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Olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens

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Abstract

Olive oil mill residues constitute a major environmental problem. Although these wastes have a high fertilizer value when applied to the soil, there is concern about their use because of their antimicrobial and phytotoxic properties. Furthermore, their effect on saprophytic growth and pathogenicity of soilborne and foliar fungi is poorly known.

In this study we investigated the effects of olive mill dry residue (DOR) on: (a) growth of the four crop species *Lepidium sativum*, *Lycopersicon esculentum*, *Lactuca sativa* and *Triticum aestivum*; (b) saprophytic growth of the four phytopathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* (FOL), *Fusarium culmorum* (FC), *Sclerotinia minor* (SM) and *Botrytis cinerea* (BC); (c) influence of soil amendment with DOR on the three plant–soilborne pathogen systems, *L. esculentum*–FOL, *T. aestivum*–FC, *L. sativa*–SM, and the two plant–foliar pathogen systems, *L. esculentum*–BC and *L. sativa*–BC.

Residues resulted phytotoxic, both in laboratory and greenhouse bioassays, for all plant species in relation to their concentrations. *L. sativum* and *L. sativa* were the most sensitive species to the residues, followed by *L. esculentum* and *T. aestivum*. In contrast with the results observed with plant species, the performances (radial growth and hyphae density) of the tested phytopathogenic fungi were positively affected by DOR. In greenhouse bioassays, *L. sativa* mortality imputable to SM increased on soil amended with DOR. BC foliar disease dramatically increased on *L. sativa* and *L. esculentum* plants grown on soil amended with DOR at all used concentrations. Differently, soil amendment with DOR did not significantly affect the disease incidence of FC on *T. aestivum* and FOL on *L. esculentum*.

Our study demonstrates that, in controlled conditions, undecomposed DOR affects the growth of crop species (phytotoxic effect) and phytopathogenic fungi (substrate effect), and that the interaction between these factors, in some cases, drives to an increase of fungal disease incidence.

Keywords: Phytotoxicity; Phytopathogenic fungi; *Botrytis cinerea*; *Fusarium oxysporum* f.sp. *lycopersici*; *Fusarium culmorum*; *Sclerotinia minor*

1. Introduction

Olive oil mill wastewater (Kotsou et al., 2004) and dry residues (DOR) (Sampedro et al., 2004) are a major environmental problem for their high organic load and antimicrobial properties, especially for Mediterranean countries where most of the world olive oil production takes place. Many studies established that these wastes have a high fertilizer value when applied to the soil for the high organic matter (OM) and nutrient content (Paredes et al., 1999). Soil amendment with DOR increases the soil OM and the concentration of inorganic elements essential for plant growth (Paredes et al., 1999). However, despite the potential agronomic value, soil amendment with DOR is also known for its antimicrobial (Capasso et al., 1995)

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and phytotoxic properties (Rodriguez et al., 1988; Martìn et al., 2002). Phytotoxic effects on several crop species after soil amendment with DOR have been reported (Bonari et al., 1993; Martìn et al., 2002), and are likely related to the high content of phenolic compounds (Sampedro et al., 2004).

Soil OM plays a pivotal role on the outcome of the plant–pathogen interactions (Hoitink and Boehm, 1999), but how OM influences soil microorganism communities is still not completely understood. Several studies demonstrated that OM affects the population dynamics of soilborne pathogens either positively, by providing substrates for saprophytic growth (Croteau and Zibilske, 1998; Manici et al., 2004), or negatively through the generation of fungistasis (Lockwood, 1977) or the release of fungitoxic compounds (Smolinska, 2000). Moreover, the decomposition level of OM critically affects the composition of bacterial taxa as well as the population and activities of biocontrol agents (Hoitink and Boehm, 1999). Soil amendment with undecomposed OM in some cases drives to an increase of diseases caused by soilborne pathogens (Hoitink and Boehm, 1999; Manici et al., 2004), while the amendment with the decomposed OM is often suppressive (Hoitink and Fahy, 1986; Szczech, 1999), although a generalization is not possible. A positive interaction between phytotoxic compounds released by roots or during the decomposition of OM and soilborne pathogens has been proposed (Patrick et al., 1963; Nigh, 1990; Zucconi, 1996), but rarely tested (Ye et al., 2004). Moreover, the plant root system under stress conditions such as water drought, low levels of dissolved oxygen, nutrient unbalance or presence of phytotoxic compounds can greatly increase the susceptibility to foliar pathogens (Agrios, 2005).

