–30 days) to evaluate the ability of container media to suppress *Fusarium* wilt of tomato. The bioassay was used to compare the suppressive effect of a compost prepared from sewage sludge and a compost-amended mix, with that of a sphagnum peat mix and that of Chateaufort soil suppressive to *Fusarium* wilt (Alabouvette, 1986). We also assessed the ability of two selected groups of *Trichoderma asperellum* (TI and TII) isolated from a suppressive compost-amended mix to induce suppression against *Fusarium* wilt of tomato in a sterilized conducive compost-amended mix and in a soil

system. The ability of these isolates was compared to that of the nonpathogenic *F. oxysporum* Fo47 and to a 10% compost amendment.

## 2. Materials and methods

### 2.1. Physical and physico-chemical properties of the substrates

A commercially available compost (Metrocompost, Castelldefels, Barcelona), made with vegetable and animal market wastes, sewage sludge and yard wastes in a tunnel system (COMPOTUNEL<sup>®</sup>) was used in this study. The chemical properties of this compost are shown in Table 1. A compost–peat mix was formulated with this compost (compost:peat:vermiculite, 2:1:1 v/v). The suppressive ability of the compost and the compost–peat mix were compared to that of a sphagnum peat mix (peat:vermiculite, 1:1 v/v) and to that of the soil from Chateaufort. Physical and physico-chemical properties of the substrates are presented in Table 2. Chemical properties of the peat and the peat–vermiculite mix are described in Ansorena (1994), and those for the Chateaufort soil in Edel et al. (2001). Electric conductivity (EC) and pH were determined in a water extract (2:1; v/v), real density was determined from ash percentage (Ansorena, 1994) and bulk density, total porous space, and water-release curves were determined by the standard method described in De Boodt et al. (1974).

### 2.2. Biological properties of the substrates

Total culturable bacterial and fungal populations in the compost, the peat mix, the peat and the compost–peat mix were determined by the serial dilution technique. Dilutions were plated on Yeast Peptone Glucose Agar adjusted to pH 7.5 with KOH for bacteria (Serra-Wittling et al., 1996), and on modified Malt Agar (malt extract 10 g l<sup>-1</sup>, chlortetracycline 50 mg l<sup>-1</sup>, Tergitol NP-10 1 ml l<sup>-1</sup>) for fungi. Petri dishes were incubated at room temperature and colonies counted after five days.

Microbial activity in the compost, the peat mix and the compost–peat mix was measured using the rate of hydrolysis of fluorescein diacetate (FDA) as described by Inbar et al. (1991). However, FDA activity was expressed as µg FDA hydrolyzed per minute and per unit of volume of substrate because of differences in bulk density among the substrates. Microbial activity of peat alone could not be measured by this technique. Standard curves and the rate of hydrolysis of FDA were performed using 10-ml samples. Background absorbance was corrected for each treatment with the control sample. Before determining the microbiological activity, all the substrates were incubated at a water tension of 1 KPa (adjusted in a weight basis) for one week at room temperature.

Table 1  
Chemical properties of the compost used in bioassays

C/N	N-NH <sub>4</sub> ( $\mu\text{g g}^{-1}$ )	N org (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (%)	Zn ( $\mu\text{g g}^{-1}$ )	Mn ( $\mu\text{g g}^{-1}$ )	Cu ( $\mu\text{g g}^{-1}$ )	Ni ( $\mu\text{g g}^{-1}$ )	Cr ( $\mu\text{g g}^{-1}$ )	Pb ( $\mu\text{g g}^{-1}$ )	Cd ( $\mu\text{g g}^{-1}$ )
15	290	2.47	1.09	0.6	6.5	0.5	0.22	0.57	598	137	274	79	101	94	1

Table 2  
Physical and physico-chemical properties of substrates used in bioassays

	PH (water)	EC <sup>a</sup> (mS cm <sup>-1</sup> )	Bulk density (g ml <sup>-1</sup> )	Real density (g ml <sup>-1</sup> )	Ash (%)	Porous Space (%vol)	EAW <sup>b</sup> (%vol)	WBC <sup>c</sup> (%vol)	DAW <sup>d</sup> (%vol)	AC <sup>e</sup> (%vol)
Compost	7.4	2.50	0.28	1.82	39.6	84.5	7.0	1.7	39.1	36.7
Peat mix	4.0	0.15	0.11	2.05	59.1	94.6	12.8	6.0	36.8	39.0
Compost-peat mix	7.0	1.81	0.20	1.88	49.0	89.4	13.5	3.5	38.4	33.4
Heat-treated	7.5	1.06	0.23	1.90	48.8	87.9	23.2	7.1	32.7	24.9

<sup>a</sup> Electrical conductivity.

