Carnation Fusarium wilt suppression in four composts

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Abstract Fusarium wilt is now a major disease of carnation crops worldwide. Methyl bromide, which is used to remedy it, is environmentally unsafe. An alternative approach integrated into biological control is to grow crops in suppressive media. Suppressiveness of seven plant growth media to *Fusarium oxysporum* f. sp. *dianthi* was evaluated in bioassays with carnation (*Dianthus cariophyllus*) cv. Medea. These media were: (1) grape marc compost, (2) cork compost, (3) olive oil husk + cotton gin trash composted and mixed with rice husk, (4) spent mushroom compost mixed with peat, (5) coir fibre, (6) light peat and (7) vermiculite. In order to look for carnation Fusarium wilt suppressiveness indicators, growth medium pH and β -glucosidase activity were

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I. Trillas Dept. Biologia Vegetal, Universitat de Barcelona, Facultat de Biologia, Avda Diagonal, 645, E-08028 Barcelona, Spain e-mail: mtrillas@ub.edu evaluated. Furthermore, *F. oxysporum* populations were measured in plant growth media at the beginning and end of bioassays. The compost media showed a range of suppressiveness in comparison with peat. Grape marc compost was the most effective plant growth medium in suppressing carnation Fusarium wilt. On the other hand coir fibre, peat and vermiculite were conducive for this disease. β -glucosidase activity and pH were positively correlated with disease severity as in other reports for tomato. Therefore, these two parameters are good indicators for carnation Fusarium wilt suppressiveness, and possibly for other *F. oxysporum* pathosystems. All composts showed similar *F. oxysporum* populations at the end of the bioassays to peat and vermiculite.

Keywords Biological control · Compost · *Dianthus cariophyllus · Fusarium oxysporum* f. sp. *dianthi* · Soilless

Introduction

The most important phytopathological problem affecting carnations (*Dianthus cariophyllus*) in most areas of the world is Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *dianthi* (Fod). Infected plants wilt readily, lower leaves become yellow and dry, the xylem tissues turn brown, and the plant may die. The infection is at first one-sided. Fusarium wilt is prevalent in SW Spain, the site of 79% of the national production area of carnations and 45% of total production (Anonymous 2006). Susceptible cultivars suffer severe yield losses, and considerable yield losses are also common in carnation cultivars previously described as resistant (Prados-Ligero et al. 2007). The crop is mainly produced as a monoculture in greenhouses; rooted cuttings are planted in late spring (May to June) and removed after 22 to 23 months before preparation of the soil for new plantings. Consequently, populations of soilborne plant pathogens often increase to unacceptable levels (Prados-Ligero et al. 2007).

Due to large crop losses, growers recently relied on soil fumigation with chemicals like methyl bromide. However, methyl bromide is banned even for critical uses, because of its environmental risks. Therefore, other culture methods have to be developed and tested. Carnations can be cultured in containers or bags using plant growth media where fumigation is not needed (Pizano 2001). However, these soilless cultures are exposed to potential inoculum sources: infected cuttings, air dispersion propagules or infested soil or pruning tools. A rapid technique to identify suppressive plant growth media before use could facilitate the choice of such media. Two of the growth media most widely used in Spain are peat and coir fibre. However, media formulated with composts can suppress different formae speciales of Fusarium wilt in comparison to peat. These formae speciales are Fusarium wilt of carnation (Cebolla and Pera 1983; Orlikowski 1983; Pera and Calvet 1989), chrysanthemum (Chef et al. 1983), cyclamen (Garibaldi 1988), flax (Chef et al. 1983), iris (Garibaldi 1988), radish (Trillas-Gay et al. 1986), sweet basil (Reuveni et al. 2002) and tomato (Szczech 1999; Cotxarrera et al. 2002). We have also previously evaluated the suppressiveness to tomato Fusarium wilt of four composts in comparison to peat and vermiculite (Trillas et al. 2002; Borrero et al. 2004, 2005). The pH and microbial activity of the composts were used to predict their suppressiveness to tomato Fusarium wilt (Borrero et al. 2004).