Antimicrobial activities of DOR are well known (Moreno et al., 1987), but only recent studies demonstrate that a large number of saprophytic fungi are able to grow on DOR and decompose it (Zervakis et al., 1996; Sampedro et al., 2004). On the other hand, the effect of DOR on the saprophytic growth and pathogenicity of soilborne and foliar fungi is an under investigated issue (see Kotsou et al., 2004). In this work we studied the effects of DOR on plant growth, saprophytic growth of phytopathogenic fungi and its role in the outcome of the plant–pathogen interactions. We analyzed three systems including plants and soilborne pathogens (1. *Lycopersicon esculentum–Fusarium oxysporum* f.sp. *lycopersici* (FOL), 2. *Triticum aestivum–Fusarium culmorum* (FC), 3. *Lactuca sativa–Sclerotinia minor* (SM)), and two systems including plants and foliar pathogens (1. *L. esculentum–Botrytis cinerea* (BC), 2. *L. sativa*–BC). Specific hypotheses were: (i) undecomposed DOR are phytotoxic to different crop species; (ii) undecomposed DOR allow saprophytic growth of phytopathogenic fungi; (iii) soil amendment with undecomposed DOR increases the incidence of disease caused both by foliar and soilborne pathogens.

2. Materials and methods

2.1. DOR preparation and phytotoxicity on plant species

Root elongation experiments were carried out to evaluate the phytotoxic effect of DOR eluates on four species: *L. esculentum* (Cultivar San Marzano), *L. sativa* (Cultivar Cambria), *T. aestivum* and *Lepidium sativum*. The last species was used because it is extremely sensitive to phytotoxic substances (Zucconi et al., 1981; Heil et al., 2002).

DOR was collected from a local manufacturer (Marche, Italy). Total C was 540 g kg^{-1}, N was 15.6 g kg^{-1} with C/N of 34.6, P_2O_5 was 3.5 g kg^{-1}, K_2O was 20.6 g kg^{-1}, Ca was 4 g kg^{-1}, Na was 1 g kg^{-1}, Fe was 1.03 g kg^{-1}, Mg was 0.5 g kg^{-1}, Cu was 138 mg kg^{-1}, Zn was 22 mg kg^{-1} and Mn was 13 mg kg^{-1}. Dry DOR was wetted by using distilled water (5% dry weight—50 g l^{-1}) and the watery suspension was collected after 5 h. The suspension was centrifuged and the eluates were sterilised by micro-filtration through 0.22 μm pore filters and stored at −20 °C until further use. The sterilized eluates were diluted with distilled water (5%, 1.5% and 0.5%) and used for bioassays. The experiments were done in a growth chamber at constant temperature (27 °C) in the dark. Twenty seeds of *Lepidium* and lettuce and 10 of tomato and wheat were placed in 9 cm Petri dishes over sterile filter paper with 4 ml of test solution. Each solution (four concentrations including distilled water as a control) was replicated 10 times for a total of 800 seeds for *Lepidium* and lettuce and 400 for tomato and wheat. Petri dishes were arranged in a growth chamber according to a totally randomised design and seedling root length was measured after 36 h (*Lepidium*), 72 h (lettuce and tomato) and 144 h (wheat). Data were expressed as percent of root length compared to the length in the control treatment.

2.2. DOR effect on phytopathogenic fungi

In this experiment the effect of DOR eluates on saprophytic growth of BC, FOL, FC and SM were tested. Agar media were prepared by mixing agar, sterile water and sterile DOR eluates to obtain four dilution levels (75%, 50%, 25% and 0%). Ten millilitres of each dilution were placed in a 9 cm Petri dish. After 24 h from substrate preparation, a 5 mm diameter plug of mycelium of each fungal species collected from the edge of the actively growing colony on PDA (Potato dextrose agar; DIFCO), was placed on the centre of the Petri dish. Radial mycelium colony growth was measured every 24 h for 1 week. 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. Mineral or organic rich substrates were not used in this test, in order to analyze if DOR eluates may sustain the saprophytic growth of phytopathogenic fungi.

2.3. Effect of soil amendment with DOR on FC, FOL, SM and BC diseases

FC is one of the causal agent of the foot-rot complex on several grasses and wheat (Smith et al., 1988). In this experiment the effect of DOR amendment on FC disease incidence was tested. Wheat seeds were planted in a sterile potting mix (twice autoclaved) and grown in seedling plug trays (plug size 2 cm ×3 cm × 5 cm, 180 plug per tray). After 10 days, seedlings were transplanted and infected by a standard root-dip inoculation method. Inoculum was prepared by growing the fungus on PDA at 25 °C. After 10 days of culture, 10 ml of sterile water were added and culture surface was scraped to remove conidia. The suspension was then filtered, centrifuged and washed twice with sterile water and adjusted to a concentration of 10^6 conidia ml^{-1} by hemocytometer. Roots of uprooted seedlings were incubated in the conidial suspension for 10 min and then planted into pots of 12 cm diameter (one plant/pot) filled with field soil. All the experiments were carried out with non-sterilized sandy-clay soil with the following characteristics: pH was 7.1, OM was 2.3%, total N was 1.42 g kg^{-1} with C/N of 9.4, P_2O_5 was 156 mg kg^{-1}, K_2O was 960 mg kg^{-1}, Ca was 1975 mg kg^{-1}, Na was 72 mg kg^{-1}, Fe was 35.6 mg kg^{-1}, Mg was 467 mg kg^{-1}, Cu was 3.5 mg kg^{-1}, Zn was 3.2 mg kg^{-1} and Mn was 18.4 mg kg^{-1}. DOR was added into the potting media as dry powdered material. Four levels of DOR (0%, 1%, 5% and 10% dry weight) and two levels of soil inoculum (no inoculum and 10^6 conidia ml^{-1}) were compared. Each treatment was replicated 15 times (a total of 120 pots) and pots were arranged in the greenhouse according to a fully randomised design. The distribution of the pots was randomized weekly to avoid microclimate effects. The plants were watered daily at field capacity and maintained in greenhouse for 25 days (22 °C day, 15 °C night). At the end of the experiment, the plants were harvested and the soil was washed off the root system. The aboveground and belowground tissue biomass was measured after drying for 72 h at 80 °C.

