

## AUTOTOXICITY OF DECAYING TOMATO RESIDUES AFFECTS SUSCEPTIBILITY OF TOMATO TO *FUSARIUM* WILT

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### SUMMARY

*Fusarium oxysporum* f.sp. *lycopersici* (FOL) induces wilting in tomato plants (*Lycopersicon esculentum*). This disease causes large losses and it increases when practices include monoculture or limited crop rotation. Under these conditions, large quantities of tomato plant residues decompose in the field and may affect growth of tomato and FOL and their interactions. Here we show that undecomposed tomato residues are phytotoxic in laboratory and greenhouse bioassays and that leaves are more phytotoxic than roots. During decomposition, phytotoxicity of leaves and roots decreases under aerobic conditions, but increases under anaerobic conditions. On the contrary, FOL radial growth and hyphal density are increased by undecomposed leaves and roots, but decreased with aerobically decomposed plant material. Tomato wilting caused by FOL increased when the soil was amended with undecomposed leaves. Our study shows that, under controlled conditions, undecomposed tomato residues affect the growth of tomato (autotoxic effect) and FOL (substrate effect), by causing an increase in incidence of the disease.

**Key words:** aerobic-anaerobic decomposition, *Fusarium oxysporum* f.sp. *lycopersici*, phytotoxicity, soil-borne pathogens.

### INTRODUCTION

Soil-borne plant pathogens that cause root and crown rots, wilts, and damping-off are among the major factors limiting the productivity of many crops. Plant-pathogen interactions are affected by soil organic matter (OM) (Hoitink and Boehm, 1999), likely by the release of allelochemical compounds during decomposition (Van der Putten *et al.*, 1997; Blum *et al.*, 1999). These compounds can be absorbed and polymerized with soil

organic matter and clay minerals (Makino *et al.*, 1996), but also transformed by the activity of soil microorganisms (Blum *et al.*, 1999; Bonanomi *et al.*, 2006a). Changes in the composition and quantity of allelochemicals can either increase or decrease the phytotoxicity of decomposing OM (An *et al.*, 2001). Phytotoxicity of decomposing plant residues has been documented for several crop and plant species under aerobic and anaerobic conditions (Patrick, 1971; Bonanomi *et al.*, 2006a). In general, phytotoxicity that is high in the early stages of decomposition remains high under anaerobic conditions, but declines in the presence of oxygen (Patrick, 1971; Bonanomi *et al.*, 2005).

Many studies report that OM may have either a positive effect on soilborne pathogens by providing the substrate for their saprophytic growth (Croteau and Zibilske, 1998; Manici *et al.*, 2004; Bonanomi *et al.*, 2006b), or a negative effect by causing fungistasis (Lockwood, 1977) or releasing fungitoxic compounds (Smolinska, 2000). Moreover, OM decomposition influences the composition of the bacterial community in the soil and the activity of biocontrol agents (Hoitink and Boehm, 1999; Mazzola, 2004). For example, soil amendment with fresh organic materials may increase the occurrence of soilborne pathogen diseases (Hoitink and Boehm, 1999; Bonanomi *et al.*, 2006b). In other cases, soil amendment with decomposed OM was found to be suppressive of disease (Hoitink and Fahy, 1986; Szczech, 1999).

The occurrence of stress conditions such as water stress, low levels of dissolved oxygen, nutrient imbalance or presence of phytotoxic compounds can greatly increase the susceptibility of plant roots to soil-borne pathogens (Agrios, 2005). Phytotoxic compounds that can be released by roots and decomposing OM, may positively affect the activity of soil-borne pathogens by reducing plant resistance (Patrick *et al.*, 1963; Nigh, 1990; Zucconi, 1996; Ye *et al.*, 2004).

*Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a serious disease both in the field and greenhouse, and control measures include the use of fumigants, soil steaming, resistant cultivars and biological control agents such as non-pathogenic soil isolates of *F. oxysporum* (Fravel *et al.*, 2003). Agronomic practices such as monoculture or limited crop ro-

tation increase the incidence of the disease, probably due to the rapid build-up of the FOL soil inoculum due to the infected plant residues. The incidence of wilt diseases depends on temperature (Larkin and Fravel 2002) and soil properties such as pH, texture, nutrient availability and OM content. However, the ecology of the saprophytic life stage of FOL in relation to OM is not clear. Soil amendments with vermicompost (Szczech, 1999) and cruciferous plant residues (Smolinska, 2000) have been found to be suppressive towards FOL, but there are no studies about the effects of tomato residues on disease development.

