

Mid-term report

for the CORE Organic II funded project

**“Innovative cropping Practices to increase soil health of organic fruit tree orchards”
BIO-INCROP**

[note to coordinators: this report covers the whole duration/first half of the project. Once approved by the funding bodies, the final report without the annexes/abstract of the mid-term report should be made publicly available on Organic Eprints]

Period covered: 01.01.2012 - 30.6.2013

Project acronym:	BIO-INCROP			
Title:	INNOVATIVE CROPPING TECHNIQUES TO INCREASE SOIL HEALTH IN ORGANIC FRUIT TREE CROPS			
Coordinator information:	Senior Researcher of CRA.			
Institution:	Consiglio Nazionale per la Ricerca e la Sperimentazione in agricoltura	CRA		
Faculty/ Department/ Section/Unit	CRA- Research Centre for Industrial Crops	CRA-CIN		
Address:	Via di Corticella 133			P.O. Box:
	Bologna	40128:	Region:	Italy:
Coordinator:	Manici	Luisa Maria :	Dr:	
Address if different from above:				
	Phone: +39 051 6316839	Fax:	E-mail: luisa.manici@entecra.it	
Start of Project:	1 January 2012	End of project:	31 December 2014	

Project partners and contact persons:

Partner no.	Country	Organisation name:	Functions*):	Involved in WP's:	Contact person with e-mail address:
1	Italy	Consiglio Nazionale per la Ricerca e la Sperimentazione in Agricoltura	PC	WP0, WP1, WP2, WP3	Luisa M. Manici luisa.manici@entecra.it
2	Italy	Laimburg Research Centre for Agriculture and Forestry	WPM	WP1, WP2; WP3, WP4	Markus Kelderer markus.kelderer@provinz.bz.it
3	Switzerland	Agroscope Changins-Wädenswil Research Station	WPM	WP1, WP2; WP3,	Andreas Naef andreas.naef@agroscope.admin.ch
4	Austria	Landwirtschaftliches Versuchszentrum Graz-Haidegg	WPM	WP1, WP2, WP3; WP4	Thomas Rühmer thomas.ruehmer@stmk.gv.at
5	Spain	Instituto Valenciano de Investigaciones Agrarias	WPM	WP1, WP3, WP4	Rodolfo Canet canet_rod@gva.es
6	Austria	University of Innsbruck-Department of Microbiology	P	WP1, WP2, WP4	Ingrid Franke-Whittle Ingrid.Whittle@uibk.ac.at

7	Germany	DLR Rheinpfalz, Center of competence	P	WP1, WP2; WP3; WP4	Gerhard Baab gerhard.baab@dlr.rlp.de
8	Turkey	Egirdir Horticulture Research Institute & Alata Horticultural Research Institute	P	WP3;	Suat Kaymak suatkaymak43@hotmail.com Hasan Pinar hpinarka@yahoo.com

*) PC: Project coordinator, WPM: Work package manager, WPCM: Work package co-manager, P: Participant

Projects website: www.bio-incrop.org

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Post/mid-term project summary suitable for web publication

(max. 1 page. - Arial, size 11) (please focus on results and conclusions, preferably in bullet points)

The main aim of BIO-INCROP is the exploitation of natural resources to increase soil health in organic fruit tree orchards of Europe. The project has two target fruit tree crops: Apple, representing temperate growing regions of Europe, and Citrus, representing Mediterranean agro-environments. The main results of the first part of the project are as follows:

APPLE

- Replant disease of apple orchards has been confirmed to have a biotic origin in a study with a bioassay on soil samples coming from three specialized apple growing areas of Germany, Austria and Italy.
- Soil fertility (nutritional and biological parameters) was not found to be a limiting factor for plant growth in replanted orchards of three main apple growing regions of Central Europe. This might be mainly due to the highest soil organic matter content characterizing growing areas of this survey.
- *Cylindrocarpon* like –species and *Pythium* spp. resulted the two soil borne fungal pathogens mainly involved in apple replant disease etiology.
- Findings on root associated microorganisms in replanted apple orchards in central Europe support the challenges of the BIO-INCROP project: the largest part of fungal endophytes which naturally colonize roots can have a commensal (null effect) or mutual (beneficial) symbiotic relationship with apple plants. Potential beneficial microbial populations of rhizosphere are the objective of the second part of the project.
- Organic amendments seem to have both positive and negative impacts on plant growth as compared to controls in a bioassay test. This effect appears to depend on

the origin of the amendment (green waste, animal manure derived; selected urban waste; other material).