<sup>b</sup> Easy Available Water: percent of water volume retained at tensions between 0.1 and 0.5 KPa per volume of substrate.

<sup>c</sup> Water Buffer Capacity: percent of water volume retained at tensions between 0.5 and 1 KPa per volume of substrate.

<sup>d</sup> Difficult Available Water: percent of water volume retained at tensions over 1 KPa per volume of substrate.

<sup>e</sup> Air Capacity: percent of air filled porosity at tension 0.1 KPa per volume of substrate.

### 2.3. Fungal strains and inoculum preparation

Two strains of *F. oxysporum* were used in this study: a pathogenic strain of *F. oxysporum* f.sp. *lycopersici* race 1 (FOL) and the non pathogenic isolate Fo47 isolated from the suppressive Chateaufrenard soil (Alabouvette et al., 1987). Erlenmeyer flasks containing 100 ml of liquid Malt Extract ( $10 \text{ g l}^{-1}$ ) were inoculated with conidial suspensions prepared from 7-day-old-cultures on Malt Agar. Liquid cultures were grown for 5 days at  $25^\circ\text{C}$  on a rotary shaker. Conidia were recovered by centrifugation ( $6000g$ , 20 min) and rinsed twice with sterile distilled water. Inoculum concentration was determined by measuring the absorbance at 600 nm.

Talc inoculum of FOL was produced by mixing a liquid inoculum with sterilized talc powder (1/3 conidia suspension, 2/3 talc, v/w). The talc–inoculum preparation was dried under sterile conditions in a laminar flow cabinet, sieved ( $200 \mu\text{m}$ ) and stored at  $4^\circ\text{C}$  (Tello-Marquina and Alabouvette, 1985). The inoculum concentration of talc was determined by the serial dilution technique on Potato Dextrose Agar (PDA Sigma–Aldrich).

Five *Trichoderma* spp. were isolated from the suppressive compost–peat mix by the serial dilution technique on modified PDA (PDA; chlortetracycline,  $50 \text{ mg l}^{-1}$ ; tergitol NP-10,  $1 \text{ ml l}^{-1}$ ; Larkin et al., 1996). Colonies of *Trichoderma* spp. were identified by stereomicroscopy (Rifai, 1969). Molecular characterization of *Trichoderma* isolates was performed from single-spore cultures. Primers ITS1 and ITS2 described by White et al. (1990) were used for amplifying and sequencing the internal transcribed spacer 1 (ITS1), adjacent to 5.8S rDNA gene. PCR amplification and sequencing conditions are described in Hermosa et al. (2000). Two different ITS1 sequences were identified among the five isolates of *Trichoderma* spp. studied. Two isolates had the ITS1 sequence accession number AJ278564 (EMBL Nucleotide Sequence Database), and were grouped as TI isolates. The other three isolates had the ITS1 sequence accession number AJ278565 (EMBL Nucleotide Sequence Database), and were grouped as TII isolates. Both ITS1 sequences are similar to those of *T. asperellum* described by Gams and Meyer (1998). A patent application has been filed for one of the isolates belonging to the TII group (T34(2), CECT No. 20417).

For the liquid inoculum of *T. asperellum*, every isolate was grown separately in Petri dishes on Malt Agar for 7 days. Sterile water (2 ml) was added to every culture and the surface was scraped to obtain a conidial suspension. The concentration was determined by counting in hemacytometer. An inoculum suspension was prepared for each of the five isolates.

### 2.4. Bioassays

Substrates were inoculated with FOL race 1, mixed vigorously, and poured in pots (9 cm diameter, 330 ml volume).

Two inoculum densities ( $5 \times 10^4$  and at  $5 \times 10^5 \text{ cfu cc}^{-1}$  of substrate) were assessed. Four tomato seedlings (2–3 true leaf stage) produced in vermiculite were transplanted into each pot with infested substrate. Pots then were placed in a growth chamber ( $25 \pm 2^\circ\text{C}$ , 16 h light and  $150\text{--}210 \mu\text{E m}^{-2} \text{ s}^{-1}$  PAR intensity) and irrigated on a rotating basis with distilled water or a complete nutrient solution every other day until day 20, and daily thereafter.

