# Efficacy of preparations based on microorganisms against apple replant disease

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**Abstract**

*Within the Core Organic 2 Project Bio-Incrop, different preparations and products based on microorganisms were tested for their activity against apple replant disease. The trials were conducted in a greenhouse on potted plantlets on M9 rootstock, which is the most commonly used rootstock in organic apple cultivations. To prove the presence of apple replant disease, potted plantlets grown on not sterilized untreated soil were used. These plantlets were used as untreated control. The effect of the microbial-based preparations on shoot length, shoot dry weight, and leaf colour of plantlets was limited.*

**Keywords:** Apple replant disease, soil treatment, rootstock M9

## Introduction

Recently soil exhaustion or apple replant disease has become a severe problem, which suppresses growth and decreases yield in apple-growing areas worldwide (Mazzola & Manici, 2012). It can be assumed that intensive apple cultivation systems with continuously increasing plant densities and unchanged spatial arrangement of plant rows due to the use of stationary support and netting structures, may be involved in disease occurrence (Kelderer *et al.*, 2012). However, possible causal agents of the disease, and especially potential non-chemical or non-synthetic control tools have not yet been investigated in detail. It is well-known that apple replant disease develops through changes in soil microbial populations (Manici *et al.*, 2003; Manici *et al.*, 2013).

Different products based on microorganisms, which may be used to restore the balance of soil microbial populations, or to control plant pathogenic fungi or to help plants to resist against infections, are available on the market. The international research Project Bio-Incrop (Innovative cropping techniques to increase soil health in organic fruit tree corps), financially supported by the European Union within the call CoreOrganic 2, aimed at investigating possible measures for the control of apple replant disease in organic farming. Within this project, a greenhouse study on potted apple plantlets grown on replant disease soil treated with different microbial-based preparations was conducted. All tested products were available on the national market, and were sold as either registered plant protection product, or fertilizer or plant growth promoter.

## Material and Methods

In 2013, the sector Organic Farming at the Research Centre Laimburg (Ora, South Tyrol, Italy) tested 12 different preparations and formulated products with biofungicide, nematicide or biostimulant as product claim and all containing active ingredient(s) allowed in organic farming (see Table 1).The preparations were added to soil taken from an orchard affected by apple replant disease at the Research Centre Laimburg. Then the soil/ product mixtures were filled into 1.4-L plastic pots, and 1 apple plantlet on M9 rootstock was transplanted into each pot in March 2013. Half of the application rate of each tested product was mixed into the soil just prior to transplanting (liquid products were applied diluted in

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water), while the other half was applied 4 weeks after transplanting. Not sterilized, untreated soil was used as control treatment. Each treatment was applied to six plantlets per replicate, and 3 replicates per treatment were used (total number of treatments: 13).

Table 1: Trade name of the tested preparations, national distributor, dosage per pot, cost (€/kg) and active ingredient(s) of the tested products.