Our study objectives were to determine: (1) the capacity of four compost plant growth media as well as peat, coir fibre and vermiculite to suppress carnation Fusarium wilt, (2) whether plant growth medium pH and microbial activity indicate carnation disease suppressiveness.

Materials and methods

Plant growth media

Four composted residues from agricultural industry waste were evaluated for carnation Fusarium wilt suppression: (1) cork compost (CC) from cork (Quercus suber) transformation, (2) grape marc compost (GMC) from the alcohol distilling industry (grape skins, seeds and stems) and (3) olive oil husk + cotton gin trash, 1:1 v/v, composted and mixed with rice husk (1:1 v/v) (OC + R). These three residues were composted according to previously described procedures (Trillas et al. 2002). The fourth plant growth medium (4) was spent mushroom compost (Recomsa, Quintanar del Rey, Spain) mixed with amended light peat (Klasmann, Valimex, Palleter, Spain) (1:1 v/v) (SM + P). Composts were compared with three plant growth media that are widely used in Spain: fertilised and amended Sphagnum light peat (Klasmann, Valimex, Palleter, Spain), coir fibre (Cocopeat, Projar, Valencia, Spain) and expanded vermiculite (Vermiculita y derivadas, Gijón, Spain). Peat was amended with 4 g l^{-1} CaCO₃ and fertilised with 0.33 g l^{-1} of K₂O (50% high solubility granulated; Compo Agricultura S.L., Barcelona, Spain) and 4.15 g l^{-1} of P₂O₅ (18% granulated; Fertiberia, Madrid, Spain). To standardise the initial conditions, the plant growth media were adjusted to 50% humidity reached at a water tension of 1,000 Pa (adjusting water content by weight) for 14 days at 25°C.

Assessment of disease severity

Disease suppressive properties of plant growth media were measured by a Fusarium wilt bioassay. The bioassays were developed with the susceptible carnation cultivar Medea and a monosporic isolate of Fod. This isolate was obtained from an infected carnation plant and stored in silica gel. The pathogenicity of this isolate was confirmed in a previous assay. Fod was grown for 7 days in AMAP culture media: agar 10 g l⁻¹, malt extract (Difco, Le Pont de Claix, France) 10 g l⁻¹, asparagine (Difco, Le Pont de Claix, France) 2 g l⁻¹, Peter's foliar feed (27 + 15 + 12; N + P₂O₅ + K₂O; and micronutrients; Scotts, Heerlen, The Netherlands) 0.5 g l⁻¹. Five ml of sterile distilled water (SDW) was added to each culture plate. The surface of the culture was scraped with a sterile spreader. The suspension of Fod was transferred to MAP liquid culture (as described above, but without agar) and grown with continuous agitation (130 rev min⁻¹) for 10 days at 25°C. After that, liquid culture was filtered with two layers of cheesecloth with the help of a vacuum pump. Conidial suspension was recovered after filtration and centrifuged at $5.000 \text{ rev min}^{-1}$, 15 min, (Eppendorf 5810 R, Hamburg, Germany) three times rinsing with SDW. The concentration of conidia was determined with a hemocytometer. The seven plant growth media were infested with Fod (6.5×10^4) conidia ml^{-1} plant growth medium), mixed vigorously and poured into plastic pots (1 1 volume). Pots of growth media without Fod were prepared as controls. Four bare-root carnation cuttings (8 to 12 true-leaf stage) grown in perlite were planted into each pot. Plants were irrigated as needed and fertilised with a nutrient solution containing: 0.5 g l^{-1} Peter's foliar feed, 0.6 g l^{-1} CaCl₂, 0.7 g l^{-1} MgSO₄.7H₂O and 0.3 g l^{-1} urea (pH 6.1). Plants were grown in a growth chamber (27°C, photosynthetically active radiation intensity 280 $\mu E s^{-1}m^{-2}$ and 16:8 h light: dark photoperiod). Bioassays were performed three times with five pots per treatment. Treatments were arranged in a randomised block design. Disease severity was monitored at 2-day intervals for 55 days after planting, and was scored on a symptom severity scale described by Baayen and Van der Plas (1992), where: 0 =asymptomatic plant (0% disease); 1 = weakly infected plant (5% disease); 2 = local base-stem symptoms (20%); 3 = unilateral and well developed symptoms (50%); 4 = strong disease symptoms throughout the plant (80%); 5 = dead plant (100%). The symptoms evaluated were wilted leaves and stems. At each assessment, mean disease severity per pot was calculated. Disease severity was expressed as the proportion of the maximum possible disease severity. The standardised area under the disease progress curve (AUDPC) per pot was calculated by disease severity integrated between symptoms onset and bioassay final time and divided by the total duration (days) of the epidemic in each bioassay; in this way, the various bioassays, which had a variety of epidemic durations, could be compared (Campbell and Madden 1990). 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 mean fresh and dry weight of leaves and stems per pot were recorded. These values were considered as replicates for each observed variable.