To determine the role of natural microflora in the suppressiveness of each substrate, the same procedure described before was followed with heated ( $60^\circ\text{C}$ , 9 days) or sterilized (autoclaved  $121^\circ\text{C}$ , 1 h, three consecutive days) compost–peat mix and Chateaufrenard soil.

Disease severity was determined every two days beginning when wilt symptoms first appeared on tomato plants, based on a symptom severity scale where: 0 = asymptomatic plants; 1 = weakly infected plants (<50% of leaves chlorotic or wilted); 2 = highly infected plants (>50% of leaves wilted but plants not dead) and 3 = dead plants. The following characteristics of tomato plants corresponding to each class were measured also: fresh biomass of the aerial portion, percent moisture content, length of stem and percentage of stem showing signs of the pathogen. The signs of the pathogen in the stem were determined by cutting the stem and measuring the length (cm) of browning of xylem tissue. Characteristics of tomato plants related to the disease severity scale established earlier are shown in Table 3.

Substrates not infested with FOL were used as controls in all bioassays. In these substrates, the plants did not show symptoms of Fusarium wilt or nutritional deficiency.

To test the ability of the natural compost microflora, of *T. asperellum* isolates and of Fo47 to restore suppression to sterilized compost–peat mix and Chateaufrenard soil, sterilized substrates were inoculated with 10% (v/v) compost or with a conidial suspension of each of the *Trichoderma* isolates or Fo47 at  $10^3 \text{ cfu cc}^{-1}$  substrate. The *T. asperellum* isolates were inoculated separately, but the results obtained were grouped according to their ITS1 sequence. The moisture content of the inoculated compost–peat mix and Chateaufrenard soil was adjusted to 1 and 10 KPa, respectively. The substrates were then incubated at room temperature in plastic boxes for 15 days. Populations of *T. asperellum* and Fo47 populations were quantified by serial dilution on acidified Malt Agar (Malt Agar,  $0.25 \text{ g l}^{-1}$  citric acid). Natural and sterilized compost–peat mix and Chateaufrenard soil were then infested with liquid inoculum of FOL race 1, and subjected to the above bioassay. The two inoculum densities  $5 \times 10^4$  and at  $5 \times 10^5 \text{ cfu cc}^{-1}$  of substrate were assessed.

Data presented in Fig. 1 correspond to compost from different batches sampled during 1997, while data presented on Fig. 2 correspond to compost sampled in 1998.

### 2.5. Experimental design and statistical analysis

Bioassays with natural and heat-treated compost–peat

Table 3  
Characteristics of tomato plants cv. Roma relating to the disease severity scale

Disease severity <sup>a</sup>	Fresh biomass/plant (g)	Length of stem (cm)	Length of stem affected <sup>b</sup> (%)	Moisture content (%)
0	13.53 ± 0.7	23.4 ± 0.6	0	93.9 ± 0.1
1	12.07 ± 1.9	17.3 ± 1.1	52 ± 0.6	93.3 ± 0.4
2	3.02 ± 0.7	9.9 ± 0.7	77.8 ± 0.6	84.8 ± 7.2
3	1.04 ± 0.2	8.9 ± 0.5	100	82.4 ± 2.1

<sup>a</sup> 0: asymptomatic plants, control included; 1: <50% of leaves chlorotic or wilted; 2: >50% of leaves wilted but plants not dead; 3: dead plants.

<sup>b</sup> Length of stem showing brown xylem vessels. Measures were performed in 25-day-old plants. Values presented are means ± SE ( $n > 15$ ).

mix substrates were performed three times (Fig. 1). Other bioassays were performed once. Each bioassay contained five pots per treatment and four plants per pot. For each rating time the mean of disease severity and percentage of symptomatic plants per pot was calculated. This mean was considered one value. The significance of differences in progress of the disease over time were assessed with a repeated measures model (Campbell and Madden, 1990). The significance of differences between biological properties of substrates and disease incidence at the end of the bioassays was assessed with an ANOVA. All the statistical analyses were performed on SPSS v. 8.0 (SPSS Inc.).