| No. | Name | Company | Dosage/pot | € Price/kg | Active ingredient/microorganisms |
|---|---|---|---|---|---|
| 1 | **Micosat F** | Geofin | 2 x 15 g | 10,00 | a consortium of beneficial soil organisms (mycorrhizal and saprophytic fungi, rhizosphere bacteria) |
| 2 | **Mycostop Biofungicide** | Bioplanet | 2 x 0,0075 g | 3200,00 | Streptomyces griseoviridis strain K61 Control several soil borne pathogens |
| 3 | **Tifi** | Italpollina | 2 x 8 g dissolved in water | 45,00 | Trichoderma atroviride |
| 4 | **Condor** | Italpollina | 2 x 4 g dissolved in water | 120,00 | Micorrize + Trichoderma |
| 5 | **OZOR** | Bioplanet | 2 x 0,45 g | 120,00 | Glomus intraradices |
| 6 | **Ekoprop nemax** | Geofin | 2 x 0,38 g | 100,00 | endomycorrhizal fungi (Glomus spp.), rhizosphere bacteria (Bacillus subtilis SN 04, Pseudomonas spp., Strptomycesspp., Arthrobothrys oligospora BL, Monacrosporium eudermatum, Myrothecium verrucaria) and Trichoderma harzianun TH 27. |
| 7 | **Nutri-Life Root-Guard** | Imported and distributed by Violmet Italia s.r.l. | 2 x 0,57 g | 100,00 | Arthrobotrys oligospora, A. conoidus, Paecilomyces fumosoroseus, P. lilacinus, Verticillium chlamydosporium and metabolites of Myrothecium verrucaria |
| 8 | **Nutri-Life 4/20** | Imported and distributed by Violmet Italia s.r.l. | 0,0142 g | 480,00 | Bacillus subtilis, Azotobacter vinelandii, Pseudomonas fluorescens, Pseudomonas putida, Rhizobium japonicum, Pseudomonas stutzeri, Trichoderma harzianum, Trichoderma viride, Pseudomonas cellulose, Azotobacter chroococcum, Azospirillum Brasilense,Polyangiu |
| 9 | **Nutri-Life 4/20** | Imported and distributed by Violmet Italia s.r.l. | 1,428 g | 480,00 | |
| 10 | **Nutri-Life Root-Guard** | Imported and distributed by Violmet Italia s.r.l. | 2 x 0,57 g | 100,00 | Arthrobotrys oligospora, A. conoidus, Paecilomyces fumosoroseus, P. lilacinus, Verticillium chlamydosporium and metabolites of Myrothecium verrucaria |
| | **Nutri-Life Tricho-Shield TM** | Imported and distributed by Violmet Italia s.r.l. | 2 x 0,57 g | 100,00 | talc-based formulation containing the beneficial fungal species Trichoderma-harzianum, T-ligonorum, Gliocladium virens |
| | **Nutri-Life Sudo-Shield TM** | Imported and distributed by Violmet Italia s.r.l. | 2 x 0,57 g | 100,00 | Pseudomonas fluorescens |
| 11 | **EM A** | EM Research Organization Inc. (EMRO) | 4 x 100ml EM-A : 10 l water = 1 watering can every 14 days // immerse before planting: 50 ml EM-A with 2 – 5 l Water (depending on demand) | 3,63 | liquid solution of Effective Microrganisms (photosynthetic bacteria, lactic acitic bacteria and yeasts) |
| | **EM Ceramic-powder** | EM Research Organization Inc. (EMRO) | 10g powder mingled with 1,5 kg soil in each pot before planting; immerse before planting: 50 g EM powder | 36,63 | ceramic powder |
| 12 | **F 1** | Geofin | 2 x 3,75ml mingled with NEM 2 | 25,00 | Micorrize Trichoderma |
| | **NEM 2** | Geofin | 2 x 3,75ml mingled with F1 | 25,00 | Fe, Mn, Zn |

The soil used in the trial was collected in an orchard affected by replant disease (Block 41) and analysed in the laboratory. The results are shown in Table 2.

Table 2: Analysis of the soil affected by replant disease used in the trial.

| | Soil texture | Humus (%) | pH | Carbonate content | salinity* mg/100g | (N) (%) | C/N ratio | P** mg/100g | K** mg/100g | Mg*** mg/100g | B** mg/kg | Fe**** g/kg Dw |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Block 41 | sandy silt | 2,4 | 7,4 | +++ | 31,5 | 0,15 | 10,0 | 11,0 | 22,5 | 17,0 | 0,6 | 29,85 |
| *KCl-extract after VDLUFA **CAL-extract after VDLUFA *** CAT-extract after VDLUFA **** KW-extract after VDLUFA | | | | | | | | | | | | |

| Heavy metals | Fe**** g/kg Dw | Al* g/kg Dw | Mn** mg/kg Dw | Cu** mg/kg Dw | Zn** mg/kg Dw | Cr** mg/kg Dw | Ni** mg/kg Dw | Pb** mg/kg Dw | Co** mg/kg Dw | Cd** mg/kg Dw | Hg*** mg/kg Dw |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Block 41 | 29,85 | 20,87 | 517,50 | 41,50 | 165,00 | 36,50 | 28,50 | 35,50 | 12,50 | 0,85 | 0,0425 |
| *CAT-extract after VDLUFA **KW-extract after VDLUFA *** DMA-extract after VDLUFA | | | | | | | | | | | |

Also all the products tested in the trial have been previously analysed in the laboratory, and results are shown in Table 3.