Fusarium oxysporum populations

The density of F. oxysporum was determined by dilution plating on a semi-selective medium (Komada's medium, Dhingra and Sinclair 1995). Samples were taken from incubated plant growth media at the beginning of the bioassays and from the rhizosphere at the end of the three bioassays. Plant growth media (0.5-1 g) were suspended in 10 ml of 0.2% water agar. The suspension was shaken and a tenfold dilution series was prepared with 0.2% water agar. Suspensions were pipetted onto three plates per dilution. Four dilutions per series were placed on plates. Colony forming units (CFU) were counted 4 days after plating and expressed as CFU ml^{-1} of plant growth media. Analyses were performed three times with one sample per plant growth medium from each of the three bioassays. Mean groups with homogeneous variance were studied separately for comparison of means.

Plant growth media characteristics

β-glucosidase activity and pH measurements were performed in incubated growth media at the beginning of the three bioassays. Plant growth medium pH was measured in a water extract (2:1; v/v), as described elsewhere (Gabriëls et al. 1991). Three samples were analysed for each growth medium. Microbial activity was estimated by measuring βglucosidase activity, following Bandick and Dick (1999). This method is based on colorimetric determination of the *p*-nitrophenol released by β -glucosidase when the plant growth medium was incubated with 4-nitrophenyl- β -D-glucopyranoside (pH 6.0). The p-nitrophenol released was extracted by filtration through Albet folded filter paper DF 413 150 (Albet, Sant Boi de LLobregat, Spain) and determined colorimetrically at 410 nm. Four samples were analysed for each plant growth medium and bioassay.

Statistical analysis

Data collected from the three bioassays were analysed with Statgraphics Plus (version 5.1; Statistical Graphics Corp., Rockville, MD, USA, 2002). The effect of growth medium on AUDPC, RLSBX, height, fresh and dry weight, pH, and microbial activity was analysed with ANOVA. Significant means were compared by appropriate tests ($P \le 0.05$). Overall relationships between AUDPC or RLSBX and continuous measured variables were analysed with regression analysis.

Results

Suppressiveness of plant growth media

Both severity indices (AUDPC and RLSBX) indicated that the four composts had suppressive effects on Fusarium wilt in comparison to peat, vermiculite, and the coir fibre, which were conducive to disease (Fig. 1). This disease was suppressed most effectively with GMC while the most conducive plant growth media were coir fibre and peat, based on AUDPC. GMC reduced disease by 99% in comparison to the conducive media (Fig. 1A). Medium OC + R was no different from GMC for disease severity measured by RLSBX, but reduced disease by 80% with respect to peat and coir fibre (Fig. 1B). Height, fresh and dry weight of carnation plants (Fig. 2) showed opposite responses compared to the severity indices (Fig. 1). The GMC medium had the tallest plants, followed by OC + R plants (Fig. 2A). However, fresh and dry weights were not significantly different in these two composts (Fig. 2B,C).