### 3. Results

#### 3.1. Suppressiveness of compost and compost mixtures to *Fusarium wilt of tomato*

The compost most effectively suppressed *Fusarium wilt* (Fig. 1). Symptoms of *Fusarium wilt* did not appear until 17 days after transplanting into the infested compost, and disease incidence was only 20% at the end of the bioassay.

In contrast, in the infested peat mix the disease was most severe. Symptoms of *Fusarium wilt* became visible after 11 days, and the severity of *Fusarium wilt* based on the repeated measures model analysis was significantly ( $P < 0.05$ ) higher than that on plants grown in the other substrates. Furthermore, after 17 days all the plants in the peat mix were symptomatic, with most of the leaves visibly affected. *Fusarium wilt* in the compost–peat mix was intermediate in severity. First symptoms became apparent after 15 days and disease incidence was 70% at the end of the bioassay (25 days). Most plants grown in compost–peat mix had less than a half of their leaves wilted at the end of the bioassay. All plants grown in the three non-infested control mixes did not develop symptoms of *Fusarium wilt*.

Additional experiments were performed with the compost–peat mix heat-treated to 60°C for 9 days to mimic self-heating which occurs during the composting process, and which causes the death of most of the microflora. Trends in disease severity and disease incidence in the natural compost–peat mix were consistent with those obtained in the experiments described above. However, heating the mix consistently increased both the severity and incidence of the disease. When compost–peat mixes

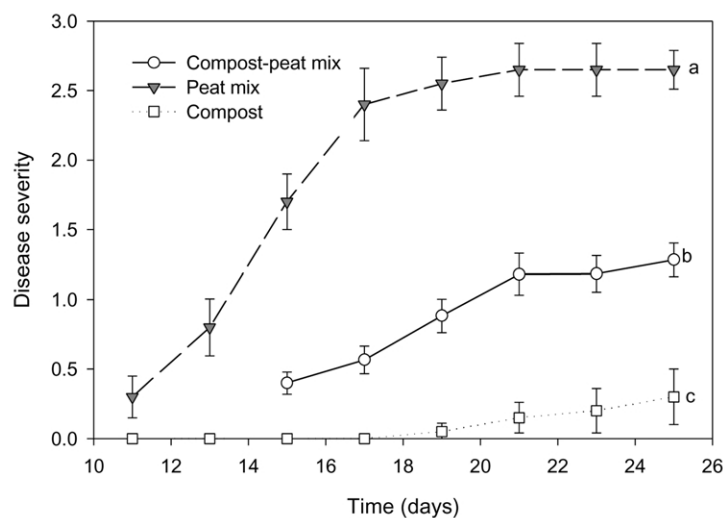


Fig. 1. *Fusarium wilt* disease progress curves for tomato plants grown in compost–peat mix, compost and peat mix. Substrates were infested with *Fusarium oxysporum* f. sp. *lycopersici* at  $5 \times 10^5$  cfu ml<sup>-1</sup>. Disease severity ranked from 0 (asymptomatic plants) to 3 (dead plants). Every curve represents the mean of at least five pots, each containing four plants. Data were analyzed by a repeated measures model. Different letters show significant differences ( $P < 0.05$ ) in a Duncan's multiple range test.