In this work we studied the effects of decomposition of tomato residues (leaves and roots) on the growth of tomato and FOL and their interaction. Based on previous work with other plant species (Bonanomi *et al.*, 2006a; Bonanomi *et al.*, 2006b), the hypotheses were: (i) undecomposed tomato residues are phytotoxic, but during decomposition their toxicity can either decrease or increase under aerobic and anaerobic conditions, respectively; (ii) undecomposed tomato residues allow saprophytic growth of FOL; (iii) soil amendment with fresh tomato residues increases the incidence of tomato wilt.

## MATERIALS AND METHODS

**Decomposition of tomato residues.** Tomato (cv San Marzano) leaves and roots were collected from the field in April 2004 (Campania Region, Southern Italy). Immediately after harvest, the material was slowly dried (30°C for 10 days), chopped with scissors (size <1 cm), and stored at room temperature.

The residues, leaves and roots separately, were decomposed in the laboratory by placing the dry plant materials in 2-l beakers with distilled water (5% dry weight - 50 g l<sup>-1</sup>) at 26±4°C for 30 days. A 10 ml soil inoculum (a 10% w/w mixture obtained by diluting in water 1 g of the field soil in which tomato plants was cultivated) was added to the organic matter. Aerobic conditions were obtained by pumping air into the mixture, while anaerobic conditions were obtained by keeping the beakers closed. Distilled water was added to compensate for evaporation. This method of decomposition was used because it is rapid, easily reproducible, and allows a standardization that avoids the effects of varying soil type (Zucconi *et al.*, 1981; Bonanomi *et al.*, 2006a). The experimental decomposition in water can be considered comparable to those occurring in the field, because the soil microbial community always operates in thin water films that surround solid particles or are inside soil aggregates (Stotzky, 1997; Nannipieri *et al.*, 2003).

Samples of the aqueous suspension were collected 5 h after the start of the experiment and then at 5, 10, 20 and 30 days. Samples were centrifuged (2935 g for 10 min), sterilised (micro-filtration with 0.22 µm pore fil-

ters), diluted in distilled water to three concentrations (50, 16.6 and 5 g l<sup>-1</sup>) and stored at -20°C. Electrolytic conductivity (EC) and pH of aqueous extracts were measured at each time to check on the progress of decomposition.

**Autotoxicity and phytotoxicity of the extracts.** The toxicity effect of the extracts was assessed by measuring root elongation of tomato (cv San Marzano) and *Lepidium sativum*. The latter species was used because it is extremely sensitive to phytotoxic substances (Zucconi *et al.*, 1981; Gehringer *et al.*, 2003).

The assays were conducted in a growth chamber at constant temperature (27°C in the dark). Twenty seeds of each species were placed on a sterile filter paper wetted with 4 ml of test solution in a 9 cm Petri dish. The experiments were done with three different concentrations (50, 16.6 and 5 g l<sup>-1</sup>) of leaf or root extracts from aerobic or anaerobic samples. Each experiment was replicated 10 times and distilled water was used as the control.

Another assay with the same experimental design tested the effects of pH (4.3, 5.5, 6.3, 7.0, 8.0 and 9.5) and EC (0.1, 1.8, 6.2, 8.1, 11.2, 14.5 and 20.5 mS/cm) on tomato root elongation. Different pHs were obtained using MES buffer (Sigma-Aldrich, Steinheim, Germany) adjusted with 1M NaOH. Different ECs were obtained by increasing the amount of NaCl in the solution (Macias *et al.*, 2000). A totally randomised experimental design was used and root length was measured after 36 hours from germination for *L. sativum* and 6 days for tomato. Data were expressed as percent of inhibition of root elongation compared to controls.

**Tomato extract effect on FOL growth.** Extracts from three sample times (5 h, 10 and 30 days) for both leaves and roots from aerobic and anaerobic conditions were tested on the saprophytic growth of FOL. Media were prepared by mixing agar, sterile water and sterile tomato residue extracts to obtain three dilution levels (50%, 10% and 0%). Fifteen ml of each medium were placed in a 9-cm Petri dish. FOL inoculum was prepared by growing the fungus on PDA (Potato dextrose agar, DIFCO) at 21°C. After 10 days, 10 ml of sterile water were added and the culture surface was scraped to remove conidia. The suspension was filtered, centrifuged, washed twice with sterile water and adjusted to a concentration of 10<sup>5</sup> conidia ml<sup>-1</sup>. After 24 hours from substrate preparation a drop of 5 µl of conidial suspension was applied on the centre of a Petri dish containing the extract media. Radial mycelium colony growth was measured every 24 h for 7 days. After 7 days, hyphal density of each colony was measured on five randomly chosen points by counting the number of hyphae crossing a 1 mm line. Five replications were used for each treatment and the experiment was repeated twice. Additional mineral or organic-rich supplements were not

used in this test in order to verify if tomato extracts could sustain FOL saprophytic growth.

