

Endophytic fungal populations acting on soil suppressiveness in fruit tree orchards

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Abstract

*Fruit production in central Europe takes place in permanent cropping systems and is affected by replant disorders, although soil organic matter content does not represent a fertility limiting factor in those growing areas. The consequent crop yield decline is mainly due to biotic causes, even if it is mediated by plant vigour. Therefore, the most appropriate strategy for controlling replant disease in organic cropping systems of central Europe is to exploit biological soil resources. To prove feasibility of this strategy, functionality of two populations of endophytic fungi isolated in apple orchards was evaluated. Antibiosis and potentiality of plant growth promotion, as observed in *Fusarium oxysporum*, as well as antagonism toward the pathogen *Cylindrocarpon* in apple tree root colonization, as observed in binucleate *Rhizoctonia*, indicate the possibility to reduce root pathogens and to increase plant growth in renewed apple orchards by exploiting microorganisms naturally present in soil.*

Introduction

The vast majority of fruit tree production in Europe takes place in intensive cropping systems and is affected by replant problems. This etiology is mainly due to biotic causes but, it is mediated by plant vigour, physiological state of plants and a-biotic factors. Therefore, yield decline is the main indicator of replant etiology for farmers. This study is a step in a line of research which focuses on exploitation of natural resources of orchard soils and aims at developing innovative cropping practices which will enable biodiversity preservation and increase soil health.

Soil fungi are well known indicators of soil functioning in both arable and permanent crops. For this reason, a study was carried out to identify the root endophytic fungal species indigenous in apple orchards of Europe, having beneficial impact on plant growth in three apple growing areas where organic management represents a consistent part of land invested with orchards.

The achievement of these objectives should lead to the third part of this research strategy aiming at defining agronomic tools suitable to increase soil inhabiting beneficial microbial populations.

Material and methods

Plant growth response was the main parameter adopted to evaluate soil health in this study. A plant growth assay was performed on soil samples coming from replanted orchards of three European apple growing areas (Italy, Germany and Austria). Rooted cuttings of clonal M9 rootstock was used as target plant. A control soil subjected to a gamma ray sanitisation cycle (corresponding to the standard treatment used in food sterilization) for each sampling site was inserted in this trial to quantify plant growth reduction caused by the replant problems. At the end of bioassay, qualitative and quantitative evaluation of fungal endophytic communities (obtained from 1944 root explants from 243 rootstock plantlets) showed that: (1) root colonization frequency reaches a total of 60%; (2) only two populations were negatively correlated to plant growth (*Cylindrocarpon*-like fungi group and *Pythium* spp.); (3) *Fusarium* and binucleate *Rhizoctonia* populations, accounting for 50% of total fungal endophytes, did not result negatively correlated to plant growth (Manici et al. 2013).

Starting from those two non pathogenic fungal populations, specific tests were performed to identify their interaction with plants to identify their functionality in relation to crop production and soil health in apple orchards.

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Results

The plant growth increase of 40% in gamma ray treated soils as compared to natural soils confirmed the biotic origin of replant disease in apple orchards (Manici et al. 2013). However, the analysis of microbial communities showed that this result seemed to be caused by a different ratio among root endophytic fungal populations, rather than by a consistent reduction of fungal root pathogens (Manici et al. 2013). Among them, *Rhizoctonia* was significantly and negatively correlated to *Cylindrocarpon* ($P < 0.05$, Coeff Corr: -0.23, for 81 paired samples; $P < 0.01$; Coeff Corr = -0.88 for 3 paired treatments consisting of 27 samples each: replanted soil, row strip soil, gamma treated soil (Fig. 1). This outcome suggests antagonism between binucleate *Rhizoctonia* sp. and *Cylindrocarpon*-like fungi in colonizing roots. Within the *Fusarium* population, a bioassay with culture filtrate with three pure colonies of *Fusarium oxysporum*, the most abundant species (33% of total *Fusarium* spp.), showed antibiosis toward *Cylindrocarpon* in poisoned media and an auxin production in 100 ml, varying from 0.9 to 2.55 mg l⁻¹ after a one-week incubation period.

Discussion

Antibiosis and growth promotion of *F. oxysporum* suggests the potentiality of soil inhabiting *Fusarium* spp. to reduce rootrot agents and increase plant growth in renewed apple orchards. This finding is consistent with the high ability of *Fusarium* genus to produce biologically active compounds (Azevedo and de Araujo 2007).

