# Replant problems in South Tyrol: role of fungal pathogens and microbial populations in conventional and organic apple orchards

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**Abstract**

South Tyrol, the main Italian apple growing area, is characterised by an highly intensive soil cultivation. Previous investigations shows the existence of replant disorders although it has not been evaluated which are the main causes. A survey has been carried out in this area with two main aims I) to evaluate the role of soil borne pathogens in apple replant disease and II) to evaluate the effect of soil management toward soil borne pathogens causing replant diseases. The experimental sites were chosen in order to obtain three couples of contiguous conventional and organic apple orchards.

Soil sickness test with young apple plants gave a significant growth reduction in all soil samples if compared to a peat control. Among all root colonising fungi (*Fusarium oxysporum, F. solani, Aphanomyces sp., Cylincrocarpon sp., Rhizoctonia sp. and Pythium sp.*) some *Rhizoctonia solani* strains and all *Pythium* spp. were the most pathogenic. In all cases organic management seems to reduce the soil sickness severity caused by root rot fungal pathogens.

**Keywords:**

Soil sickness, organic orchards, soil-borne pathogens, *Pythium* spp., *Rhizoctonia solani*, micoflora.

## Introduction

South Tyrol is the main apple producing area in Italy, with 18.000 ha under conventional and 700 ha under organic management.

In South Tyrol the most part of apple orchard sites have been replanting for at list twenty-thirty years both for the high land cost and the continuous looking for new competitive varieties in Italian and European market.

Most of the sites have been planted with apple trees for at least 50 years, renewing the orchards continuously, replacing old varieties with new competitive varieties on the Italian and European market.

Although previous investigations show the existence of replant disorders, typical symptoms, such as stunting or tree mortality, are not frequently observed in producing orchards. In addition, the role of replant problems on yield losses in South Tyrol is difficult to be assessed: since pre-planting treatments with fumigants are not allowed.

The etiology of replant disease has never been fully explained; usually, many secondary, rather than primary factors, are involved: toxic chemical compounds released by decomposing roots, unbalanced nutrient supply, cold and drought stress are some causal factors often suggested, but literature also frequently indicates that soil borne pathogens play a fundamental role in apple replant disease (May Sewell *et al*, 1981; Jeffee *at al.*, 1982; Mazzola, 1998).

A survey has been carried out in Adige Valley in South Tyrol with the following aims 1) to evaluate the role of soil-borne pathogens in apple replant disease 2) to evaluate the effect of conventional and organic management on soil-borne pathogens causing replant disease in apple orchards.

## Material and Methods

The experimental sites were selected to obtain three couples of contiguous conventional and organic apple orchards (Dezini, Moscon, Terzer) with different varieties grafted on the rootstock M9. The organic orchards were managed according to the criteria of biodynamic agriculture. Soil samples in each orchard were collected at four opposite points 50 cm from the trunk of five trees, chosen at random. Soil samples of each orchard were mixed and subjected to the following test:

1. Growth test with seedling assay
2. Total root colonising fungi and pathogenicity test of the most frequent fungal strains
3. Microbial parameters

*1. Growth test with seedling apple assay*

Apple seeds (cv. Granny Smith) were stratified at +3 $^{o}C$ for 40 days, then they were seeded in sterile peat/sand (3/1) mixture and maintained at 12–14 $^{o}C$ for 21 days. Seedlings were transplanted to soil samples into 60 hole (7 cm deep, 4 cm dia.) thermo-formed trays. The trial was arranged in a randomised block design with three replicates, each of 30 plants; peat control were also inserted in the trail. Seedlings were grown at 24–28 $^{o}C$ in greenhouse for 40 days, then harvested. Plant height was measured and health assessed giving scores from 0–3 (0: dead plant; 1–3: from weak to healthy plant).

The growth index was calculated as follows: height of above ground part of apple plant x health score.

Data were subjected to one way analysis of variance and to LSD mean separation test.