**Effect on FOL wilt of amending soil with tomato residues.** In this experiment the effect of soil amendment with undecomposed tomato leaves on FOL disease incidence was tested. Tomato seeds of the susceptible cultivar San Marzano were planted in a sterile potting mix (autoclaved twice) and grown in seedling plug trays (plug size  $2 \times 3 \times 5$  cm, 180 plug per tray). Inoculum was prepared as described above and the concentration adjusted to  $10^5$  and  $10^6$  conidia  $\text{ml}^{-1}$ . After 10 days, seedlings were transplanted and infected by a standard root-dip inoculation method (Larkin and Fravel, 2002). Roots of uprooted seedlings were submerged in the conidial suspensions for 10 min and then planted in pots (12 cm diameter, three plants/pot) filled with field soil. All experiments were carried out with non-sterilized sandy-clay soil with the following characteristics: pH = 7.1, OM = 2.3%, total N =  $1.42 \text{ g kg}^{-1}$  with C/N of 9.4,  $\text{P}_2\text{O}_5$  =  $156 \text{ mg kg}^{-1}$ ,  $\text{K}_2\text{O}$  =  $960 \text{ mg kg}^{-1}$ , Ca =  $1975 \text{ mg kg}^{-1}$ , Na =  $72 \text{ mg kg}^{-1}$ , Fe =  $35.6 \text{ mg kg}^{-1}$ , Mg =  $467 \text{ mg kg}^{-1}$ , Cu =  $3.5 \text{ mg kg}^{-1}$ , Zn =  $3.2 \text{ mg kg}^{-1}$  and Mn =  $18.4 \text{ mg kg}^{-1}$ . Tomato leaves were added to the potting media as dry material. The experimental design included soil amended with four levels of dry leaves (0%, 1%, 3% and 5% w/w) and three levels of soil inoculum (no inoculum,  $10^5$  and  $10^6$  conidia  $\text{ml}^{-1}$ ). Each treatment was replicated ten times for a total of 120 pots, arranged in a greenhouse to a fully randomised design. The distribution was randomized again weekly to avoid microclimate effects. Plants were watered daily to field capacity and grown for 25 days ( $22^\circ\text{C}$  day,  $15^\circ\text{C}$  night). Disease symptoms were monitored at 10, 17 and 21 days after inoculation and plants were classified into three groups, healthy, wilted and dead. After 25 days the plants were harvested and the root system washed under tap water. Total plant weight (root and shoot) was measured after drying for 72 h at  $80^\circ\text{C}$ .

**Data analysis.** Data were analysed statistically using standard analysis of variance (ANOVA) and significance was evaluated at  $P < 0.05$ . Four-way ANOVA was performed to test the main effects and interactions of decomposition conditions (aerobic vs. anaerobic), plant materials (leaves vs. roots), decomposition time and extract concentration on the inhibition of *L. sativum* and tomato root growth separately. The same four-way ANOVA was applied to test the main effects and interactions on FOL hyphal density. One-way ANOVA was applied to test the effects of pH and EC on tomato root elongation. Finally, two-way ANOVA was performed to test the main effects and interactions of FOL inoculum (three levels) and addition of tomato residues (four levels) on the growth of tomato plants in the pot bioassay.