FOL is the causal agent of the wilt disease of tomato (Larkin and Fravel, 2002). In order to test the effect of the amendment with DOR on the incidence of FOL disease, tomato plants were prepared as described for wheat. After 10 days, the tomato seedlings were infected by a standard root-dip inoculation method (Larkin and Fravel, 2002). FOL inoculum, plants inoculation and growth conditions were done as described above for FC. DOR was added into the potting media as dry powdered material. The experimental design compared three levels of DOR amendment (0%, 1%, and 3% dry weight) and two levels of FOL inoculum (no inoculum and 10^6 conidia ml^{-1}). The lower DOR amendment percentages were used for tomato because of the higher sensitivity of this plant compared to wheat. Each treatment was replicated eight times (total of 48 pots) and pots were arranged in a greenhouse as described for wheat. Plants were watered daily at field capacity and monitored for 21 days (27 °C day, 20 °C night). Other experimental procedures were as described for wheat.

SM is the causal agent of soft rot of a wide range of hosts including lettuce (Smith et al., 1988). The effect of the amendment with DOR on the incidence of SC disease was tested. Ten-day-old lettuce seedlings were transplanted in the soil previously infected with SM (one plant/pot; pot of 12 cm diameter). Common millet seeds in 0.5 l flasks, were inoculated with SM cultured on PDA and saturated with a Czapeck solution (1/10). The flasks were incubated for 21 days at 21 °C. The resulting SM millet inoculum was air-dried for 3 days and added up to 0.5% (dry weight) to the pots. In the control, non-inoculated common millet was added to pot medium. Other experimental procedures were conduced as described above for wheat and tomato.

BC causes the grey mould on several plant species (Agrios, 2005), and the effect of the amendment with DOR on the incidence of BC disease was tested on tomato and lettuce. The plants of both species were prepared as described above. Soil was amended with DOR at three levels (0%, 1% and 3% dry weight). After 20 days of plant growth under these conditions, young completely expanded leaves were excised and immediately inoculated with BC. The inoculum was prepared by obtaining the growing mycelia on malt extract agar (FLUKA) at 21 °C. After 10 days of culture, 10 ml of sterile water were added and the culture surface was scraped to remove conidia. The suspension was filtered, centrifuged, twice washed with sterile water and adjusted to a concentration of 10^5 conidia ml^{-1} by hemocytometer. Conidia were pre-germinated for 5 h (pre-germination solution: 20 mM sucrose, 10 mM K_2PO_4 at pH 6.5 and 10 ppm of Vitamin B5). A 5 μl of suspension with pre-germinated conidia was applied on lettuce and tomato leaves (20 leaves for each treatment for a total of 120 leaves for both species). The leaves were incubated in the laboratory at 21 °C and 100% of RH to facilitate BC growth. Leaf disease incidence was observed after 60 and 90 h from inoculation by digital photography and the area of diseased leaf was measured by image analyser software (LUCIA; Laboratory Imaging Ltd. version 4.51).

2.4. Statistical analyses

One-way analysis of variance (ANOVA) was applied to test the effect of DOR extract concentration on root length of the four plant species and on fungal diametric growth rate and hyphal density. Two-way ANOVA was applied to test the main effects and interaction of FC inoculum and soil amendment with DOR on wheat S/R ratio and seedling biomass. The same statistical analysis was applied for the experiments on lettuce inoculated with SM and tomato with FOL. Finally, two-way ANOVA was done to test the main

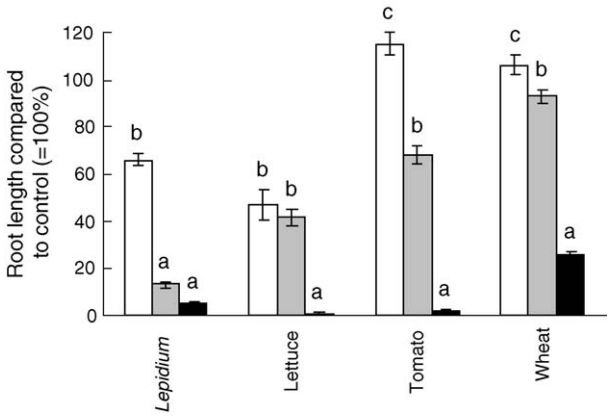

Fig. 1. Effect of DOR eluates at three concentrations (5% black, 1.5% grey and 0.5% open bars) on root length of *Lepidium*, lettuce, tomato and wheat compared to control (=100%; water). Different letters indicate significant differences (comparison only within species; Duncan test, $P < 0.05$). Data are averages (±1S.E.) of 10 replicates.

effects and interactions of soil amendment with DOR and timing from inoculation on foliar diseases of tomato and lettuce. Significance was evaluated at $P < 0.05$ for all tests.