*2. Total root colonising fungi and their aggressiveness toward apple seedlings.*

Young apple plants were washed under running water for two hours, then sterilised for 2 minutes with 1% sodium hypo-chlorite water solution and rinsed with sterile water. Three root explants for each plant and soil sample were placed on water agar. After an incubation period of 4 days, colonies grown from root segments were transferred to Sucrose Potato Agar + 200 mg $l^{-1}$ streptomycin sulphate mg $l^{-1}$. Colonies were identified to the standard taxonomic references, *Pythium* sp. identification was done by Centraalbureau voor Schimmelcultures.

The pathogenicity of 14 more frequent fungal isolates was assessed. Twenty days old seedling cv Golden, obtained as below, were transplanted in pot with artifi-

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cially inoculated peat, 5 /1 (v/v) peat / sand-meal inoculum (Nene *et al.*, 1981). Twelve plants for each strain (four per pot) were grown in greenhouse for twenty-five days after transplanting. The number of dead plants of each treatment were recorded.

*3. Microbial parameters.*

Total fungi, culturable bacteria and fluorescent bacteria were recorded by soil dilution plate method using appropriate selective media (respectively: Water Agar (WA), Thornton, King's B) . Data were expresses as Colony Forming Units (CFU) $g^{-1}$ soil.

Data were subjected to one way analysis of variance and to LSD mean separation test.

## Results

The mortality of the apple plants from the six applied treatments varied widely ($P \leq 0.01$), in all cases soils from organic orchards gave a lower mortality than adjacent conventional orchards, but only in one case this difference was statistically significant (Fig. 1).

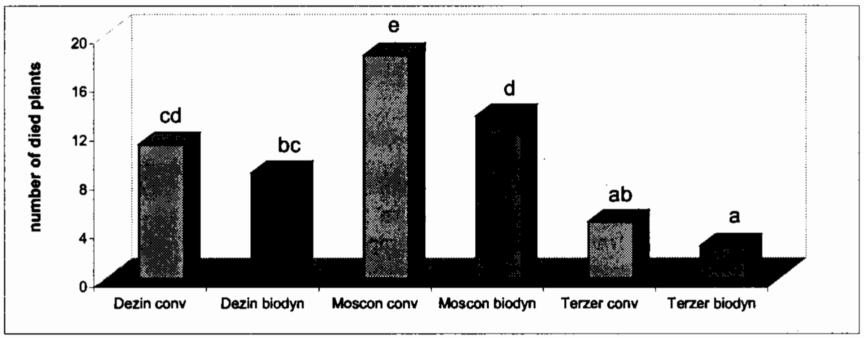



**Figure 1.** Number of young apple plant died in growth test. LSD test $P < 0.05$ *

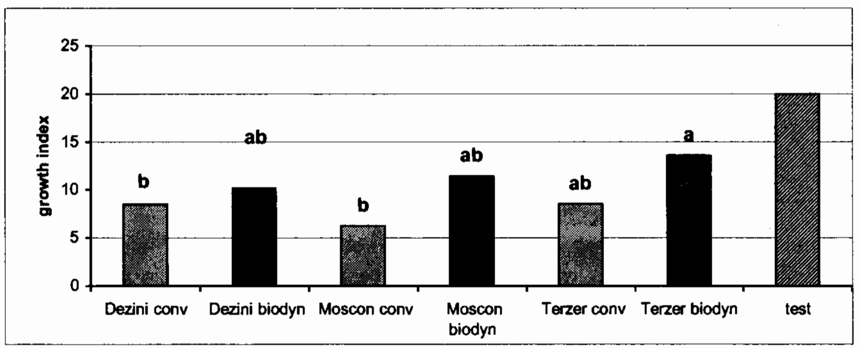



**Figure 2.** Growth index by apple seedlings test. LSD test $P < 0.05$ *



Seedling grown on natural soils coming from 6 apple orchards sites, in all cases, gave a significantly lower growth index than the control on peat in which no dead plants were recorded.

Concerning the growth index, there was no significant difference between the soils of the six sites, only growth index on peat, where no plants died was significantly different. In all cases young apple plants grown on soil from organic orchards showed a higher index than adjacent conventional orchards, but it did not differ in a significant way (Fig. 2).