## RESULTS

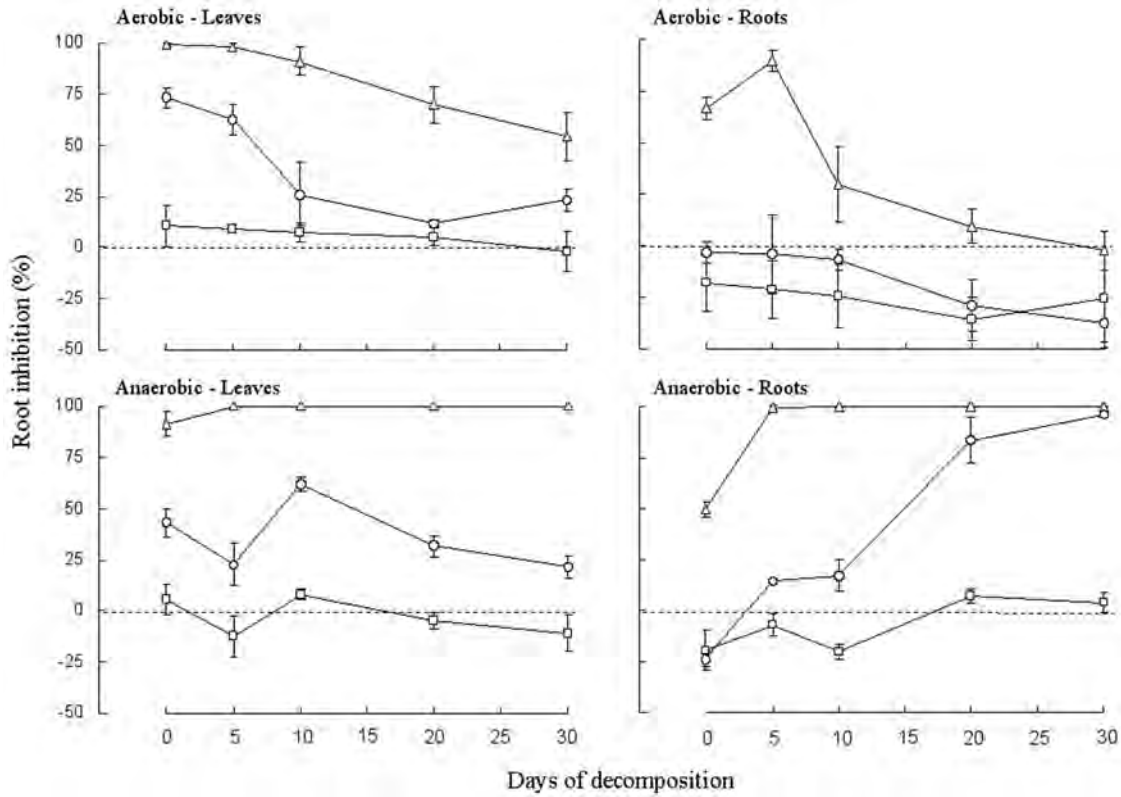
**Autotoxicity and phytotoxicity of the extracts.** Inhibition of root growth of *L. sativum* and tomato was significantly affected by the decomposition conditions, plant material, decomposition time and extract concentration (Table 1). In both species, an increase in extract concentration caused a proportional increase in root growth inhibition (Figs. 1-2).

Aerobic and anaerobic decomposition conditions produced differences in phytotoxicity. At the start of decomposition, phytotoxicity was high for both *L. sativum* and tomato. However, in aerobic conditions phytotoxicity and autotoxicity progressively decreased during decomposition for both the leaf and root materials (Figs. 1-2). Undecomposed leaf extracts were slightly more phytotoxic and their phytotoxicity decreased more slowly than that of roots (Figs. 1-2). In contrast, when conditions were anaerobic, toxicity remained very high for concentrated extracts, and increased for lower extract concentrations during decomposition for roots (Figs. 1-2). However, in anaerobic conditions phytotoxicity and autotoxicity were high for both plant materials reaching comparable levels after thirty days.

