Predictive Factors for the Suppression of Fusarium Wilt of Tomato in Plant Growth Media

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ABSTRACT

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Fusarium wilts are economically important diseases for which there are no effective chemical control measures. However, biological control and fertility management are becoming efficient alternatives for controlling this disease. Growth media formulated with composts that are able to suppress Fusarium wilt of tomato provide a control system that integrates both strategies. The aim of this study was to predict Fusarium wilt suppression of growth media using abiotic and biotic variables. Grape marc compost was the most effective medium used to suppress Fusarium wilt. Cork compost was intermediate, and light peat and expanded vermiculite were the most conducive growth media. The growth media evaluated

were in a pH range of 6.26 to 7.97. Both composts had high β -glucosidase activity. When pH and β -glucosidase activity were taken into account as predictive variables, more than 91% of the variation in severity of Fusarium wilt was explained. This relationship illustrates the effect of nutrient availability and the degree of microbiostasis, two key factors in this pathosystem. Microbial populations involved in suppressiveness were cellulolytic and oligotrophic actinomycetes, fungi, and the ratios cellulolytic actinomycetes/cellulolytic bacteria, oligotrophic bacteria/copiotrophic bacteria, and oligotrophic actinomycetes/oligotrophic bacteria. Based on community level physiological profiles, different community structures were evident among growth media evaluated.

Additional keywords: *Fusarium oxysporum* f. sp. *lycopersici*, *Lycopersicon esculentum*, microbial activity.

Fusarium wilt diseases, caused by pathogenic formae speciales of the soil-inhabiting fungus *Fusarium oxysporum* (Sacc.) W. C. Snyder & H. N. Hans., cause severe losses in a wide variety of crop plants, including tomato (*Lycopersicon esculentum* Mill.). This pathogen occurs throughout most tomato-growing areas and devastates the crop. Fusarium-resistant cultivars have been available for decades and provide some degree of control; however, the occurrence and development of new pathogenic races is a continuing problem. There are no commercially acceptable tomato cultivars with adequate resistance (27). In addition, the resurgent interest in planting heirloom and other susceptible tomato cultivars has increased the incidence of Fusarium wilt. Effective soil fungicide treatments for this disease also are unavailable. Difficulties in controlling Fusarium wilt have stimulated the search for biocontrol systems. The nature of soils and composts that are suppressive to Fusarium wilt is the result of complex interactions between the abiotic characteristics of the media and microbial populations (1,2,21,60). Numerous biocontrol agents have been identified in these soils and composts (21,60). Unfortunately, the introduction of biocontrol agents has provided inconsistent results (59). Growth media formulated with composts such as hardwood bark (9,55), pine bark (8,42,44), poplar bark (16), wood shavings (8), cork (54), grape marc (54), olive pumice (44), cattle manure (45), sewage sludges (11), and vermicompost (16,51,52) can suppress Fusarium wilt. Several of these have been used successfully for consistent biological control, particularly for containerized crops (21,22). The microbiological nature of Fusarium wilt-suppressiveness for most of these compost-amended growth media

has been demonstrated (8,9,11,16,22,42,43,45,47,51,54,55). The role of nutritional factors in wilt-suppressiveness also has been suggested (11,22,42,55). For some composts, it has been demonstrated that fungitoxic substances are not involved (43,45,47,51).

The main purpose of this study was to determine the relationship between biotic and abiotic properties of growth media and the severity of Fusarium wilt in order to predict disease suppressiveness of growth media. In addition, nutrient availability, nutrient status of plants, and microbial diversity were evaluated at the conclusion of the experiments in order to understand possible mechanisms involved in the suppressive phenomena. For this purpose, two Fusarium-suppressive composts, which can be used as plant growth media, were compared with two standard growth media.

MATERIALS AND METHODS

Plant growth media. Two residues from agricultural and industrial wastes were composted and evaluated for Fusarium wilt suppression: cork compost (CC) from cork (*Quercus suber* L.) production and grape marc compost (GMC) from the alcohol-producing industry (grape skins, seed, and stems). Both wastes were composted as described elsewhere (54). Composts were compared with *Sphagnum* light peat (P) (Klasmann, Valinex, Pallete, Spain) and expanded vermiculite (V) (Vermiculita y derivadas, Gijón). Peat was neutralized with CaCO_3 at 4 g/liter. To determine the role of microflora in suppression, the two composts also were heated to 60°C for 6 days, (CC-60 and GMC-60).