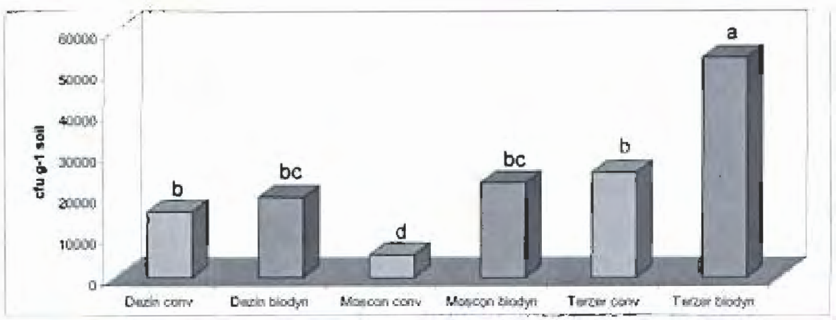
Root colonisation varied from 20 to 60 %. The most frequent colonizing fungi belonged to *Rhizoctonia*, *Fusarium*, *Aphanomyces* and *Pythium* genus (Tab.1). *Rhizoctonia solani* and *Pythium* strains were the most pathogenic, *F. solani* and *Cylindrocarpon* strains showed a low pathogenicity, while others assessed strains did not give disease symptoms (Tab.1).

**Table 1.** Root colonising fungi and their pathogenicity on artificially inoculated apple seedlings.

| | | Pathogenicity test | |
|---|---|---|---|
| Fungal species | Frequency (%) | Number of assessed strain | pathogenicity |
| *Rhizoctonia solani* | 23.8 | 5 | 2 no path, 1 low, 2 high |
| *Fusarium solani* | 24.6 | 1 | Low |
| *Aphanomyces* sp. | 18.4 | 2 | No |
| *Fusarium oxysporum* | 16.2 | 1 | No |
| *Cylindrocarpon* sp. | 3.8 | 1 | Low |
| *Pythium intermedium* | 6.3 | 3 | 1 medium, 2 high |
| *Fusarium* spp. | 6.3 | | |
| *Trichoderma* sp. | 1.5 | | |

Regarding the microbial soil parameters, total culturable bacteria and fluorescent bacteria, they varied respectively from 120.000 cfu $g^{-1}$ soil to 220.000 cfu $g^{-1}$ soil and from 800.000 to 3.000.000 cfu $g^{-1}$ soil; no differences of bacteria level were observed between conventional and organic management.

Total fungi in the six soils varied widely. Organic orchard soils showed in all cases a higher level than the contiguous conventional orchards (Fig. 3).




*Means followed by a common letter are not significantly different.

**Figure 3.** Total fungi in soil samples coming from 6 orchard. LSD test $P < 0.05$ *

## Discussion

All root colonizing fungi of young apple plants in growth assays on soil samples coming from South Tyrol orchards, belonged to the fungi complex, which has been reported to have a causal role in the development of apple replant disease by several authors in U.S.A (Mazzola, 1998), Canada (Brown, 1995), Australia (Dullaide *et al.*, 1994) and Great Britain ( Sewell, 1981). The high frequency and the aggressiveness toward young apple plants of *Pythium* and *Rhizoctonia* indicates that the apple replant disease can be widely influenced by soil microbial balance. In fact, these two pathogens are able to survive saprophytically in soil, therefore they are strongly affected by microbial activity both by feeding competition and by antibiotic activity.

The high content of organic matter of the apple orchard soils (between 2 % and 5 %) in South Tyrol is probably the main reason why replant disease is not so evident. This high organic matter content is due to a common approach in the management of apple orchards tending to maintain and increase organic matter content in soil, the base of soil fertility. Nevertheless, the better growth index and the lower mortality in soil coming from organic as compared to conventional orchards (Fig. 1 and 2), and the higher level of micoflora in organic than in conventional soils (Fig. 3), indicate that soil management tending to microbial balance can suppress soil borne pathogens. It is interesting to emphasise that in the Moscon site, the only one in which plant mortality in conventional management was significantly higher than in organic (Fig. 1), the soil micoflora level also significantly differed between organic and conventional management (Fig. 3). In addition, the site labelled Terzer, giving the lower plant mortality in growing test, showed the highest micoflora mean level among three observed sites, with a micoflora level in soil from organic orchard significantly higher than all others soil samples (Fig. 3).



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