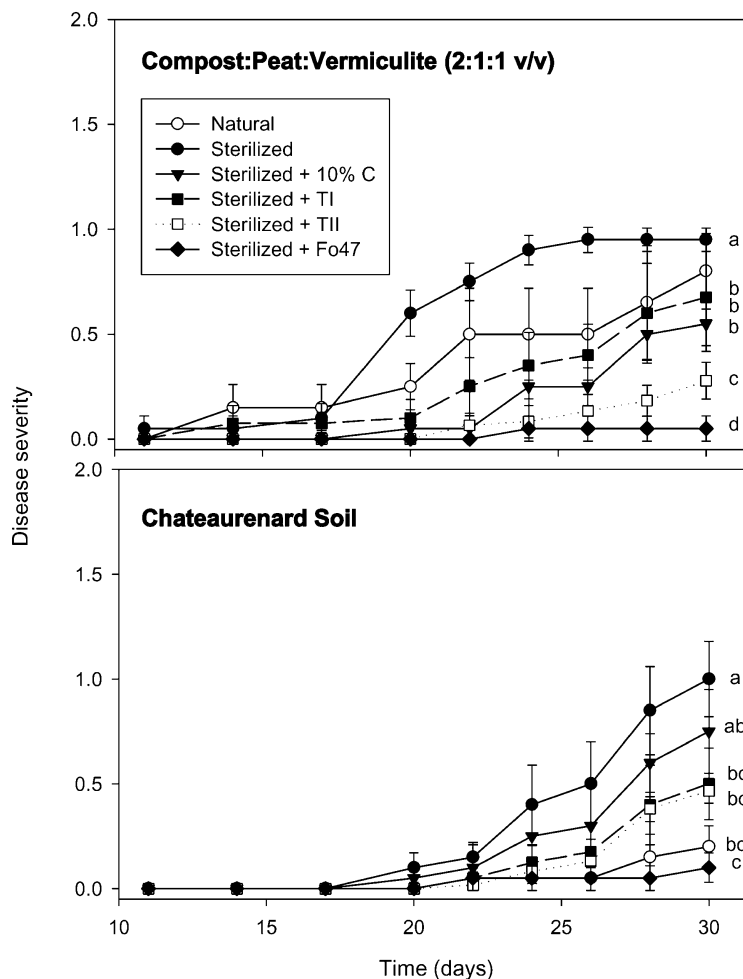


Fig. 2. *Fusarium* wilt disease progress curves for tomato plants grown in compost–peat mix or in Chateaufrenard soil, natural or sterilized, amended with 10% compost (10% C) or inoculated with Fo47 or *Trichoderma asperellum* isolates from group TI or TII. Substrates were infested with *Fusarium oxysporum* f. sp. *lycopersici* at  $5 \times 10^5$  cfu ml<sup>-1</sup>. Disease severity ranked from 0 (asymptomatic plants) to 3 (dead plants). Every curve represents the mean of five pots, each containing four plants. Data were analyzed by a repeated measures model. Different letters show significant differences ( $P < 0.05$ ) in a Duncan's multiple range test.

were infested at  $5 \times 10^5$  cfu cc<sup>-1</sup> potting mix the mean disease severity after 25 days was 1.2 in the natural mix, and 2 in the heated mix. Heating the mix increased the disease incidence from 70 to 90%. Similar trends were obtained in compost–peat mixes infested at a lower concentration, although values obtained for disease severity and disease incidence were lower. When inoculated at  $5 \times 10^4$  cfu cc<sup>-1</sup> potting mix the mean disease severity reached 0.1 and 0.8 in the natural mix and the heated mix, respectively. In this case, heating increased the disease incidence from 10 to 38%.

Physical properties of the substrates were between or near to the optimum ranges established for potting media (Ansorena, 1994). The pH and EC differed drastically among the substrates (Table 2). The peat mix, for instance, was acidic and had a low EC, whereas the compost was basic and had a high EC. The compost–peat mix had a similar pH to the compost. However, EC in this mix was approximately half that of the compost. The heavy metal

concentrations in the compost were below the limits established by Spanish legislation (RD877/91).

Fungal populations were not significantly ( $P < 0.05$ ) different among substrates. Bacterial populations were significantly ( $P < 0.05$ ) lower in peat and the peat mix. Microbial activity, as measured by hydrolysis of FDA per unit of volume, was significantly ( $P < 0.05$ ) higher in the compost and compost-mix than in the peat mix (Table 4).

### 3.2. Efficacy of *Trichoderma* isolates in biocontrol

The ability of the two different groups of *T. asperellum* to induce biocontrol of *Fusarium* wilt of tomato in sterilized compost–peat mix or Chateaufrenard soil, compared to Fo47 or a 10% of naturally suppressive compost amendment is presented in Fig. 2. The mean trends in disease severity for the treatments with TII isolates represent overall means for the data obtained with three such isolates tested separately. The mean trends for the treatments with TI isolates

Table 4

Microbiological properties of the substrates used in bioassays (ND, not determined. Different letters show significant differences ( $P < 0.05$ ) in a Duncan's multiple range test)