Assessment of disease severity. Disease-suppressive properties of plant growth media were measured by a Fusarium wilt bioassay described elsewhere (11). To standardize initial conditions, the growth media were incubated at a water tension of 1,000 Pa (adjusted for weight) for 14 days at 25°C. Liquid culture (500 ml

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of 1% malt extract; Sigma-Aldrich, St Louis) of *Fusarium oxysporum* f. sp. *lycopersici* race 2, isolate RAF 70 obtained from an infected tomato plant (FOL-2), was prepared and grown with continuous agitation (130 rpm) for 10 days at 25°C. Conidia were recovered by centrifugation at 5,000 rpm for 15 min (Selecta Medifriger BL-S, Abrera, Spain) and rinsed twice with sterile distilled water. The concentration of conidia was determined with a hemocytometer. The six plant growth media were infested with FOL-2 (10^5 microconidia/cm³ of plant growth medium), mixed vigorously, and poured into 9-cm-diameter plastic pots (330 cm³ volume). Pots of growth media without FOL-2 were prepared as controls. Four tomato seedlings (one- to two-true-leaf stage) grown in sterile vermiculite were transplanted into each pot. Plants were irrigated as needed and fertilized with a nutrient solution containing Peter's foliar feed 27-15-12 at 0.5 g/liter (Scotts, Heerlen, The Netherlands), CaCl₂ at 0.6 g/liter, and MgSO₄·7H₂O (pH 5.68) at 0.696 g/liter. Plants were grown in a growth chamber (25°C, photosynthetically active radiation (PAR) intensity 280 µE/m²·s, photoperiod 16 h light and 8 h dark, and 60% relative humidity). Bioassays were repeated at least three times with four plants per pot and five pots per treatment. Treatments were arranged in a randomized block design. Disease severity was monitored at 2-day intervals for 25 days after planting, and was scored 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 (11). Disease severity was expressed as proportion of the maximum possible disease severity. At each disease assessment, the mean of disease severity per pot was calculated. The area under the disease progress curve standardized (AUDPCs) per pot was calculated by disease severity integrated between symptoms onset and bioassay final time and dividing by the total duration (days) of the epidemic in each bioassay, in order to compare the various bioassays, which had a variety of epidemic durations. At the end of the bioassays, mean values per pot (four plants) of the relative length of the stem with brown xylem (RLSBX), and the fresh and dry weight of leaves and stems means per pot were recorded. These values per pot were considered as replicates for each observed variable.

Physical and physicochemical properties of growth media. The bulk density (BD) for the plant growth media was determined according to De Boot et al. (13). Electrical conductivity (EC) and pH of media were measured in a water extract (2:1, vol/vol) as described by Bunt (6) and Gabriëls et al. (15), respectively. Physical and chemical variables of the growth media were determined at the beginning of the bioassays. The pH also was measured at the end of each bioassay. Three samples were analyzed for each growth medium.

Nutrient availability and nutrient status of plants. Plant growth media samples (50 g) were collected from the pots and extracted with 0.5 M ammonium acetate (pH 4.65). Plant samples (0.25 g dry weight) from all treatments were incinerated at 550°C for 8 h and dissolved in 20 ml of 1 N HCl, filtered as necessary, and stored at 4°C until analysis. Calcium, magnesium, iron, copper, manganese, and zinc were determined by atomic absorption spectroscopy (63) and sodium and potassium were analyzed by atomic emission spectrometry (63). Phosphorous was measured with colorimetry (37), nitrate (plant growth media) with reflectometry (RQ flex Merck, Darmstadt, Germany) (50), and boron (plants) with colorimetry (31). Total nitrogen in plants was measured with Kjeldahl digestion, modified to include nitrates, and ammonium subsequently was determined with colorimetry (36). All analyses were performed with three samples for plants and two samples of plant growth media from one of the three bioassays, at the end of the experiment.

Biological characteristics of growth media. All determinations were performed in incubated growth media at the beginning of bioassays, except for microbial diversity.

Microbial biomass. Three techniques were used to assess microbial biomass: (i) phosphates from phospholipids (10), with digestion as described by Van Veldhoven and Mannaerts (57) and phosphate determination with colorimetry (37); (ii) fumigation-extraction, based on the estimation of cell carbon by extraction with saline solution of fumigated and nonfumigated samples and their conversion to biomass (46); and (iii) acridine orange direct counting (AODC) technique, which was performed by counting cells from an aqueous extraction with an epifluorescence microscope (30). The aqueous extraction was filtered through 0.2-µm pore size black polycarbonate membranes (Isopore, Millipore Iberica S.A., Madrid, Spain). Counting was performed at ×1,250 with a minimum of 400 cells per filter. Controls were counted also in sterile water. Three samples were analyzed for each plant growth medium, except for those that were heated.

Microbial activity. Microbial activity was estimated by measuring β-glucosidase activity. This method is based on colorimetric determination of the *p*-nitrophenol released by β-glucosidase when the plant growth medium was incubated with *p*-nitrophenyl-β-D-glucoside (pH 6.0). The *p*-nitrophenol released was extracted by filtration and determined colorimetrically. β-Glucosidase activity was measured according to Bandick and Dick (4). Two samples were analyzed for each plant growth medium.