3. Results

3.1. Phytotoxicity of DOR on plant species

Root length of all species was significantly affected by the concentration of DOR eluates (ANOVA, $P < 0.01$ for all species), and the inhibition increased with concentration (Fig. 1). *Lepidium* and lettuce were the most sensitive species, with a significant inhibition also at the lowest eluate concentration (0.5%), followed by tomato and wheat. Repeating the experiment we observed a similar result (data not shown).

3.2. DOR effects on phytopathogenic fungi

Diameter growth rate was significantly affected by DOR eluates for FC and SM (ANOVA, $P < 0.01$ in both species), while there was no effect for FOL and BC (ANOVA, $P = 0.92$ and 0.59, respectively). Diametric growth rate increased with DOR extract concentration for SM, while it decreased for FC (Fig. 2a). Hyphal density was significantly increased by DOR eluate concentrations for all species (ANOVA, $P < 0.01$ in all cases; Fig. 2b). Repeating the experiment we observed a similar result (data not shown).

3.3. Effect of soil amendment with DOR on FC, FOL, SM and BC disease

Soil amendment with DOR significantly affected wheat seedling S/R ratio and biomass (ANOVA, $P < 0.01$ in both cases), while FC inoculum did not affect the two parameters (ANOVA, $P = 0.26$ and 0.36, respectively). Furthermore, the

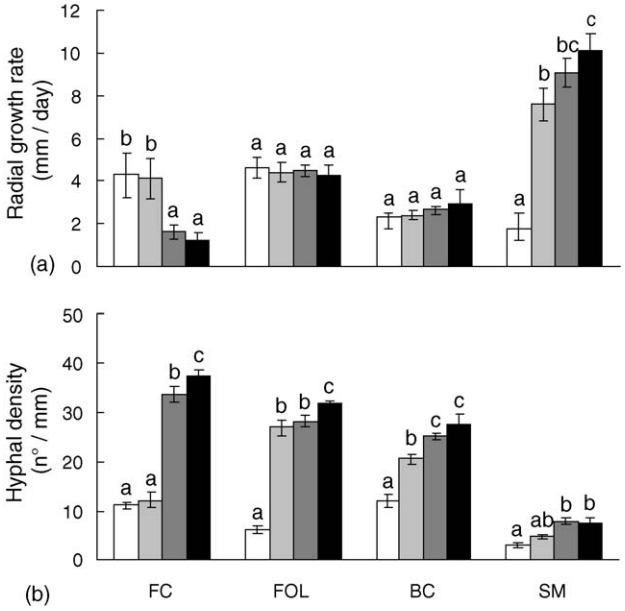

Fig. 2. Effect of DOR eluates at four concentrations (75% black, 50% dark grey, 25% grey and 0% open bars) on radial growth rate (a) and hyphal density (b) of *F. culmorum* (FC), *F. oxysporum* f.sp. *lycopersici* (FOL), *B. cinerea* (BC) and *S. minor* (SM). Different letters indicate significant differences (comparison only within species; Duncan test, $P < 0.05$). Data are averages (±1S.E.) of five replicates.

interaction between inoculum and soil amendment was not statistically significant (ANOVA, $P = 0.16$ for S/R ratio and $P = 0.84$ for seedling biomass). Amendment at 5% and 10% reduced the seedling S/R ratio from 4.4 in the control to 1.2 (Fig. 3a). Seedling biomass was reduced at the highest

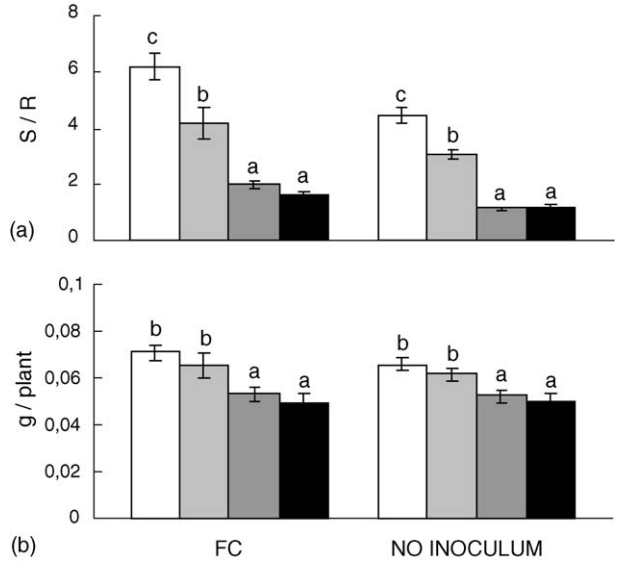

Fig. 3. Effect of soil amended with DOR at four concentrations (10% black, 5% dark grey, 1% grey and 0% open bars) and *F. culmorum* (FC) at two levels on wheat shoot/root ratio (a) and seedling dry biomass (b). Different letters indicate significant differences (comparison only within inoculum level; Duncan test, $P < 0.05$), data are averages (±1S.E.) of 15 replicates.