Plants grown in plant growth media not inoculated with Fod did not show signs of weaknesses or toxicity during cultivation, and they developed properly. There were no significant differences in plant height between plant growth media (Table 1). For plant dry weight, there were only differences between plants grown in CC and coir fibre (Table 1). For fresh weight, only plants grown in peat and coir fibre had a higher fresh weight than CC, SM + P or vermiculite (Table 1). Except for these cases there were no differences in agronomic parameters between plant growth media. However, non-inoculated peat and coir fibre showed good height, fresh and dry weight while inoculated peat and coir fibre gave poor plant growth. This indicates that the most limiting factor for plant development in inoculated media is Fusarium wilt severity. None of the plants grown in the seven noninfested control growth media developed symptoms of Fusarium wilt.

The strongest negative correlations between disease severity variables (AUDPC and RLSBX) and the



Fig. 1 A, B Standardised AUDPC and RLSBX for carnation plants. *CC* composted cork, *GMC* grape marc compost, *OC* + *R* olive oil husk + cotton gin trash composted and mixed with rice husk, *SM* + *P* spent mushroom compost mixed with peat. Plant growth media were infested with *F. oxysporum* f. sp. *dianthi*. Disease severity scale was from θ asymptomatic plants, to 5 dead plants. Data for AUDPC and RLSBX were transformed for analysis with the arcsine \sqrt{x} . For each square, bars with the same letter were not significantly different according to Tukey's test at *P*<0.05. SE of the mean (*n*=5) is indicated by a vertical line

height, fresh and dry weight of leaves and stems (data not shown) were obtained for RLSBX. These correlations were found between RLSBX and the square root of height (R^2 =0.79, P<0.001, n=105), RLSBX and the square root of fresh weight (R^2 =0.91, P<0.001, n=105) and RLSBX and the square root of dry weight (R^2 =0.84, P<0.001, n=105). Consequently, RLSBX was selected for further correlation analysis with the different variables measured.

Fusarium oxysporum population densities

Composts showed significantly lower *F. oxysporum* densities at the end of the bioassays than at the



Fig. 2 A–C. Height, fresh and dry weight for carnation plants. For abbreviations, see Fig. 1. Plant growth media were infested with *F. oxysporum* f. sp. *dianthi*. Data for fresh and dry weights were transformed for analysis with the \sqrt{x} . For each square, bars with the same letter were not significantly different according to Tukey's test at *P*<0.05. SE of the mean (*n*=5) is indicated by a vertical line

beginning, unlike coir fibre, peat and vermiculite, in which the population density did not change (Fig. 3). Vermiculite had a low density of *F. oxysporum* at the beginning of the bioassay while SM + P had the highest population (Fig. 3). At the end of the bioassay coir fibre had the highest *F. oxysporum* population of

all the media, except peat (Fig. 3). There was a positive correlation between AUDPC and *F. oxy-sporum* population density at the end of the bioassays $(R^2=0.68, P<0.001, n=21, AUDPC=1.35436-9336.48/F. oxysporum density).$

Plant growth media properties before the bioassays

Composts had higher pH and β -glucosidase activity than the other media (Table 2). Coir fibre and peat had the lowest pH and low β -glucosidase activity (Table 2). The OC + R medium had the highest and SM + P the lowest β -glucosidase activity of the four composts tested (Table 2). A multiple linear regression was found between RLSBX, pH and β -glucosidase activity in the seven plant growth media. These two properties explained >67% of the variation in RLSBX (*P*<0.000, *n*=21). The resulting equation was RLSBX=2.457– 0.000458 microbial activity–0.267 pH.

Discussion

The correlation between pH and β -glucosidase activity with respect to tomato Fusarium wilt severity (AUDPC) reported by Borrero et al. (2004) is similar to that found here with RLSBX and carnation Fusarium wilt. This confirms and generalises the value of pH and β -glucosidase activity as suppression indicators for plant growth media.

The suppression of Fusarium wilt of carnation in CC and GMC media in comparison to peat and vermiculite is similar to that reported for tomato and two races of F. oxysporum f. sp. lycopersici (Borrero et al. 2004, 2006). In the present study, the suppressiveness of these composts was extended to another formae speciales (F. oxysporum f. sp. dianthi). For both Fusarium wilts, GMC was the most suppressive. The carnation Fusarium wilt suppressiveness registered for OC + R and SM + P was also established for tomato Fusarium wilt (Borrero et al. 2005). Our results are consistent with those of other studies, where peat was found to be conducive for several Fusarium wilts, while successful disease suppression was provided by composts (Noble and Coventry 2005).