Microbial diversity. The density of cultivable groups of bacteria and fungi associated with biocontrol phenomena was determined by dilution plating on semiselective media according to Tuitert et al. (56) with modifications. Samples were taken from the rhizosphere at the end of bioassays. Plant growth media (5 to 10 g) were suspended in 250 ml of 0.1% sodium pyrophosphate. The suspension was shaken and 10-fold dilution series were prepared with 0.1% water agar. Suspensions were pipetted onto three plates per culture medium and dilution. Four or five dilutions per series were placed on plates. For isolation of copiotrophic bacteria, *Bacillus* spp., fluorescent *Pseudomonas* spp., oligotrophic and cellulolytic bacteria, and actinomycetes, 100 µg of cycloheximide was substituted for benomyl (Energía e Industrias Aragonesas, S.A., Madrid) at 10 µg/ml and Previcur (Propamocarb, 72.2%, Schering, Alcácer, Spain) at 0.3 µl/ml. Fungal counts were made on potato dextrose agar amended with Tergitol-7 (Fluka Chemie AGB, Buchs, Switzerland) at 1,000 ppm and 50 µg of oxytetracycline hydrochloride (Sigma-Aldrich) (10). Data for population density was normalized by natural log (Ln) transformations. Analyses were performed twice with one sample per each control and each infested plant growth medium from each of the two bioassays.

Community level physiological profiles. Eco-microplates (Biolog Inc., Hayward, CA) were used to determine differences in physiological abilities of the microbial communities from different plant growth media and the similarities between these communities and the pathogen. The microplates contained 96 wells with 31 carbon sources plus control, with three replicates each, as has been proposed for community characterization in environmental samples (25). The growth media extraction method was performed according to Insam et al. (26). Population cell density was determined by AODC to standardize the inoculum density. The density of FOL-2 conidia in suspension was determined by hemocytometer. Five eco-microplates were inoculated for plant growth medium suspensions and three for FOL-2 conidial suspension. The microplates were incubated in darkness at 25°C for 5 days. Color formation was measured with a microplate reader (LabSystem Multiskan MS, Helsinki, Finland) at 590 nm and readings were made at 12-h intervals. For the analysis, the average absorbance of the control wells in each plate was subtracted from that of wells containing the same C-source. Negative values were considered as zero. Microbial community diversity was calculated with these data using Shannon (48) and Gini (19) indices to compare functional diversity of microbial communities. For further analysis of Biolog data, a Riemann's sum for each C-source was calculated from all readings taken during the 5 days of incubation (49). The

transformed data were subjected to principal component analysis (PCA) (17), and distances in Euclidean space from the first five principal components were calculated (24).

Statistical analysis. Data collected from all trials were analyzed with Statgraphics Plus (version 5.1; Statistical Graphics Corp., Rockville, MD). The effect of growth medium on AUDPCs, pH, EC, BD, nutrient availability, nutrient status in plants, and biological characteristics of container medium were analyzed with analysis of variance. Significant means were compared by Tukey's whole significant difference test ($P = 0.05$). Overall relationships between AUDPCs and continuous measured variables were analyzed with regression analysis.

RESULTS

Suppressiveness of plant growth media. Fusarium wilt was suppressed most effectively with GMC, with more than a 92% reduction in AUDPCs and RLSBX compared with peat or vermiculite. Media CC, GMC-60, and CC-60 were intermediate, and P and V were the most conducive growth media (Fig. 1A). The heated composts had reduced suppressive ability; however, GMC-60 maintained moderate levels of suppressiveness compared with P and V (Fig. 1A and B). Medium CC-60 was not different from P for disease severity measured by RLSBX (Fig. 1B). At an

Fig. 1. A, Standardized area under disease progress curve (AUDPCs) and **B,** relative length of stem with brown xylem (RLSBX) for tomato plants in six growth media: CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; and GMC-60, heated grape marc compost. Disease severity scale was 0 = asymptomatic plants, 1 = $\leq 50\%$ affected leaves, 2 = $> 50\%$ affected leaves, and 3 = dead plants. Plant growth media were infested with *Fusarium oxysporum* f. sp. *lycopersici* race 2 at a dose of 1×10^5 microconidia/cm³. Data for AUDPCs and RLSBX were transformed for analysis with the arcsine $\sqrt{x}$. Bars with the same letter are not significantly different according to Tukey's test at $P < 0.05$. Standard error of the mean is indicated by vertical line.

inoculum concentration of 1×10^4 microconidia/cm³ of plant growth medium, the AUDPCs were similar to that for 1×10^5 microconidia/cm³ of plant growth medium but with lower disease levels (data not shown). The best correlation between measured disease-related variables and the fresh and dry weight of leaves and stems (data not shown) was between AUDPCs and the square root of fresh weight ($R^2 = 72.49$, $P < 0.001$). RLSBX and the square root of dry weight had the lowest correlation ($R^2 = 20.94$, $P < 0.001$). Consequently, AUDPCs was selected to make further correlation analysis with the different measured variables. None of the plants grown in the six noninfested control growth media developed symptoms of Fusarium wilt.