Table 3: Analysis of the products tested in the trial.

| | Dry matter (%) | Humidity (%) | Ash (% FM) | Organic Substance (% FM) | Nitrogen (N) (% m/m) | C/N-ratio |
|---|---|---|---|---|---|---|
| Mycosat F | 98,7 | 1,3 | 94,2 | 4,5 | 0,1 | 33,0 |
| Mycostop Biofungicide | 96,1 | 3,9 | 1,9 | 94,2 | 1,9 | 29,0 |
| Tifi | 91,2 | 8,8 | 5,5 | 85,7 | 0,1 | 355,0 |
| Condor | 91,3 | 8,7 | 10,1 | 81,2 | 0,3 | 139,0 |
| Ozor | 76,5 | 23,5 | 40,1 | 36,4 | 1,3 | 16,0 |
| Ekoprop nemax | 95,0 | 5,0 | 8,2 | 86,8 | 5,5 | 9,0 |
| F 1 | 10,5 | 89,5 | 1,9 | 8,6 | 2,2 | 2,0 |
| Nem 2 | 19,9 | 80,1 | 5,0 | 14,9 | 0,0 | 576,0 |
| EM-A | 1,2 | 98,8 | 0,3 | 0,9 | 0,0 | 27,0 |
| EM-Ceramic | 97,5 | 2,5 | 93,1 | 4,4 | 0,1 | 55,0 |
| Ozor 2013 | 96,6 | 3,4 | 49,1 | 47,5 | 1,6 | 17,0 |
| Nutri-Life Root-Guard | 96,4 | 3,6 | 96,3 | 0,1 | 0,0 | 2,0 |
| Nutri-Life Sudo-Shield | 93,3 | 6,7 | 92,8 | 0,5 | 0,1 | 5,0 |
| Nutri-Life Tricho-Shield | 97,3 | 2,7 | 96,9 | 0,4 | 0,0 | 6,0 |
| Nutri-Life 4/20 | 88,8 | 11,2 | 20,9 | 67,9 | 1,1 | 35,0 |

Plantlets were allowed to grow for a period of 14 weeks, and the following parameters were assessed on the plantlets:

Leaf colour: at the beginning of May leaf colour was measured on 25 leaves per treatment using a Minolta CR-300 Chroma Meter.

Shoot length: shoot length was measured at the end of May, 14 weeks after transplanting of the plantlets; the result is reported as sum of the length of all shoots per plantlet.

Dry weight of shoots: at the end of May, the fresh and dry weight of the shoots was measured.

To verify whether apple replant disease was actually present in the soil used in the trial, a preliminary trial was carried out: a replant disease soil sample was sterilized for 12 hours

at 100°C in a drying cabinet. Then apple plantlets on M9 rootstock were planted into this soil and their growth was compared to that of plantlets grown in the not sterilized, untreated replant disease soil. The optimal time and heat for the sterilization of the soil samples had been identified previously in a trial, in which the following treatments had been compared: (a) exposure to 80°C, uncovered soil; (b) exposure to 80°C, soil closed into plastic bags; (c) exposure to 100°C, soil closed into plastic bags; (d) untreated control. A visual analysis of the plantlets grown in soil exposed to these 4 treatments had shown that the best results for soil sterilization are obtained with (c) exposure to 100°C, soil closed into plastic bags.

Statistical analysis

The data assessed in the trial were compared across treatments using 1-way ANOVAs, followed by Tukey's test for post-hoc comparisons of means ($p<0.05$), while a one-sample T-test was used to compare data between treatments (sterilized soil and not sterilized untreated soil) in the preliminary trial. All analyses were performed using the statistics program PASW 17.