The high Fusarium wilt severity in carnations grown in coir fibre was similar to that found in

Plant growth medium ^a	Height (cm) ^b	Fresh weight (g) ^b	Dry weight (g) ^b
Coir fibre	28.3±2.3 a	13.59±0.80 a	2.00±0.10 a
Peat	25.4±2.6 a	12.14±0.67 a	1.87±0.11 ab
Vermiculite	23.9±2.5 a	8.47±0.90 b	1.64±0.12 ab
CC	18.4±2.5 a	7.85±0.68 b	1.46±0.11 b
GMC	24.8±1.9 a	10.71±0.46 ab	1.86±0.08 ab
OC + R	21.8±1.6 a	10.48±0.37 ab	1.89±0.07 ab
SM + P	17.1±1.5 a	8.10±0.41 b	1.53±0.09 ab

Table 1 Plant height and fresh and dry weight at the end of the bioassays for six growth media without pathogen inoculation

^a For abbreviations, see Fig. 1.

^b Mean values and standard errors followed by different letters are significantly different based on Tukey's test at P < 0.05, n=15. Data were transformed for analysis with the log(x) function.

tomato (Borrero et al. 2005). Coir fibre mixed with peat was also conducive to cyclamen Fusarium wilt (O'-Neill and Finlay 1996). This indicates that coir fibre, instead of being a usual alternative to peat, does not prevent Fusarium wilt due to the low pH and β -glucosidase activity, as in peat and vermiculite. In this sense, the lower microbial activity and pH in SM + P in comparison with the other composts may be due to the proportion of peat in the mixture (1:1, ν/ν).

At the end of composting, the olive oil husk + cotton gin trash had a high ammonium/nitrate ratio (data not published). The severity of Fusarium wilts decreases with NO₃–N and increases with NH₄–N fertilisation (Jones et al. 1993). Bark composts



Fig. 3 *Fusarium oxysporum* populations at the beginning and end of the bioassays. For abbreviations, see Fig. 1. Plant growth media were infested with *F. oxysporum* f. sp. *dianthi*. Points with the same letter were not significantly different according to LSD test at P < 0.05. SE of the mean (n=3) is indicated by a vertical line

immobilise nitrogen, preferentially as ammonia. Most of the nitrogen available for plant growth is thus in the form of nitrates. The impact of bark composts on nutrition therefore affects the severity of Fusarium wilt (Hoitink et al. 1991). This nitrogen dynamics characterises CC and GMC (Carmona et al. 2004). Sludge composts release ammonia upon mineralisation and enhance Fusarium wilts (Hoitink et al. 1987). The OC + R also release ammonia (Carmona et al. 2004), so Fusarium wilt is favoured with respect to the other composts. In the present study OC + Rshowed the highest β -glucosidase activity and one of the highest pH values. With these properties OC + Rcould be expected to be the most suppressive plant growth medium, but it was not. This can be explained by the high ammonium/nitrate ratio.

One consequence of suppressiveness in composts is low pathogen activity. This is reflected in the lower inoculum density of the phytopathogen at the end of the bioassays than at the beginning (this was not the case in the conducive plant growth media) (Fig. 3). However, propagules do not disappear in composts, even in the case of the more suppressive compost (GMC). This phenomenon indicates that the biocontrol mechanism in composts may be associated with microbiostasis. This mechanism is related to a slow reduction of the survival of propagules (Lockwood 1988). This interpretation is consistent with the model proposed.

High values of microbial activity and biomass are associated with microbiostasis (Hoitink et al. 1996). This phenomenon prevents germination of spores of nutrient-dependent germination pathogens (Lockwood 1988). Furthermore, the microbial activity of the media is related to competition (Hoitink et al. 1991;

lant growth medium ^a pH ^b		β -glucosidase activity ^b (µg hydrolysed <i>p</i> -nitrophenol cm ⁻³ h ⁻¹)	
Coir fibre	5.66±0.01 e	32.562±2.184 d	
Peat	5.29±0.19 e	30.252±3.152 d	
Vermiculite	6.54±0.09 d	1.567±0.603 e	
CC	7.76±0.01 ab	91.772±10.268 b	
GMC	7.47±0.00 bc	83.998±3.113 b	
OC + R	7.92±0.01 a	252.201±4.401 a	
SM + P	7.14±0.02 c	59.103±8.655 c	

Table 2 Plant growth media pH and β -glucosidase activity at the beginning of the bioassays

^a For abbreviations, see Fig. 1.