Physical, chemical, and biological properties of growth media at the beginning of bioassays. The BD was higher in both composts than in peat and vermiculite (Table 1). The GMC medium had the highest pH. After heat treatment, the pH rose transiently for both composts. Vermiculite and peat had the lowest EC, followed by GMC and CC. The CC medium had the highest β -glucosidase activity, followed by GMC; when heated, both composts had enzyme activity similar to that of peat (Table 2). Similar trends were obtained from biomass determinations: phosphate and fumigation extraction enabled separation of V from GMC, and AODC differentiated the two composts from each other and from P and V (Table 2). No significant relationships were observed among parameters (BD, pH, EC, β -glucosidase activity, PBP, FE biomass, and AODC) studied before the bioassay and AUDPCs. However when the pH and the β -glucosidase activity were taken into account as predictive variables (multiple linear regression), more than 91% of the variation in AUDPCs was explained ($P < 0.05$; Fig. 2).

The results of the PCA performed for community level physiological profiles (CLPPs) on the whole community Biolog pattern from growth media and FOL-2 are depicted graphically (Fig. 3). Based on the scatter in the coordinate score, there was a clear separation between growth media samples along the PC1 axis ($P < 0.05$), accounting for 77% of the variance in the data. There also was a clear separation between GMC and the other growth media along the PC2 axis ($P < 0.05$). The PC2 explained 16% of the variance in the data. Among the growth media, there were different C-metabolic profiles and, therefore, different community structures. The first five principal components explained 98% of the variance in the data. Distances in Euclidean space, formed by the first five principal components between FOL-2 and growth media whole community patterns, revealed the shortest distance to be between FOL-2 and CC. This means that the CC microbial community had the catabolic potential most qualitatively similar to FOL-2. Diversity indices, however, revealed that CC had the lowest functional diversity, while GMC had a higher Gini index than P, with Shannon indices being similar (Table 3).

Nutrient availability, nutrient status of plants, and rhizosphere microbial diversity at the end of the bioassays. The

TABLE 1. Physical and physicochemical properties of plant growth media evaluated in bioassays^w

Plant growth medium ^x	Bulk density (g/cm ³) ^y	pH ^y	pH ^z	Electrical conductivity (mS/cm) ^y
CC	0.22 b	6.26 d	5.74 b	1.53 a
CC-60	ND	6.71 c	5.85 b	1.62 a
P	0.09 c	6.28 d	5.79 b	0.24 c
V	0.13 c	6.48 dc	6.07 b	0.05 c
GMC	0.41 a	7.35 b	6.72 a	1.00 b
GMC-60	ND	7.97 a	6.86 a	0.94 b

^w Within each column, values with different letters are significantly different ($P < 0.05$) according to Tukey's test. ND = not determined.

^x CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; GMC-60, heated grape marc compost.

^y At beginning of bioassay.

^z At end of bioassay.

GMC medium maintained a high pH at the end of bioassays and heat treatment did not affect the final pH for either CC or GMC (Table 1). During the bioassay, plants showed no symptoms of nutrient deficiency. However, at the end of the bioassay, nutrient availability was different in infested growth media (Table 4). Nutrient status of plants also differed in infested media (Table 5). In the pH range used in this study, growth media per se conditioned the availability of nutrients, even when the same fertigation was used.

Infested P and V had higher levels of Cu than either compost. Infested P and V had the lowest levels of Na, Ca, Fe, and Mn. The GMC showed the highest levels of Na, Ca, Mg, P, and K (Table 4). There was a significant positive correlation between the availability of Cu for all growth media and the AUDPCs in infested pots (Table 6) and a negative correlation between the availability of Na and Ca for all growth media and the AUDPCs (Table 6). The nutrient status of plants in unheated GMC had the lowest level of N and Mn (Table 5). Plants grown in infested vermiculite had the highest level of Na. Plants grown in GMCs had higher levels of K than those grown in CC, peat, and vermiculite (Table 5). Based on chemical analysis, plants grown in infested peat had higher levels of N, P, and Fe and lower levels of Mg than those grown in CC and GMC (Table 5). Plants grown in

Fig. 3. Ordinate plot of principal components (PC1 and PC2) of microbial communities of four plant growth media at beginning of bioassay and *Fusarium oxysporum* f. sp. *lycopersici* (FOL-2) according to Biolog pattern. CC, composted cork; P, peat; V, vermiculite; and GMC, grape marc compost.

TABLE 2. β -Glucosidase activity and microbial biomass of six plant growth media at the beginning of bioassays evaluated by three different techniques: phosphate biomass from phospholipids (PBP), fumigation extraction (FE), and acridine orange direct counting (AODC)^y

Plant growth medium ^z	β -Glucosidase (μ g hydrolyzed <i>p</i> -nitrophenol/cm ³)	PBP (μ moles P/cm ³)	FE biomass (μ moles C/g)	AODC (number of cells)
CC	172.95 a	0.032 ab	251.68 ab	4.44×10^6 a
CC-60	54.96 c	ND	ND	ND
P	35.07 c	0.014 ab	272.11 ab	3.73×10^5 c
V	0.59 d	0.006 b	158.36 b	8.44×10^4 c
GMC	110.04 b	0.036 a	475.71 a	1.94×10^6 b
GMC-60	40.34 c	ND	ND	ND

^y Within each column, values followed by different letters are significantly different based on Tukey's test at $P < 0.05$. ND = not determined.