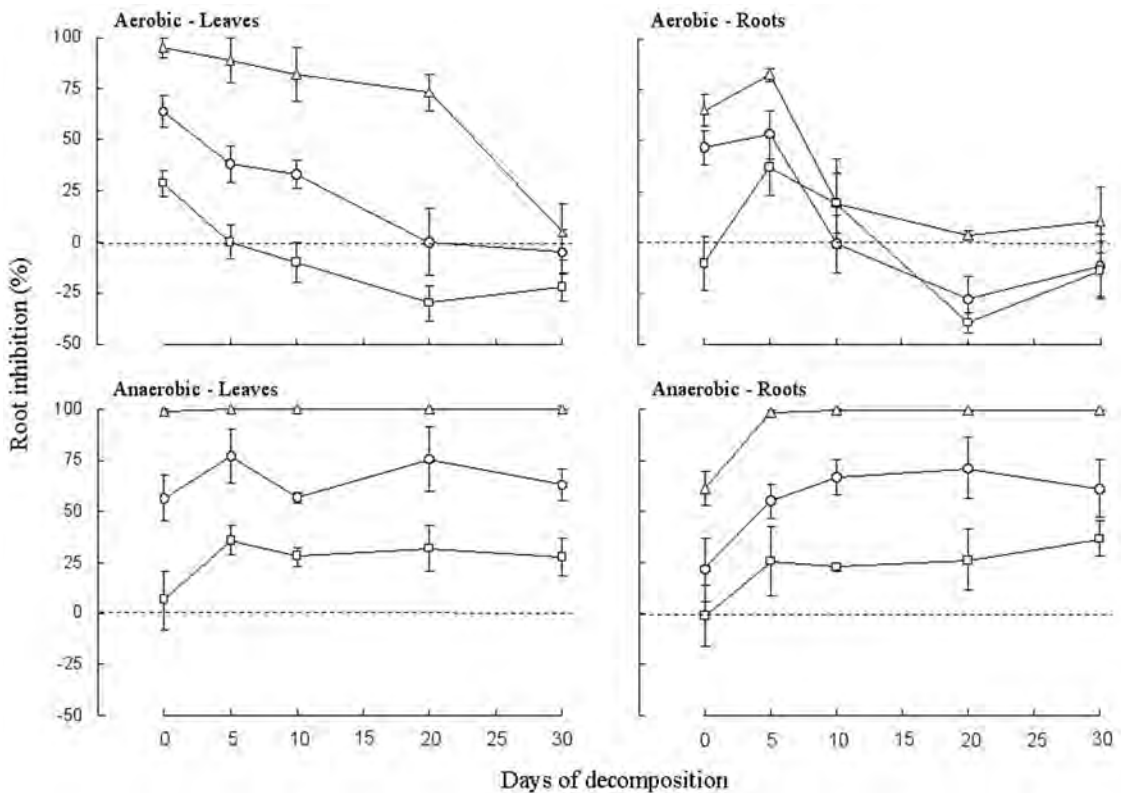
The pH of the extracts from both leaves and roots rapidly increased during aerobic decomposition whereas it remained constant in anaerobic conditions (Fig. 3). The EC of the extracts ranged from 6.9 mS/cm for undecomposed leaves to 1.1 mS/cm for roots after 30 days of decomposition in aerobic conditions (Fig. 3). During decomposition the EC values obtained with leaves were always higher than those with roots; moreover they decreased slightly with roots and rapidly with leaves (Fig. 3).

Tomato root growth was significantly affected both by the pH (ANOVA  $F=7.04$ ,  $P=0.01$ ; Table 2) and the EC within the ranges tested (ANOVA  $F=229.5$ ,  $P=0.001$ ; Table 2). Root growth was inhibited by 26.7% at the lowest pH level, and by 100% at the EC value of 20.5 mS/cm (Table 2). However, pH and EC values did not affect tomato root growth within the ranges measured for both leaf and root extracts (compare Table 2 with Fig. 3).

**Tomato extract effect on FOL growth.** FOL radial mycelium growth and hyphal density were significantly affected by tomato residues, decomposition conditions, time, and extract concentrations (respectively ANOVA  $F=14.0$   $P < 0.001$ ;  $F=27.1$   $P < 0.001$ ;  $F=806.1$   $P < 0.001$  and  $F=21.8$   $P=0.01$ ). The statistical interactions between conditions x time and material x time were highly significant ( $P < 0.001$  in all cases). The highest hyphal density was observed on undecomposed extracts of both leaves and roots (Fig. 4). Hyphal density remained almost constant during anaerobic decomposition, but clearly decreased in aerobic conditions (Fig. 4). Similar behaviour was observed for mycelium radial growth rate (data not shown).



**Fig. 1.** Inhibition of root elongation compared to control (=0%) of *Lepidium sativum* by aqueous extracts of *Lycopersicon esculentum* leaves and roots at three concentrations (50, 16.6 and 5 g l<sup>-1</sup>, triangles, circles and squares, respectively), during aerobic and anaerobic decomposition. Data are averages of ten replicates, bars indicate ±1SE.



**Fig. 2.** Inhibition of root elongation compared to control (=0%) of *Lycopersicon esculentum* by aqueous extracts of *L. esculentum* leaves and roots at three concentrations (50, 16.6 and 5 g l<sup>-1</sup>, triangles, circles and squares, respectively), during aerobic and anaerobic decomposition. Data are averages of ten replicates, bars indicate ±1SE.