	Total fungi log (cfu cc <sup>-1</sup> )	Total bacteria log (cfu cc <sup>-1</sup> )	FDA activity ( $\mu\text{g FDA cc}^{-1} \text{ min}^{-1}$ )
Compost	6.98 a	9.60 c	1.72 b
Peat	6.53 a	6.55 b	ND
Peat mix	6.08 a	4.65 a	1.30 a
Compost–peat mix	6.39 a	9.53 c	1.47 ab

represent overall means for two isolates. Results presented in Fig. 2 show that in the sterilized compost–peat mix the TII isolates of *T. asperellum* were not significantly different in efficacy from Fo47 until 26 days after transplanting into the infested mix. Thereafter, more disease developed on the plants in the mix inoculated with the TII isolates ( $P < 0.05$ ). TI isolates were not significantly different in efficacy from either Fo47 or TII isolates until 24 days after transplanting. Interestingly, TII isolates were significantly more effective than (i) the natural compost–peat mix from which they were isolated, and (ii) the 10% amendment with natural compost. However, trends in disease severity of Fusarium wilt for the natural compost–peat mix, the sterilized peat–compost mix amended with 10% naturally suppressive compost or the TI isolates did not differ significantly (Fig. 2). Values for disease incidence at the end of the bioassay (after 30 days from transplanting) also reveal the higher efficacy of TII isolates in the sterilized compost–peat mix (Table 5). Hence, disease incidence was decreased from 55% in the natural mix to 28% in the sterilized mix inoculated with TII isolates. However, in the sterilized mix inoculated with TI isolates disease incidence was not significantly different ( $P < 0.05$ ) from that obtained in the natural mix.

In the Chateaufrenard soil no differences in efficacy among TI and TII isolates were detected, whether based on disease severity (Fig. 2) or disease incidence (Table 5). The amendment with 10% naturally suppressive compost was as effective as *T. asperellum* isolates, but less effective than Fo47.

Table 5

Fusarium wilt incidence (%) on tomato plants var. Roma after 30 days of culture in compost–peat mix or in Chateaufrenard soil natural or sterilized, amended with 10% (v/v) natural compost or inoculated with Fo47 or *Trichoderma asperellum* isolates from group TI or TII (Substrates were infested with *Fusarium oxysporum* f. sp. *lycopersici* at  $5 \times 10^5$  cfu ml<sup>-1</sup>). Data were analyzed by ANOVA. Values with different letters are statistically different ( $P < 0.05$ ) in a Duncan's multiple range test)

	Compost:peat:vermiculite (2:1:1 v/v)	Chateaufrenard soil
Natural	55 c	15 a
Sterilized	90 d	90 c
Sterilized + 10% compost	45 bc	65 bc
Sterilized + TI	53 bc	45 b
Sterilized + TII	28 b	43 b
Sterilized + Fo47	5 a	10 a

Finally, severe disease developed in plants transplanted into the infested sterilized compost–peat mix and Chateaufrenard soil. The highest values for disease severity (Fig. 2) and incidence (Table 5) were obtained in these sterilized substrates. In the compost–peat mix, sterilization increased disease incidence at the end of the bioassay (30 days after transplanting) 35% from the natural mix. In the Chateaufrenard soil the equivalent increase in disease incidence was 75%. Plants in all control non-infested mixes remained free of symptoms.

In a second experiment but with a lower inoculum density of FOL ( $5 \times 10^4$  cfu cc<sup>-1</sup>) trends among all treatments in the compost–peat mix and Chateaufrenard soil were similar to those presented in Fig. 2. The disease severity and incidence of Fusarium wilt in the sterilized mixes again was high (90% in the sterilized compost–peat mix, 55% in the sterilized Chateaufrenard soil). However, in natural mixes disease severity and incidence of Fusarium wilt was very low (25% in the compost–peat mix, no disease in the Chateaufrenard soil). Both in the compost–peat mix and in the Chateaufrenard soil, disease severity and incidence of Fusarium wilt was significantly ( $P < 0.05$ ) lower in the mixes inoculated with *T. asperellum* isolates or Fo47 than that on plants transplanted into the sterilized mixes. In the sterilized compost–peat mix, TI and TII isolates reduced the disease incidence from 90 to 13 and 5%, respectively. No disease was observed in the sterilized mix inoculated with Fo47. In the Chateaufrenard soil, the reduction was from 55 to 23 and 22% for TI and TII isolates, and again no disease was recorded in the soil inoculated with Fo47. Efficacy of TI and TII isolates at this low inoculum density of FOL did not differ significantly from those obtained for the natural mixes and the 10% compost amendment. Differences in disease severity were not detected among the treatments with TI and TII isolates of *T. asperellum* and Fo47 in the compost–peat mix. However, both disease severity and incidence were higher for TI and TII isolates than for Fo47 in the Chateaufrenard soil. At this inoculum density, symptoms of disease did not develop on any plant in the sterilized mix that was inoculated with Fo47 nor in the natural Chateaufrenard soil.