^z CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; GMC-60, heated grape marc compost.

Fig. 2. Correlation (multiple) among standardized area under disease progress curve (AUDPCs), β -glucosidase activity, and pH, recovered from six plant growth media at the beginning of bioassays. These growth media were infested with *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 1×10^5 microconidia/cm³. CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; and GMC-60, heated grape marc compost. Disease severity scale: 0 = asymptomatic plants, 1 = $\leq 50\%$ affected leaves, 2 = $> 50\%$ affected leaves, and 3 = dead plants.

GMC had lower levels of Cu than V and peat (Table 5). The levels of Fe, N, Cu, P, and Na in plants grown in the various growth media were positively correlated with the AUDPCs (Table 6).

Among noninfested media, P and V had the lowest populations of *Bacillus* spp. and oligotrophic actinomycetes (Table 7). Medium CC had the highest population of fluorescent *Pseudomonas* spp., while GMC had the highest number of oligotrophic actinomycetes. The heat-treated CC and GMC media had higher populations of *Bacillus* spp. and lower densities of fluorescent *Pseudomonas* spp. than unheated. There also was a reduction in the population densities of oligotrophic actinomycetes and fungi for GMC-60.

Populations of copiotrophic bacteria, cellulolytic bacteria, and oligotrophic bacteria were not significantly different among infested growth media (Table 7). The population densities of *Bacillus* spp. remained high for GMC in the presence of the pathogen. Numbers of oligotrophic and cellulolytic actinomycetes and fungi were highest in infested GMC (Table 7). These three populations of microorganisms were negatively correlated with AUDPCs (Table 8). Vermiculite had the lowest number of oligotrophic actinomycetes (Table 7). A negative correlation was found between the oligotrophic actinomycetes/oligotrophic bacteria ratio and AUDPCs (Table 8). The ratios for cellulolytic actinomycetes/cellulolytic bacteria and oligotrophic bacteria/copiotrophic bacteria also were negatively correlated with AUDPCs (Table 8).

TABLE 3. Gini (1-G) and Shannon (H') diversity indices of microbial communities of plant growth media at beginning of bioassay according to Biolog pattern

Plant growth medium ^y	Diversity index ^z	
	1-G	H'
CC	0.492 c	2.950 b
P	0.637 b	3.181 a
V	0.714 a	3.254 a
GMC	0.697 a	3.124 a

^y CC, composted cork; P, peat; V, vermiculite; GMC, grape marc compost.

^z Within each column, values followed by different letters are significantly different based on Tukey's test at $P < 0.05$.

TABLE 4. Nutrient availability of six plant growth media at the end of bioassay^x

Plant growth medium ^y	Nutrient/growth medium (mg/cm ³) ^z									
	NO ³	P	K	Mg	Ca	Na	Fe (10 ⁻²)	Cu (10 ⁻²)	Zn (10 ⁻²)	Mn (10 ⁻²)
CC	0.123 a	0.196 c	0.196 c	0.842 e	4.787 b	0.139 b	0.31 bc	0.02 d	0.24 b	0.86 b
CC-60	0.085 ab	0.352 b	0.352 b	0.983 c	5.561 b	0.136 b	0.38 abc	0.05 c	0.42 a	1.96 a
P	0.060 ab	0.189 c	0.189 c	0.643 f	2.595 c	0.078 c	0.19 d	0.09 b	0.10 cd	0.13 c
V	0.023 b	0.059 d	0.059 d	0.895 d	1.668 c	0.028 d	0.28 cd	0.14 a	0.05 d	0.18 c
GMC	0.025 b	0.429 a	0.429 a	1.316 a	9.983 a	0.216 a	0.41 ab	0.01 d	0.09 cd	0.81 b
GMC-60	0.025 b	0.431 a	0.431 a	1.177 b	9.462 a	0.201 a	0.44 a	0.01 d	0.12 c	0.90 b

^x Plant growth media were infested with *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 1×10^5 microconidia/cm³.

^y CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; GMC-60, heated grape marc compost.

^z Analysis of variance was performed with transformed data: x^{-2} for K and Mg to obtain a constant variance. Within each column, values followed by different letters are significantly different based on Tukey's test at $P < 0.05$.