**Effect on FOL wilt of amending soil with tomato residues.** Residues and inoculum levels significantly affected plant growth, while the interaction between these factors was not significant (respectively ANOVA  $F=29.6$   $P<0.001$ ;  $F=11.9$   $P<0.001$ ;  $F=0.93$   $P=0.47$ ). Plant growth was reduced by amendment with tomato residues and FOL inoculum, and the effect was dependent on the concentration of these two factors (Fig. 5a).

The percentage of wilted plants was significantly affected by tomato residue concentrations, inoculum levels, time from inoculum and all interactions between the three terms (three-way ANOVA:  $P$  values always  $<0.01$ ). In soil amended with tomato residues, wilt symptoms appeared immediately after transplanting (Fig. 5b), but only at high concentrations (3% and 5%). With the 3% residue amendment, tomato plants recovered from a 25% wilting after 10 days to a value of 8% after 17 days (Fig. 5b). On the FOL-inoculated plants, wilt symptoms appeared 17 days post-inoculation (Fig. 5b). Significant interactions occurred between FOL inoculum and residue amendments. Combinations of residues at 3% and FOL at concentrations of  $10^5$  and  $10^6$ , produced more wilted plants than the single factors (Fig. 5b). When residues were used at 5%, wilted plants reached the value of 100% after 21 days irrespective of the FOL inoculum concentration (Fig. 5b).

## DISCUSSION

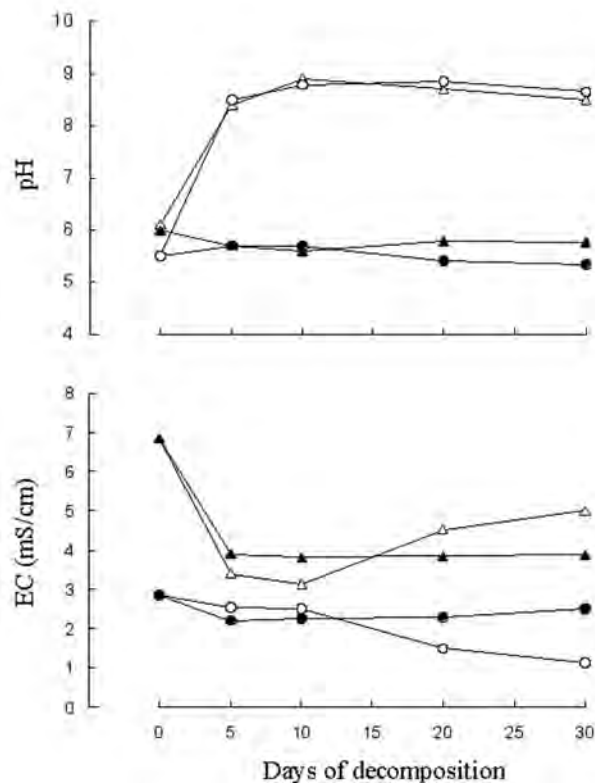
The ecology of the saprophytic life phase of soil-borne pathogens and its role on the incidence of diseases are not completely understood (Zucconi, 1996; Hoitink and Boehm, 1999). Our study showed that decomposition of tomato leaves and roots has different effects on tomato plants and the phytopathogenic fungus FOL. The phytotoxicity of tomato residues was significantly affected by the type of plant material (leaves and roots) and by the conditions of decomposition. Moreover, soil amendment with undecomposed tomato leaves significantly increased the incidence of FOL wilt.

The initial phase of decomposition basically consists of plant tissue breakdown and subsequent release of cell content. At this stage, extracts of tomato leaves and roots were phytotoxic and autotoxic. As in previous studies, leaf tissue was more phytotoxic than roots (Bonanomi *et al.*, 2006a). After this initial high level, phytotoxicity during aerobic decomposition steadily decreased for both leaves and roots. However, under anaerobic conditions phytotoxicity increased during decomposition. These results agree with previous observations on both crop and plant species that showed similar behaviour during decomposition (Patrick, 1971; Bonanomi *et al.*, 2006a).

The observed toxicity cannot be explained by different values of pH and EC because root growth of both

*Lepidium* (Bonanomi *et al.*, 2006a) and tomato plants (Table 2) were not directly affected by these parameters within the ranges observed during the decomposition experiments (Fig. 3). However, pH can influence the dissociation of allelochemical compounds such as organic acids thus increasing their toxicity in acidic conditions (Armstrong and Armstrong, 1999). This is consistent with our results that pH did not have a direct effect on the plants tested (Table 2), but that phytotoxicity was greater at the lowest pH values in anaerobic conditions. Moreover, although aqueous solutions with EC values above 8.1 mS/cm (Table 2) had a negative effect on tomato root growth, autotoxicity cannot be attributed to EC because after 30 days in anaerobic conditions its value was still below 4 mS/cm, a level that did not affect tomato root growth.