## Results

The results of the preliminary trial showed that the soil used in the trail was actually affected by apple replant disease. In fact, both shoot length and shoot dry weight values of plantlets grown on sterilized soil were significantly higher than those of plantlets grown on not sterilized untreated soil (Table 4).

Table 4: Shoot length and shoot dry weight of plantlets grown on sterilized and not sterilized untreated soil (preliminary trial).

| | Shoot length (cm) | | Dry weight of the shoots (g) | |
|---|---|---|---|---|
| | Mean | stat. | Mean | stat |
| **Sterilized soil** | 37,71 | a | 3,06 | a |
| **Untreated control** | 21,54 | b | 1,71 | b |

After 12 weeks of growth, the highest hue leaf colour code value was recorded for Treatment n. 12, F1 + NEM 2 (Table 5). Shoot length was highest for Treatment 11,EM A + Em ceramic powder, followed by Treatment n. 9, Nutri-Life 40/20 at 500g/ha, while the highest shoot dry weight value was registered for Treatment n. 7, Nutri-Life Root-Guard

Table 5: Shoot length, dry weight of the shoots and hue code h° of the tested compost treatments.

| | treatments | shoot length (cm) | | dry weight of shoots (g) | | Leaf color h° | |
|---|---|---|---|---|---|---|---|
| 1 | **Micosat F** | 24,86 | ab | 2,38 | abc | 125,32 | ab |
| 2 | **Mycostop Biofungicide** | 22,32 | a | 1,86 | ab | 125,02 | ab |
| 3 | **Tifi** | 25,39 | ab | 2,37 | abc | 123,68 | ab |
| 4 | **Condor** | 22,21 | a | 1,99 | abc | 123,16 | ab |
| 5 | **OZOR** | 24,38 | ab | 2,09 | abc | 124,06 | ab |
| 6 | **Ekoprop nemax** | 23,35 | ab | 1,73 | a | 122,39 | a |
| 7 | **Nutri-Life Root-Guard** | 22,44 | a | 1,98 | abc | 123,03 | ab |
| 8 | **Nutri-Life 4/20 50g/ha** | 22,08 | a | 2,06 | abc | 125,66 | ab |
| 9 | **Nutri-Life 4/20 500g/ha** | 28,78 | ab | 2,54 | bc | 125,59 | ab |
| 10 | **Nutri-Life Tricho-/Sudo-Shield+Root Gua** | 23,97 | ab | 2,28 | abc | 124,97 | ab |
| 11 | **EM A + Em ceramic powder** | 29,89 | **b** | 2,35 | abc | 122,48 | a |
| 12 | **F1 + NEM 2** | 26,08 | ab | 2,62 | **c** | 126,72 | b |
| 13 | **Untreated control** | 27,86 | ab | 2,61 | bc | 122,34 | a |

Considering the application rates of the different products tested in the trial (Table 1) and the nitrogen content of the products determined via laboratory analysis (Table 3), no significant increase in the nitrogen content of treated soil samples was expected.

## Discussion

The best method for soil sterilization had been established in preliminary studies: among the 4 tested treatments, heating of soil samples closed into plastic bags in a drying cabinet at 100°C for 10 hours proved to be the most effective method (results not reported). This method of soil sterilization was therefore then used in the preliminary trial aiming at verifying whether apple replant disease was actually present in the soil used in the trial.

Based on the nitrogen content of the tested products determined via laboratory analysis, biasing of data due to addition of nitrogen compounds can be excluded.

Even though significant differences in shoot length and shoot dry weight of M9 rootstock plantlets grown on treated and untreated soil emerged, these results can not be considered of relevance in practice. In fact, most of the tested treatments resulted in reduced shoot length and shoot dry weight values in comparison to the untreated control, and a similar trend was observed for leaf colour. And there are other 2 interesting points to for discussion. Treatment 9 was ten times higher concentrated then treatment 8 and showed a significant better performance as treatment 8. In a separated not reported pot-trail three preparations were sterilized and compared with the original preparations. There was no difference in the performance of the plantlets.

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