TABLE 5. Nutrient status of tomato leaves and stems grown in six plant growth media at the end of bioassay^x

Medium ^y	Nutrient/dry plant (mg/g) ^z										
	N	P	K	Mg	Ca	Na	Fe	Cu (10 ⁻²)	Zn	Mn	B (10 ⁻²)
CC	15.588 bc	21.106 bc	24.261 cd	7.964 ab	31.398 a	1.931 b	0.112 cd	1.173 ab	0.071 bc	0.125 bc	4.129 b
CC-60	17.248 abc	24.588 a	27.893 bc	6.110 cd	31.237 a	1.930 b	0.157 bc	1.601 a	0.127 a	0.306 a	4.469 ab
P	22.775 a	24.567 a	22.483 cd	4.590 d	26.552 ab	2.030 b	0.310 a	1.543 a	0.094 ab	0.120 bc	4.155 b
V	21.616 ab	23.235 ab	20.025 d	8.953 a	22.076 b	3.276 a	0.240 ab	1.806 a	0.062 c	0.103 c	4.208 ab
GMC	9.728 d	17.849 c	34.268 ab	7.176 bc	25.965 ab	1.166 c	0.093 d	0.897 b	0.072 bc	0.084 d	4.155 b
GMC-60	14.357 c	20.603 bc	41.617 a	7.133 bc	27.149 ab	1.507 bc	0.125 cd	1.425 a	0.125 a	0.145 b	4.704 a

^x Plant growth media were infested with *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 1×10^5 microconidia/cm³.

^y CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; GMC-60, heated grape marc compost.

^z Analysis of variance was performed with transformed data: $1/x$ for Fe, Cu, and Mn and $\ln(x)$ for N to obtain a constant variance. Within each column, values followed by different letters are significantly different based on Tukey's test at $P < 0.05$.

DISCUSSION

Based on the correlation between AUDPCs and plant fresh weight, *Fusarium* wilt was the main growth-limiting factor in infested growth media. This relationship has been demonstrated in studies on *Fusarium* wilt of tomato and basil (11,40,45). Our results are consistent with those of other studies where peat was found to be a conducive plant growth medium for several *Fusarium* wilts, whereas successful disease suppression was provided by composts (8,9,11,16,42,44,45,52). Biocide treatment applied to composts eliminated or reduced disease suppression (9,11,16,45,55). In the present study, GMC medium maintained a moderate disease suppression despite heat treatment (60°C), and the same occurred for CC, albeit at a lower level.

The beneficial effects of high pH in reducing disease caused by *Fusarium* spp. in a number of crops, including tomato, are well established (28,61). The growth media evaluated in this study were within a pH range of slightly acid (6.26) to slightly alkaline

TABLE 6. Correlation (simple) among standardized area under the disease progress curve (AUDPCs) and nutrient availability of plant growth media (milligrams of nutrients per cubic centimeters of plant growth medium) and tomato plants (milligrams of nutrients per gram of dry weight) at the end of the bioassay

AUDPCs, element ^y	R ²	P ^z	Equation
Plant growth media			
Na	81.36	*	AUDPCs = $0.54 - 2.04 \times \text{Na}$
Cu	77.23	*	AUDPCs = $-0.006 + 12.96 \times \sqrt{\text{Cu}}$
Ca	77.16	*	AUDPCs = $0.58 - 0.20 \times \ln(\text{Ca})$
Tomato plants			
Fe	98.54	***	AUDPCs = $0.65 - 0.06/\text{Fe}$
N	97.24	**	AUDPCs = $e^{(1.29 - 45.14/\text{N})}$
Cu	93.01	**	AUDPCs = $e^{(1.85 - 0.05/\text{Cu})}$
P	87.69	**	AUDPCs = $e^{(5.56 - 155.53/\text{P})}$
Na	78.74	*	AUDPCs = $e^{(1.02 - 4.68/\text{Na})}$

^y The six plant growth media were inoculated with *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 1×10^5 microconidia/cm³.

^z Significance levels: *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively.

(7.97) at the beginning of the bioassays. The carbon source requirements of *F. oxysporum* have been identified (61). Availability of these carbon sources, and even the loss of endogenous reserves from chlamydospores of *Fusarium* spp., will depend on both their presence in the media and microbial competition for their use (35). The microbial activity of the media is an index related to competition (20,60) and has provided meaningful insights into the microbial carrying capacity of organic soil with respect to the biological control of diseases caused by several soilborne plant pathogens sensitive to microbiostasis (5). Some *Fusarium* wilt-suppressive soils have been characterized for their high degree of microbiostasis derived from high microbial activity (1,53). For the six plant growth media used in this study, when pH and β -glucosidase activity were taken into account as predictive variables (multiple linear regression), more than 91% of the variation in *Fusarium* wilt severity (as measured by AUDPCs) was explained ($P < 0.05$) (Fig. 2). The high correlation coefficient obtained from the integration of a chemical environment, such as pH, and a microbial parameter, such as β -glucosidase activity, with respect to the occurrence of *Fusarium* wilt illustrates their importance in the growth media studied and their predictive potential.