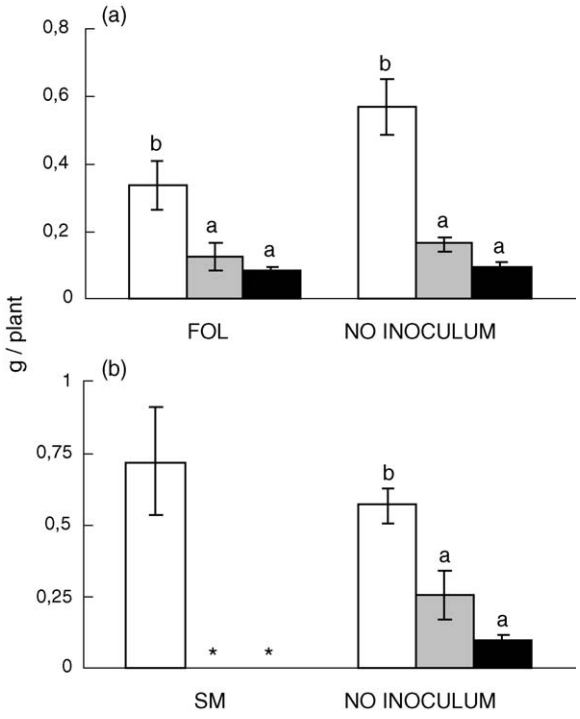

Fig. 4. Effect of soil amended with DOR at three concentrations (3% black, 1% grey and 0% open bars) and *F. oxysporum* f.sp. *lycopersici* (FOL) or *S. minor* (SM) inoculum at two levels on tomato (a) and lettuce (b) seedling dry biomass. Different letters indicate significant differences (comparison only within inoculum level; Duncan test, $P < 0.05$). Asterisks on SM inoculated lettuce indicate 100% mortality, data are averages (±1S.E.) of eight replicates.

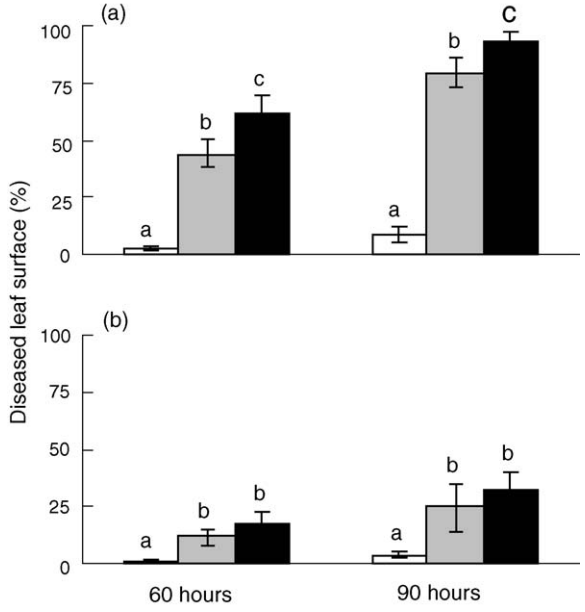

Fig. 5. Effect of soil amended with DOR at three concentrations (3% black, 1% grey and 0% open bars) and *B. cinerea* (BC) foliar inoculum on tomato (a) and lettuce (b) foliar disease after 60 and 90 h from the inoculation. Different letters indicate significant differences (comparison only within the same time; Duncan test, $P < 0.05$). Data are averages (±1S.E.) of 20 replicates.

concentration from 65.8 mg/plant in the control to 49.9 mg/plant (Fig. 3b).

Amendment significantly reduced tomato seedling biomass (Fig. 4a; ANOVA, $P < 0.01$), while FOL inoculum and its interaction with soil amendment was not significant (ANOVA, $P = 0.057$ and 0.052, respectively). Seedling biomass was reduced from 56.5 mg/plant in the control to 9.8 mg/plant at the highest DOR concentration (Fig. 4a).

Lettuce seedling biomass was also significantly reduced by the amendment (Fig. 4b; ANOVA, $P < 0.01$), while SM inoculum alone did not affect lettuce biomass (ANOVA, $P = 0.09$). However, the interaction between inoculum and soil amendment was significant (ANOVA, $P = 0.022$). Soil amendment did not affect lettuce mortality (ANOVA, $P = 1$), while SM inoculum and its interaction with soil amendment was significant (ANOVA, $P < 0.05$ in both cases). Mortality was 0% for non-inoculated plants, while it rose to 70% for inoculated but not amended plants and 100% for inoculated and amended plants at both DOR concentrations.