#### 4. Discussion

It is interesting that the compost used in this work, which

contained sewage sludge as raw material, was suppressive to *Fusarium* wilt of tomato. Earlier reports show that composts with low C/N values, such as those prepared from sewage sludge do not suppress *Fusarium* wilts even when inoculated with effective biocontrol agents (Hoitink et al., 1987). Similar observations for composted sewage sludge have been made in California (Quarles and Grossman, 1995). It has been proposed that lack of biological suppressiveness of *Fusarium* wilt in such low C/N composts is due to the release of high concentration of ammonium (Hoitink et al., 1987). Application of fertilizers with high ammonium aggravates *Fusarium* wilts whereas fertilizers with high nitrate nitrogen and high calcium suppress *Fusarium* wilts (Jones and Woltz, 1981). The compost used in this work, even though it contained sewage sludge as a raw material, was low in available ammonium (Table 1). This may have been a direct result of the higher C/N materials (yard wastes) that were also included with the feedstock from which the compost was prepared. This would decrease the ammonium concentrations in the compost (Hoitink et al., 1993) and thus eliminate the negative effect of this compound on *Fusarium* wilt. The level of suppression of *Fusarium* wilt obtained using this compost as a substrate was close to that obtained using Chateaufort soil, reported as a highly suppressive soil to *Fusarium* wilts (Alabouvette, 1986).

The high pH values observed in the studied compost and the compost–peat mix, as well in the Chateaufort soil, might also contribute to their ability to suppress *Fusarium* wilt. High pH reduces the availability of micronutrients such as Fe, Cu and Zn. This unavailability of micronutrients can limit growth, sporulation and pathogenicity of *F. oxysporum* f. sp. *lycopersici* (Jones and Woltz, 1981). Low iron availability can also induce siderophore production and iron competition, a mechanism used by certain antagonists of *Fusarium* wilt (Alabouvette, 1999). Finally, high EC or salt content, also observed in compost and compost mixtures, can reduce the survival of the pathogen and therefore decrease the inoculum potential of soils (Amir and Riba, 1990).

The heavy metal concentrations of the compost were below the Spanish limits established for the higher quality compost (RD877/91) and, in general, below the concentrations described to have a fungicidal effect on *F. oxysporum* (Gabr et al., 1998). In addition, the high pH of the compost and the compost–peat mix could reduce the availability of these metals. Only the effect of copper on the pathogen cannot be completely ruled out, since concentrations of this element approximately double those obtained in the compost were reported to have a strong inhibitory effect on *F. oxysporum* (Gabr et al., 1998).

On the other hand, since disease incidence and disease severity increased when the compost–peat mix was heated or sterilized, suppression of this disease can also be related to the microflora present in the natural mix. Other studies have reported the involvement of biotic properties in *Fusarium* wilt suppression with composts (Chef et al.,

1983; Trillas-Gay et al., 1986). The comparison among major biological properties of the suppressive compost, the compost–peat mix and the conductive peat mix shows that bacterial populations are significantly higher in the suppressive than in conductive substrates used in this work (Table 4). This suggests that bacteria may play a role in the suppression of *Fusarium* wilt by compost. Similar results were reported by Larkin et al. (1993) who compared microbial populations in suppressive soils to those in conductive soils to *Fusarium* wilt. Bacterial groups such as fluorescent pseudomonads contribute to suppressiveness of soils suppressive to *Fusarium* wilts (Lemanceau and Alabouvette, 1993). De Britto Alvarez et al. (1995) also showed that the addition of some composts to soil increased the incidence in the tomato rhizosphere of bacteria exhibiting antagonism towards *F. oxysporum* f. sp. *radicis-lycopersici*, and other pathogenic fungi. Further research is needed to determine whether total bacteria or specific populations are involved.