The pH of plant growth medium, as a determinant of *Fusarium* wilt severity, is associated with the availability of macro- and micro-nutrients, important for either growth, sporulation, or virulence of *F. oxysporum* (28). In our study, there was a negative correlation between Ca availability and AUDPCs; this is consistent with other studies (28). The pH reduces the availability of nutrients such P, Mg, Mn, Cu, Zn, and Fe in organic growth media (18), the pathogen being more vulnerable than the host plant (61). In this study, there was a significant positive correlation between AUDPCs and final availability in growth media of Cu, and final nutrient status in plants of Fe, Cu, and P. These results are consistent with the importance of pH as a chemical environmental index of *Fusarium* wilt in tomato (2,11,28,61). In general, more disease is associated with higher rates of nitrogen application (28,61). The correlations between N in plant tissue and AUDPCs are in agreement with the above-mentioned studies. In terms of the relationship between form of nitrogen (nitrates versus ammonia) and *Fusarium* wilt of tomato, several considerations should be noted: (i) daily fertigation was mostly with the ammonium form, (ii) the nitrification process was rapid in all growth media (data not shown), and (iii) the immobilization process by microorganisms was low and similar for the two studied composts (data not shown). Consequently, nitrogen source was a

controlled variable in this study. High Na content in plants is associated with increased disease severity of *Fusarium* wilt in tomato (12). Sodium competes and replaces Ca ions of the polygalacturonate chains of the cell membrane, making the plant more susceptible to *Fusarium* wilt disease (29). In this study, the correlation between Na in plant tissue and AUDPCs was consistent with previous studies (12).

The stages of growth, decline, or persistence of a population of *Fusarium* in soil depend on the ecological balance and nutrient availability (61). The most critical limitation is chemical energy and competition for energy sources present in soil (33). Other microorganisms (bacteria, actinomycetes, and fungi) compete according to the magnitude of their genetically controlled reproductive capacity. Suppression of *F. oxysporum* by competitive microorganisms seems to be mainly due to depletion of available carbon sources, but also to antibiotic production (34). Furthermore, actinomycetes and bacterial populations are favored by high soil pH (58). For the growth media studied, at the end of bioassays a strong negative correlation between populations of different microorganisms and AUDPCs was found for cellulolytic actinomycetes and the ratio of cellulolytic actinomycetes/cellulolytic bacteria. These results are in agreement with the biological control of diseases in growth media formulated with stabilized organic components as determined by the microbial carrying capacity, this being dependent upon the concentration of slow release sources of nutrients for growth and activity such as carbohydrates (hemicellulose, cellulose, and so on) (21,23). Boehm et

TABLE 8. Correlation (simple) among standardized area under the disease progress curve (AUDPCs) and different microorganisms recovered from growth media (CFU/cm³ of plant growth medium)

Microorganism group ^y	R ²	P ^z	Equation
OA	99.31	***	AUDPCs = 1/(2.8635 + 5.8 10 ⁻⁶ ·OA)
OA/OB	99.12	***	AUDPCs = 1/(2.6899 + 2,586.12·OA/OB)
CA	98.50	***	AUDPCs = 1/(3.694 + 0.172 10 ⁻³ ·CA)
CA/CB	98.50	***	AUDPCs = 1/(3.6939 + 8,829.72·CA/CB)
F	80.68	*	AUDPCs = e ^(-0.6941 - 0.0000064 · F)
OB/C	68.90	*	AUDPCs = e ^(-0.7444 - 3.1961 · OB/C)

^y The six plant growth media were infested with *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 1 × 10⁵ microconidia/cm³. OA = oligotrophic actinomycetes, OB = oligotrophic bacteria, CA = cellulolytic actinomycetes, CB = cellulolytic bacteria, F = fungi, and C = copiotrophic bacteria.

^z Significance levels: *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively.

TABLE 7. Microbiological properties of plant growth media at the end of the bioassay^x

Growth media ^y	×10 ⁵ CFU/cm ³ of plant growth medium ^z							
	CA	B	C	FP	CB	OA	OB	F
Controls								
CC	0.1120 ab	3.111 c	2,863 ab	16.17 a	336.3 ab	2.596 b	1,329 a	0.3523 bc
CC-60	0 b	33.63 b	3,686 ab	0.0075 c	1,093 a	9.263 b	3,519 a	1.993 ab
P	0.0373 ab	0.122 d	1,902 b	0.0886 bc	227.5 ab	0.8007 c	504.1 a	1.574 ab
V	0 b	7.480 d	5,956 ab	0.2689 bc	135.2 b	0.0787 d	2,299 a	0.5135 bc
GMC	4.824 a	19.99 b	3,781 ab	0.3026 b	2,127 a	44.21 a	2,728 a	7.270 a
GMC-60	ND	137 a	9,225 a	0.0238 c	402.7 ab	2.878 b	2,687 a	0.0923 c
Infested								
CC	0 b	8.766 ab	6,646	1.109 ab	1,083	2.651 b	3,679	0.9636 b
CC-60	0 b	32.07 ab	9,311	0.7774 ab	688.0	1.354 b	6,206	0.4951 b
P	0 b	1.478 bc	4,526	0.0290 b	671.4	1.082 b	2,089	0.5765 b
V	0 b	0.123 c	1,799	0.0638 ab	227.0	0.0023 c	1,377	0.2788 b
GMC	1.599 a	25.31 a	3,734	0.2308 ab	512.5	49.24 a	4,454	3.706 a
GMC-60	0 b	90.62 a	5,494	2.5614 a	597.6	1.973 b	4,201	2.434 b

^x Control = noninfested media and infested = *Fusarium oxysporum* f. sp. *lycopersici* race 2 at 1 × 10⁵ microconidia/cm³.