BC disease incidence on tomato and lettuce was significantly increased in soil amended with DOR at both concentrations (Fig. 5a and b; ANOVA, $P < 0.001$ in both cases). Moreover, there were significant effects of the time from inoculation and the statistical interaction between soil amendment and time (ANOVA, $P < 0.001$ in both cases). After 90 h, disease severity was low on lettuce control (3.6% of leaf surface became necrotic), but it increased to 32.4% in soil amended with 3% of DOR. The same effect was observed on tomato, but with higher disease severity. After 90 h, necrotic leaf surface was 8.1% on control, but it rose to 79.5% and 93.6% in amended soil with 1% and 3% of DOR, respectively. Tomato and lettuce plant growth was significantly affected by soil DOR amendment as in previous experiments (see Fig. 4).

4. Discussion

The ecology of the saprophytic life phase of soilborne pathogens, and its role on disease incidence is still not completely understood (Hoitink and Boehm, 1999). In this context, our study demonstrates that DOR have a very contrasting effect on crop species and phytopathogenic fungi, and that soil amendment with DOR significantly affects host–pathogen interactions.

4.1. DOR phytotoxicity

DOR eluates were phytotoxic for all plant species in relation to their concentrations. *Lepidium* and lettuce were the most sensitive species, followed by tomato and wheat (Fig. 1). Soil amendment with DOR depressed plant growth in all greenhouse experiments. Tomato and lettuce were more sensitive than wheat to DOR amendment at a concentration as low as 1%, which significantly reduced their growth (Figs. 3b and 4). Wheat seedlings were less

sensitive to DOR phytotoxicity, although the S/R ratio was clearly reduced, thus indicating a progressive difficulty for that plant to use the amended soil (Marschner, 1995; Neri et al., 1996; Zucconi, 1996). Depression of plant growth after addition of organic residues has been reported (Patrick, 1971; Hodge et al., 1998; Blum et al., 1999; Neri et al., 2002; Bonanomi et al., 2006) and often related to N net immobilization by microbial competition (Seligman et al., 1986; Michelsen et al., 1995). However, this supposed N starvation is not a plausible mechanism to explain growth depression in N rich conditions (Hodge et al., 1998), such as in our case where OM have a C/N ratio of 34.6. Our results agree with previous studies that demonstrate the phytotoxic nature of undecomposed DOR (Sampedro et al., 2004) and, in addition, show that sensitivity to DOR is plant species-specific.

4.2. DOR effect on phytopathogenic fungi

In contrast with the response observed for plant species, the radial growth and hyphal density of the tested phytopathogenic fungi increased with the DOR concentration (Fig. 2a and b), except for the FC diametric growth rate (Fig. 2a). These results suggest that the presence of undecomposed DOR in the soil may provide energy and nutrients for saprophytic growth of fungi and, consequently, the building of soil inoculum. *Fusarium* species are often described as aggressive saprophytes (Gordon and Okamoto, 1990), and pathogenic species as well have the ability to colonize crop residues (Gordon and Martyn, 1997). Previous studies investigated FOL saprophytic growth in relation with the availability of organic carbon provided as purified substances (Steinberg et al., 1999), but there are only few reports on the effects of complex OM such as crop debris or root exudates (Steinberg et al., 1999). Smolinska (2000) demonstrated that cruciferous residues (*Brassica juncea* and *Brassica napus*) have negative effects on FOL survival in soil. Comparing this study with our results (Fig. 2), FOL response appears to be dependent on the type of OM used for amendment, although more investigations with other OMs are needed to support this hypothesis. Concerning BC, FC and SM to our knowledge, no data are present in recent literature that can be compared with our results. Finally, our results extend to phytopathogenic fungi the ability of saprophytic fungi to utilize DOR (Zervakis et al., 1996; Sampedro et al., 2004).

4.3. DOR effect on diseases induced by phytopathogenic fungi

The hypothesis that undecomposed DOR can increase the ability of phytopathogenic fungi to cause diseases is only partially supported by our greenhouse assays.

Soil amendment with DOR does not significantly affect the disease incidence of FC on wheat and FOL on tomato (Figs. 3b and 4a). Differently, lettuce mortality due to SM increases with soil amended at both concentrations (Fig. 4b). This result clearly shows that reduction of lettuce growth induced by SM was further increased by undecomposed DOR, and indicates that SM inoculum and soil amendment might influence plant growth additively or synergistically. The mechanisms through which soil amendment with DOR increase the ability of SM to cause diseases are still unknown. At least three hypotheses can be proposed: (i) phytotoxicity of DOR produces root lesions, such as root browning (data not shown) that may facilitate SM penetration; (ii) DOR phytotoxicity by reducing plant growth, may also weaken plants, thus facilitating the pathogen attack and/or the process of infection; (iii) the presence of DOR residues in soil may improve SM survival, growth (Fig. 2a and b) and sporulation by causing the build-up of a larger inoculum potential. However, further studies are needed to unravel the role of these processes in SM disease incidence.