The autotoxicity of undecomposed tomato leaves was demonstrated by the soil bioassay. Depression of plant growth after addition of organic residues has been reported (Hodge *et al.*, 1998; Blum *et al.*, 1999; Conklin *et al.*, 2002) and related to N, and in some cases to P, net immobilization by microbial competition (Seligman *et al.*, 1986; Michelsen *et al.*, 1995). However, N starvation does not seem a plausible mechanism to explain growth depression in N rich conditions, i.e. for litter with a C:N ratio lower than 30 (Miller, 1996; Hodge *et*



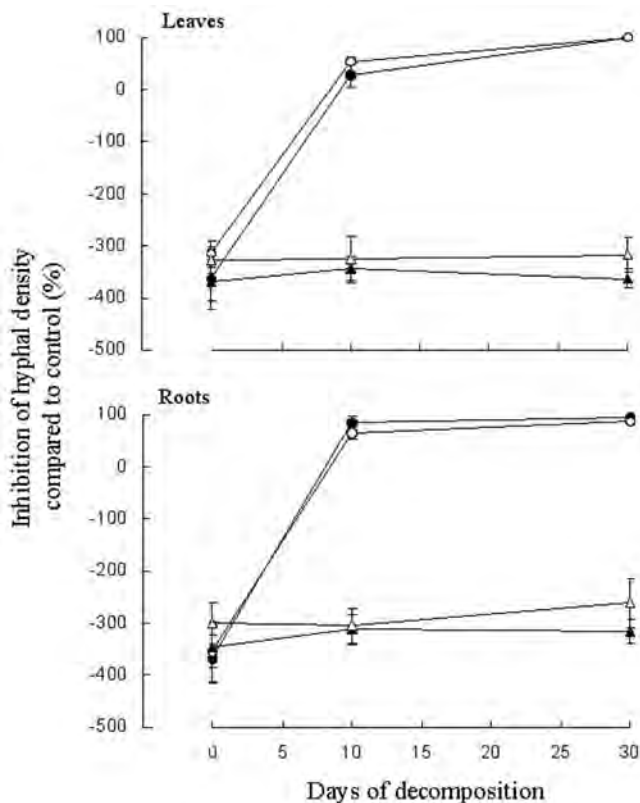
**Fig. 3.** Variation of pH (above) and EC (below) of aqueous extracts (concentrations of  $50 \text{ g l}^{-1}$ ) from *Lycopersicon esculentum* leaves (triangles) and roots (circles), in aerobic (open symbols) and anaerobic (closed symbols) decomposition conditions.

*al.*, 1998) as for tomato residues (Murchiea *et al.*, 1999).

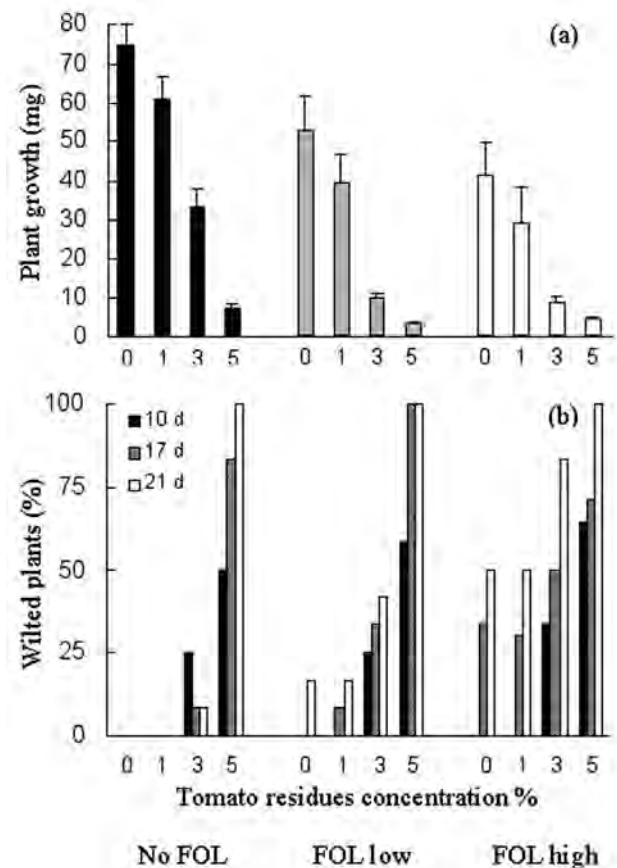
In contrast with plant responses, FOL radial growth and hyphal density were high on undecomposed tomato residues, but decreased in the presence of materials derived from the aerobic decomposition processes that also induce an abundant production of chlamydospores (data not shown). An explanation for the observed FOL behaviour may be related to its ability to use the energy and nutrients released during decomposition of residues. *Fusarium* species have often been described as aggressive saprophytes (Gordon and Okamoto, 1990), and pathogenic species maintain the ability to colonize crop residues (Gordon and Martyn, 1997). In a previous study, FOL saprophytic growth was investigated in the presence of organic carbon provided as purified substances (Steinberg *et al.*, 1999a), while the effects of complex OM such as crop debris or root exudates have been described only in a few reports (Steinberg *et al.*, 1999b). Smolinska (2000) showed that cruciferous residues (*Brassica juncea* and *Brassica napus*) have negative effects on FOL survival in soil, whereas Bonanomi *et al.* (2006b) showed that this fungus is able to use un-

