Available online at www.sciencedirect.com
SCIENCE DIRECT
Agriculture, Ecosystems and Environment 115 (2006) 194–200
Agriculture Ecosystems & Environment
www.elsevier.com/locate/agee

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

Giuliano Bonanomi [a,*], Veronica Giorgi [b], Del Sorbo Giovanni [a], Davide Neri [b], Felice Scala [a]

[a] *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, University of Naples Federico II, via Università 100, Portici 80055 (NA), Italy*
[b] *Dipartimento di Energetica, Polytechnic University of Marche, via Brecce Bianche, Ancona (AN), Italy*

Received 12 July 2005; received in revised form 17 December 2005; accepted 10 January 2006
Available online 21 February 2006

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)

* Corresponding author. Tel.: +39 03396919212; fax: +39 0817755320.
E-mail addresses: giulianobonanomi@hotmail.com (G. Bonanomi), d.neri@univpm.it (D. Neri), scala@unina.it (F. Scala).

0167-8809/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.agee.2006.01.002

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.