^y CC, composted cork; CC-60, heated composted cork; P, peat; V, vermiculite; GMC, grape marc compost; GMC-60, heated grape marc compost.

^z CA = cellulolytic actinomycetes, B = *Bacillus* spp., C = copiotrophic bacteria, FP = fluorescent *Pseudomonas* spp., CB = cellulolytic bacteria, OA = oligotrophic actinomycetes, OB = oligotrophic bacteria, F = fungi. Within each column of controls or infested media, values followed by different letters are significantly different based on Tukey's test at $P < 0.05$. Analysis of variance was performed with transformed data in Ln(x). For cellulolytic actinomycetes (2m)⁻¹, m = number of replicate added to obtain normalized data. ND = not determined.

al. (5) have shown that the concentration of carbohydrates in a suppressive light peat mix decreases as suppressiveness is lost. Tuitert et al. (56) also found that high populations of cellulolytic actinomycetes and the ratio of cellulolytic actinomycetes/cellulolytic bacteria were able to differentiate between organic household waste composts which were conducive to or suppressive of *Rhizoctonia solani*. Workneh and Van Bruggen (62) also recorded positive correlations between the total number of actinomycetes and the proportion of cellulolytic ones with suppressiveness of soil to *Pyrenochaeta lycopersici*. Other strong negative correlations related to suppressive phenomena found in this study involved oligotrophic actinomycetes, oligotrophic actinomycetes/oligotrophic bacteria, and oligotrophic bacteria/copiotrophic bacteria. Tuitert et al. (56) found higher oligotrophic populations in growth media that were suppressive of, rather than conducive to, *R. solani*. Ohta and Hatory (41) suggested that oligotroph life is associated with cellulose decomposers on organic debris. Our results regarding the negative correlation of fungal populations and AUDPCs also are in agreement with those for organic household waste composts suppressive of *R. solani* (56). On the other hand, Kuter et al. (32) found no differences in total numbers of fungi for hardwood bark composts that were suppressive and nonsuppressive of *Rhizoctonia damping-off*; however, there were quantitative differences in the relative abundance of certain taxa which separated suppressive from conducive media amended with hardwood bark compost. The high population densities of *Bacillus* spp. associated with the two composts, either natural or heated, may account for the suppressiveness in our composts. Similarly in a study on potting mix amended with chicken manure, high population densities of *Bacillus* spp. were associated with the survival of seedlings infested with *Phytophthora cinnamomi* (3). The high population densities of fluorescent *Pseudomonas* spp. in CC (controls) may be involved in the suppressiveness of this material (2,14).

The community structures of the plant growth media studied were different at the beginning of the bioassays, based on CLPPs. The similar catabolic potential of CC microbial communities and FOL-2, together with the lower diversity index and the higher β -glucosidase activity and AODC microbial biomass, suggest a high competition for C-sources. These results, along with the low level of disease suppression of heated CC, suggest that control is biological, and basically concerns competition between *F. oxysporum* and endogenous microbial communities. Although the high biological diversity found in vermiculite is noteworthy, its very low microbial activity and biomass should not be overlooked. The high diversity found for this inorganic material can be explained by the fact that vermiculite is almost totally sterile prior to use. The primary source of microorganisms in this medium probably is handling processes, although aerial transmission also may play a role (7). Higher diversity cannot explain disease control in this study, as evidenced by the Shannon diversity index between the conducive peat and the suppressive GMC and the lower 1-G and Shannon index of the suppressive CC with respect to peat. Thus, our results are in agreement with those of Boehm et al. (5) for control of *Pythium* root rot, but not with those of Nitta (39) or Toyota et al. (53), where lower diversity was associated with conduciveness to brown stem rot of bean and *Fusarium* wilt of radish, respectively.

In conclusion, the strong relationship found at the beginning of the bioassays for AUDPCs with pH of growth media and β -glucosidase activity of microbial populations supports its predictive value. The pH reflected the availability of inorganic nutrients in this multiple correlation. For *Fusarium* wilt control, the importance of availability of specific nutrients is demonstrated by fertility management (28) and by the biocontrol induced for Fe competition by fluorescent *Pseudomonas* spp. (2,14). Based on our results, microbial competition (measured as β -glucosidase activity), especially for the C-source present in the media and root exudates

(33,38,60), plays a significant role in disease suppression. The microbial community acts as a nutrient sink that is independent of the microbial community structure in this study. Future research may provide additional information that would allow us to generalize the relationships between pH and β -glucosidase activity as predictive variables for the suppressiveness of other growth media and other *Fusarium* wilt diseases.

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