decomposed olive mill residues for its saprophytic growth. These studies and our present results suggest that the fungal response is dependent on the type of OM used for amendment.

Our greenhouse assays showed that undecomposed tomato leaves increased the incidence of FOL wilt when soil was amended with tomato leaves at 1% and 3%. Soil amendment with undecomposed tomato leaves produced symptoms on tomato plants such as stunting, leaf yellowing and wilting, similar to those caused by FOL. However, these two factors could be distinguished by the timing of symptom development. Symptoms caused by undecomposed leaves were evident immediately after transplantation, but during the experiment the plants partially recovered from wilting (Fig. 5b). Generally, allelochemicals such as those released during decomposition show their negative effects on plants as soon as they come into contact with the roots, probably because they reduce water uptake (Blum *et al.*, 1999). In contrast, FOL symptoms appeared only after 17 days and increased in the speed of appearance when both FOL and leaf amendment were present.



**Fig. 4.** Inhibition of FOL hyphal density after 7 days by two concentrations (50% = open symbols; 10% = closed symbols) of extracts from *Lycopersicon esculentum* leaves and roots obtained in aerobic (circles) and anaerobic (triangles) conditions during the decomposition. Positive values indicate inhibition, negative values stimulation compared to control (water agar); bars indicate  $\pm 1$ SE. Elongation of hyphae on extracts from leaves decomposed in aerobic conditions for 30 days was arrested immediately after germination of conidia.



**Fig. 5.** Plant growth (a) and wilting (b) of *Lycopersicon esculentum* in soil amended with four concentrations (0, 1, 3 and 5% w/w) of undecomposed leaves of *L. esculentum* and three concentrations (no inoculum,  $10^5$  = low and  $10^6$  conidia/ml<sup>-1</sup> = high) of FOL inoculum. Values are the average of ten replicates; bars indicate  $\pm 1$ SE.

To explain these results, three hypotheses may be proposed: (i) leaf autotoxicity could produce root lesions (root browning was observed, data not shown) that may facilitate an active FOL penetration (Olivain and Alabouvette, 1999); (ii) leaf autotoxicity may reduce plant growth and weakens the plants, which then become more susceptible to pathogen attack (Di Pietro *et al.*, 2003); (iii) the presence of undecomposed leaves in soil may improve FOL survival, growth (Fig. 4) and sporulation, causing a build-up of a larger inoculum potential.

Our study suggests that agronomic management that includes removal of tomato residues from the field could reduce FOL wilt incidence. However, the autotoxic effect could be avoided by promoting decomposition before transplantation. Furthermore, conditions like water-logging that produce anaerobiosis and consequently increase autotoxicity should be avoided. Our results support previous studies that reported an increase of root diseases induced by crop residues (Blok and Bollen, 1993), complex OM (Bonanomi *et al.*, 2006b), or purified phytotoxic compounds (Ye *et al.*, 2004). Further studies are needed to improve our understanding of the saprophytic ecology of soilborne pathogens, as an essential step to obtain their control.

## ACKNOWLEDGEMENTS

We thank Prof. Franco Zucchini for the very useful discussions on the subject of phytotoxicity of organic matter.

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Received October 12, 2006

Accepted February 2, 2007