At the same time the colonizing ability of the binucleate *Rhizoctonia* population, having a non pathogenic behaviour, seems to act antagonistically to *Cylindrocarpon*-like fungi, having a role in growth reduction of replanted orchards. This finding is consistent with what has previously been observed in fungal ecology applied to apple and forest crops (Grönberg et al. 2006; Kelderer et al. 2012).

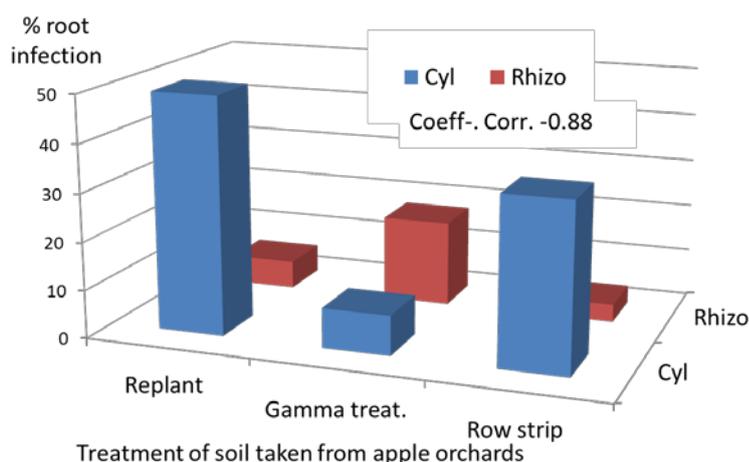


Fig. 1. Abundance of pathogenic population of *Cylindrocarpon*-like fungi (Cyl.) as compared to binucleate *Rhizoctonia* sp. (Rhizo) in soil from 9 replanted apple orchards in Europe. The negative correlation between those two populations revealed their antagonism in colonizing apple roots.

Functionality of two common root colonizing fungi occurring in apple orchards, as observed in this study, supports the existence of soil biological resources acting as

additional beneficial factors in soil suppressiveness and suggests the potential advantages to exploit these natural resources for developing innovative cropping practices in organic agriculture. Indeed, soil fungi living saprophytically in soil are the primary microbial contributors to the carbon metabolism process (Bailey et al. 2002). In addition, soil fungi represent the part of microbial biomass most susceptible to plant residue incorporation into the soil, as well as to cropping practices (van der Wal et al. 2006). Finally, soil fungal community composition is strongly affected by vegetative cover (Broeckling et al. 2008).

Findings so far obtained support the original challenges of the project, namely that microbial populations naturally present in orchard soils are a resource to be exploited for increasing soil health and sustaining high quality fruit production.

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References

- Azevedo J L, Araújo W L (2007) Diversity and applications of endophytic fungi isolated from tropical plants, p.189-207. In Ganguli B N, Deshmukh S K (eds) *Fungi: multifaceted microbes*. Boca Raton, CRC press, 321p.
- Bailey VL, Smith JL, Bolton Jr H (2002) Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biology and Biochemistry* 34, 997–1007.
- Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root Exudates Regulate Soil Fungal Community Composition and Diversity *Applied Environmental Microbiology* 74,738–744.
- Garbeva P, van Veen JA, van Elsas JD (2004) Microbial diversity in soil: selection of the microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* 42,243-270.
- Grönberg H, Kaparakis G, Sen R (2006) Binucleate *Rhizoctonia* (*Ceratohiza* spp.) as non-mycorrhizal endophytes alter *Pinus sylvestris* L. seedling root architecture and affect growth of rooted cuttings. *Scandinavian Journal of Forest Research* 21, 450-457.
- Kelderer M, Manici LM, Caputo F, Thalheimer M (2012) Planting in the 'inter-row' to overcome replant disease in apple orchards: a study on the effectiveness of the practice based on microbial indicators. *Plant and Soil* 357, 381-393.
- Manici LM, Kelderer M, Franke-Whittle I, Rühmer T, Baab G, Nicoletti F, Caputo F, Topp A, Insam H, Naef A (2013) Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe. *Applied Soil Ecology* 72,207-214.
- van der Wal A, van Veen JA, Smant W, Boschker HTS, Bloem J, Kardol P, van der Putten WH, de Boer W (2006) Fungal biomass development in a chronosequence of land abandonment. *Soil Biology and Biochemistry* 38,51–60.