BC foliar disease dramatically increased on lettuce and tomato plants when soil was previously amended with DOR, at all concentrations (Fig. 5a and b). It is noteworthy that BC disease incidence increased also where soil was amended with the lowest level of DOR (1% equivalent to 10 g kg^{-1}). A general weakness of plant defence mechanisms toward pathogen invasion after growth in the presence of phytotoxic DOR could be a plausible explanation for the observed effects. To our knowledge, this is the first paper reporting the increase of a foliar disease incidence after soil amendment with DOR.

5. Conclusion

Our study demonstrates that in controlled conditions undecomposed DOR affects in contrasting ways crop species (phytotoxic effect) and phytopathogenic fungi (substrate effect). Our results indicate that soil amendments with DOR do not affect FC and FOL disease, but increases those caused by SM and BC. Moreover, it is important point out that with BC the disease increased also when the DOR dosage tested was comparable with that used in field applications. This means that a correct agronomic management of DOR is strongly necessary, taking into account the plant–pathogen system. For example, the application of undecomposed DOR in lettuce crops should be carried out at low dosages, lower than the recommended 4 kg m^{-2} (Ocampo et al., 1975), which is equivalent to 20 g kg^{-1} in our study. In alternative, DOR could be applied to soil only after appropriate decomposition processes such as composting that reduce phytotoxicity (Tsioulpas et al., 2002; Sampedro et al., 2004). In conclusion, DOR management must: (i) reduce its direct phytotoxic effects on crops, (ii) limit the increase of crop susceptibility to foliar and soilborne pathogens, and (iii) reduce the possible build-up of soil inoculum of phytopathogenic fungi. Further studies with other crops and pathogens could be carried out to extend our knowledge about the management of DOR for soil applications.

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References

Agrios, G.N., 2005. Plant Pathology, 5th ed. Elsevier Academic Press.

Blum, U., Shafer, S.R., Lehman, M.E., 1999. Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs. an experimental model. Crit. Rev. Plant Sci. 18, 673–693.

Bonanomi, G., Sicurezza, M.G., Caporaso, S., Esposito, A., Mazzoleni, S., 2006. Phytotoxicity dynamics of decaying plant materials. New Phytol. 169, 571–578.

Bonari, E., Macchia, M., Angelini, L.G., Ceccarini, L., 1993. The waste waters from olive oil extraction: their influence on the germinative characteristics of some cultivated and weed species. Agric. Med. 123, 273–280.

Capasso, R., Evidente, A., Schivo, L., Orru, G., Marcialis, M.A., Cristinzio, G., 1995. Antibacterial polyphenols from olive oil mill waste waters. J. Appl. Bacteriol. 79, 393–398.

Croteau, G.A., Zibilske, L.M., 1998. Influence of papermill processing residuals on saprophytic growth and disease caused by *Rhizoctonia solani*. Appl. Soil Ecol. 10, 103–115.

Gordon, T.R., Okamoto, D., 1990. Colonization of crop residue by *Fusarium oxysporum* f.sp. melonis and other species of *Fusarium*. Phytopathology 80, 381–386.

Gordon, T.R., Martyn, R.D., 1997. The evolutionary biology of *Fusarium oxysporum*. Annu. Rev. Phytopathol. 35, 111–128.

Heil, M., Delsinne, T., Hilpert, A., Schürkens, S., Andary, C., Linsenmair, K.E., Sousa, S.M., Mckey, D., 2002. Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. Oikos 99, 457–468.

Hodge, H., Stewart, J., Robinson, D., Griffiths, B.S., Fitter, A.H., 1998. Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. New Phytol. 139, 479–494.

Hoitink, H.A.J., Fahy, P.C., 1986. Basis for the control of soilborne plant pathogens with composts. Annu. Rev. Phytopathol. 24, 93–114.

Hoitink, H.A.J., Boehm, M.J., 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu. Rev. Phytopathol. 37, 427–446.

Kotsou, M., Mari, I., Lasaridi, K., Chatzipavlidis, I., Balis, C., Kyriacou, A., 2004. The effect of olive oil mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. Appl. Soil Ecol. 26, 113–121.

Larkin, R.P., Fravel, D.R., 2002. Effects of varying environmental conditions on biological control of *Fusarium* wilt of tomato by non-pathogenic *Fusarium* spp. Phytopathology 92, 1160–1166.

Lockwood, J.L., 1977. Fungistasis in soil. Biol. Rev. 52, 1–43.

Manici, L.M., Caputo, F., Bambini, V., 2004. Effect of green manure on *Pythium* spp. population and microbial communities in intensive cropping systems. Plant Soil 263, 133–142.