Compost and compost–peat mix showed a high microbial activity. Values obtained for this substrates were higher than that reported in composted hardwood bark media (Chen et al., 1988), composted pine bark media (Boehm and Hoitink, 1992), or a bark compost media (Hoitink et al., 1993). The peat mix also showed a microbial activity higher than that reported in other peat media (Inbar et al., 1991; Boehm and Hoitink, 1992; Hoitink et al., 1993). However, values for microbial activity in the peat mix were lower than for the compost and compost–peat mix. Microbial activity has been reported to be a key factor in suppression of *Fusarium* wilt (Serra-Wittling et al., 1996). A positive correlation between microbial activity of substrates and suppression of *Fusarium* wilt of tomato can be established from our results. Similar correlation has also been reported for the suppressive ability of potting media or organic matter-amended field soils to suppress diseases caused by many soilborne plant pathogens (Hoitink and Boehm, 1999), such as *Pythium ultimum* (Inbar et al., 1991; Craft and Nelson, 1996), *Phytophthora* spp. (Workneh et al., 1993; You and Sivasithamparam, 1994) and *Pyrenochaeta lycopersici* (Workneh et al., 1993). In all the substrates used in this work, the microbial activity was beyond the threshold established for the suppression of *Pythium* spp. (Boehm and Hoitink, 1992).

The increase in the suppressive ability of the peat mix after the addition of compost demonstrates that suppressiveness can be transferred from a highly suppressive compost to a conductive substrate. Compost added at 10% (v/v) to the sterilized compost–peat mix was adequate to restore suppressiveness against *Fusarium* wilt. However, the suppressive ability of the natural Chateaufort soil was higher than that induced in the sterilized soil after inoculation with compost, which suggests that soil microflora was more effective than compost microflora in suppressing *Fusarium* wilt. These results confirm the relative importance of compost microflora in suppressiveness, and agree with

those reported by Serra-Wittling et al. (1996) with *Fusarium* wilt of flax. In our case, the presence of indigenous saprophytic *F. oxysporum* in the natural Chateaurnaud soil may account for higher disease suppression in this soil (Alabouvette, 1986).

The isolates of *T. asperellum* isolated from the suppressive compost–peat mix were able to induce suppression in both compost and soil. However, some of them seemed to be more effective when reintroduced into the compost media from which they were isolated. These results could be related to poor colonization of the soil by the *Trichoderma* isolates or to changes in their metabolism affecting their interactions with the pathogen (Hoitink and Boehm, 1999). The efficacy of the isolates of *T. asperellum* studied to suppress *Fusarium* wilt seem to be higher than other *Trichoderma* spp. reported as biocontrol agents (Trillas-Gay et al., 1986; Larkin and Fravel 1998). For instance, a combined inoculation with *T. hamatum* and *Flavobacterium balustinum* was needed to control *Fusarium* wilt of radish (Trillas-Gay et al., 1986). These results demonstrate the potential of these isolates as biocontrol agents.

In summary, our results show that the use of some composted sewage sludge in growing media can be effective for suppression of *Fusarium* wilt. Amendment with 10% of such compost may be adequate to induce suppressiveness either in disease conducive compost or soil environments. Several isolates of *T. asperellum* from a suppressive mix prepared with this compost showed a high ability to suppress *Fusarium* wilt also in both compost and soil environments. Some of these isolates showed an ability to induce suppression to *Fusarium* wilt better than a 10% compost amendment. However, the nonpathogenic *F. oxysporum* Fo47 was even more effective than these isolates. Suppression of *Fusarium* wilt with such compost seems to be the result of a combination of abiotic and biotic factors such as low ammonium, slightly high pH, high EC, large bacterial populations and high microbial activity.

## Acknowledgements

This research was supported, in part, by Universitat de Barcelona through a Predoctoral Fellowship, by the Ministerio de Educación y Ciencia, Spain, and by the I.N.R.A. Dijon, France. We thank METROCOMPOST S.A. for providing compost, Dr Soliva and Dr Martinez for his support in this project, Dr Tello for providing the isolate of *F. oxysporum* f. sp. *lycopersici*, and FITO S.A. for providing tomato seeds. We also thank Dr Monte and Dr Hermosa of the Universidad de Salamanca for helping us to identify *Trichoderma* isolates, and Dr H.A.J. Hoitink and Dr M.S. Krause for kindly reviewing the manuscript.

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