^b Mean values and standard errors followed by different letters are significantly different based on Tukey's test at P < 0.05, n=3 for pH and n=12 for β -glucosidase activity.

Weller et al. 2002) and may reduce disease intensity caused by several soilborne plant pathogens (Boehm et al. 1997; Borrero et al. 2004). Some Fusarium wilt-suppressive soils have shown a high degree of microbiostasis derived from high microbial activity (Alabouvette et al. 1996). High β -glucosidase activity can indicate competition for carbon compounds and may therefore be a microbiostasis indicator. Termorshuizen et al. (2006), working with amendment of compost to a steam-sterilised loamy soil found disease suppression for *F. oxysporum* f. sp. *lini*/flax for 18 different composts, except for that with the lowest level of basal respiration. However, some of the composts/soils that are suppressive also have very low levels of basal respiration.

The stages of growth, decline or persistence of a population of Fusarium in soil depend on the ecological balance and nutrient availability (Jones et al. 1993). Our results indicate that environmental conditions in composts are unfavourable to this pathogen. In this sense, it was confirmed that high pH is a favourable condition for Fusarium wilt suppression (Jones et al. 1993; Borrero et al. 2004). In the pH range used in this study, growth media per se conditioned the availability of nutrients, even when the same fertilisation regime was used. At high pH, nutrients important for Fusarium, such as Cu, Fe, P or Mg, are less available (Handreck and Black 1991; Jones et al. 1993; Borrero et al. 2004). The pH of plant growth media, as a determinant of Fusarium wilt severity, is associated with the availability of macroand micro-nutrients, important for growth, sporulation or virulence of F. oxysporum (Jones et al. 1993). Furthermore, actinomycetes and bacterial populations (related to suppressiveness) are favoured by high soil pH (Jones et al. 1993). These results are consistent with the importance of pH as an environmental index of Fusarium wilt (Jones et al. 1993; Alabouvette et al. 1996; Cotxarrera et al. 2002). In contrast, Termorshuizen et al. (2006) did not find a relationship between flax Fusarium wilt suppressiveness and pH of soil amended with composts. Van der Gaag et al. (2007) also did not find a relationship between cyclamen and begonia Fusarium wilt and pH working with composts, although these authors attempted to match the pH of peat mixes working in a narrow pH range (5.0–5.9 in water extract 1:1.5 v/v).

These kinds of suppressiveness mechanisms, which are associated with abiotic factors and total microbial activity in plant growth media or soil, promote general suppression. On the other hand specific suppressiveness is promoted by one or only a few microorganisms (Weller et al. 2002). General and specific mechanisms of suppression of Fusarium wilts have been reported (Alabouvette et al. 1996). In this sense, three composts (CC, GMC and SM + P) have shown both general and specific suppression, partially lessening tomato Fusarium wilt suppression by heat treatment (Borrero et al. 2004, 2005). This specific suppression is not always related to high pH and β -glucosidase activity. This explains why the multiple correlation found was not higher.

The multiple correlation found may provide information that will allow us to generalise on the relationships between pH and β -glucosidase activity as indicative variables for the suppressiveness of plant growth media to Fusarium wilt diseases. Soilless cultures with peat or coir fibre are, as stated above, susceptible to Fusarium wilt. Furthermore, these plant growth media in Spain and other areas are imported, and peat comes from valuable ecosystems. On the other hand, the management of wastes generated by agriculture is a complex problem. Composting is one of the most desirable techniques for reducing waste volume. Therefore, the use of suppressive compost for plant growth media can solve these problems. The pH and β -glucosidase activity could become useful tools for the identification of Fusarium wilt suppressive composts.

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