Marschner, H., 1995. Mineral Nutrition in Higher Plants. Academic Press, New York.

Martìn, J., Sampedro, I., García-Romera, I., García-Garrido, J.M., Ocampo, J.A., 2002. Arbuscular mycorrhizal colonization and growth of soybean (Glycine max) and lettuce (*Lactuca sativa*) and phytotoxic effects of olive mill residues. Soil Biol. Biochem. 34, 1769–1775.

Michelsen, A., Schmidt, I.K., Jonasson, S., Dighton, J., Jones, H.E., Callaghan, T.V., 1995. Inhibition of growth, and effects on nutrient uptake of artic graminoids leaf extracts—allelopathy or resource competition between plants and microbes? Oecologia 103, 407–418.

Moreno, E., Perez, J., Ramos-Cormenzana, A., Martinez, J., 1987. Antimicrobial effect of waste water from olive oil extraction plants selecting soil bacteria after incubation with diluted waste. Microbios 51, 169–174.

Neri, D., Nobuo, S., Inujima, A., 2002. Effects of organic residues on strawberry root growth. Acta Hort. 567, 503–506.

Neri, D., Madia, T., Zucconi, F., Guardagli, G., 1996. Root growth of rice seedlings in relation to crop-residues metabolism. In: Proceedings of the International Workshop: Dynamics of Roots and Nitrogen in Cropping System of the Semi-arid Tropics, Patancheru, Andhra Pradesh, India, 21–25 November, pp. 102–128.

Nigh Jr., C.L., 1990. Stress factors influencing *Fusarium* infection in asparagus. Acta Hort. 271, 315–322.

Ocampo, J.A., Barea, J.M., Montoya, E., 1975. Interaction between Azotobacter and phosphobacteria and their establishment in the rhizosphere as affected by soil fertility. Can. J. Microbiol. 21, 1160–1165.

Paredes, C., Ceggara, J., Roing, A., Sánchez-Monedero, M.A., Bernal, M.P., 1999. Characterization of olive mill wastewater (alpechin) and its sludge for agricultural purposes. Biores. Technol. 67, 111–115.

Patrick, Z.A., Toussoun, T.A., Snyder, W.C., 1963. Phytotoxicity substance in arable soils associated with decomposition of plant residues. Phytopathology 53, 152–161.

Patrick, Z.A., 1971. Phytotoxic substance associated with the decomposition in soil of plant residues. Soil Sci. 111, 13–18.

Rodriguez, M.M., Perez, J., Ramos-Cormenzana, A., Martinez, J., 1988. Effect of extracts obtained from olive oil mill waste waters on *Bacillus megaterium* ATTC 33085. J. Appl. Bacteriol. 64, 219–226.

Sampedro, I., Aranda, E., Martìn, J., Garcìa-Garrido, J.M., Garcìa-Romera, I., Ocampo, J.A., 2004. Saprobic fungi decrease plant toxicity caused by olive mill residues. Appl. Soil Ecol. 26, 149–156.

Seligman, N.G., Feigenbaum, S., Feinerman, D., Benjamin, R.W., 1986. Uptake of nitrogen from high C-to-N ratio. 15N-labelled organic residues by spring wheat grown under semi-arid conditions. Soil Biol. Biochem. 18, 303–307.

Smith, I.M., Dunez, J., Lelliott, R.A., Phillips, D.H., Archer, S.A., 1988. European Handbook of Plant Diseases. Blackwell Scientific Publications, Oxford.

Smolinska, U., 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. J. Phytopathol. 148, 343–349.

Steinberg, C., Whipps, J.M., Wood, D.A., Fenlon, J., Alabouvette, C., 1999. Effects of nutritional sources on growth of one non-pathogenic strain and four strains of *Fusarium oxysporum* pathogenic on tomato. Mycol. Res. 103, 1210–1216.

Szczech, M.M., 1999. Suppressiveness of vermicompost against *Fusarium* wilt of tomato. J. Phytopathol. 147, 155–161.

Tsioulpas, A., Dimou, D., Iconomou, D., Aggelis, G., 2002. Phenolic removal in olive oil mill wastewater by strains of *Pleurotus* spp. in respect to their phenol oxidase (laccase) activity. Biores. Technol. 84, 251–257.

Ye, S.F., Yu, J.Q., Peng, J.H., Zheng, J.H., Zou, L.Y., 2004. Incidence of *Fusarium* wilt in *Cucumis sativus* L. is promoted by cinnamic acid, an autotoxin in root exudates. Plant Soil 263, 143–150.

Zervakis, G., Panayiotis, Y., Balis, C., 1996. Edible mushrooms from olive oil mill wastes. Int. Biodeteriorat. Bioremediat. 237–243.

Zucconi, F., Forte, M., Monaco, A., De Bertoldi, M., 1981. Evaluating toxicity of immature compost. BioCycle 22, 54–57.

Zucconi, F., 1996. Declino del suolo e stanchezza del terreno. Ed. Spazio Verde, Padova.