- Impact of biologically active formulates on plant growth varied according to the origin of soil samples used in a bioassay test. Results obtained thus far in three research stations suggest:
 - Untreated controls never yielded the poorest plant growth when compared to ten commercially available bioformulates in the bioassay.
 - Four biological formulates out of ten under evaluation did not give any growth increase as compared to the untreated control, while a positive effect for at least three bioformulates was observed in each bioassay test. A similar result was obtained also by the Turkish partner in a bioassay test using soil samples artificially infected by *Phytophthora cactorum*.

CITRUS

- Among soil treatments evaluated in a field trial located in the Valencia region (E), solarization resulted up to now the main promoter of changes. However, the concurrent use of manure seemed to have a stronger influence on microbial communities than it did in biochemical parameters
- Clear effects of the treatments (green manure with *Brassicaceae*, organic amendments, solarization) on the microbial populations in soil were found, although the statistical significance of the results was negatively affected by the high variability of biological parameters in agricultural soils.

Pre-project summary

Multiannual crops such as fruit tree crops are affected by soil sickness or yield decline. “Replant disease” is the main biological component of this problem due to the eco-functional intensification of growing areas specialising in fruit production. The severity of this etiology is mediated by plant vigour, physiological state of plants and abiotic factors, therefore its occurrence is actually an indicator for fruit growers of the degraded status of a soil's biological processes for its crops.

The project aims to develop innovative management options able to increase soil biological functioning, focusing the attention on soil suppressiveness: the natural ability of soil to control soil-borne pathogenic agents of replant disease. The activities are planned on two target crops: citrus and apple, representing two main agro-environments of Europe. Cover crops taken from local germoplasm collections or from the natural vegetative covers and recycled high quality organic materials are the natural resources upon which the project focuses, in order to develop innovative cropping practices which will enable soil biodiversity preservation and exploit its biological features.

The early evaluation of plant response and the use of advanced methodologies to evaluate microbial response toward the inputs are the strategies used to identify natural resources and techniques capable of increasing microbial biomass and diversity and selectively affecting beneficial and pathogenic microbial populations.

The resulting innovative cropping practices are also easily transferred to other crops such as stone fruits and strawberries. Each country's activities are planned in close cooperation with regional agricultural research centres working on organic farming and laboratories with specific expertise. The dissemination plan of the BIO-INCRP project should increase grower's awareness of soil biodiversity as a resource for developing new technologies.

1. Main results, conclusions and fulfilment of objectives

1.1 Summary of main results and conclusions

Note: this should cover the whole/first half of the project duration

(max. 5 pages – Arial size 11).

Activities performed in the first part of BIO-INCROP project concern five of the eight main objectives fixed in the project proposal. They are:

- Evaluation of soil borne pest and pathogens involved in replant disease
- Role of rhizospheric bacterial and fungal communities in plant health
- Selection of naturally available resources to increase microbial diversity and biomass
- Compost and organic amendments
- Evaluation of biologically active formulates

The further three objectives will be pursued based to results and material (strains of microorganisms, selected organic amendments, selected bio-formulates, etc) obtained in the first part of the project

- Cover crops and wild plants selected on basis of plant/microorganisms interaction
- Survey of available means for controlling replant disease
- Investigation of new low input agronomical options

The first step in evaluation of soil borne pathogens and pest involved in replant disease was performed in a survey on specialized apple growing regions of Germany (Rhineland Palatinate), Austria (Styria) and Italy (South Tyrol). A bioassay test on soil samples from replanted apple orchards of those regions and the corresponding sterilized soil samples was combined with the evaluation of root associated microorganisms as well as soil physical-chemical and biological parameters. Findings confirmed that:

WP1-A. As sterilized soil samples gave plant growth significantly higher than that obtained on soil samples taken from replanted orchards, replant disease of apple orchards in Europe has been confirmed to have a biotic origin.

WP1-B. The lack of correlation among plant growth, soil nutritional and biological parameters indicates that soil fertility was not a limiting factor in the European apple orchards which were considered in this survey.

WP1-C. *Cylindrocarpon* like –species and *Pythium* spp. results the only two groups of fungal endophytes negatively correlated to plant growth.

WP1-D. Given that only two root endophytic fungal genera (40%) of the five most abundant ones isolated from apple rootstocks (92%) gave negative correlations to plant growth; findings support the challenges of the BIO-INCROP project: the largest part of fungal endophytes which naturally colonize roots can have a commensal (null effect) or mutual (beneficial) symbiotic relationship with apple plants.

In conclusion, this first study which is concluded (see submission of manuscript) confirms the biotic origin of replant disease in apple orchards and suggests that soil microbial populations indigenous to apple orchards can actually represent a resource to be exploited for increasing plant growth/soil health.

The next step of this ongoing research program is focused on composition of rhizosphere bacterial and fungal communities, which will be analyzed through several molecular methods (Pyrosequencing and DGGE and other methods), to investigate their interaction with plant growth and root endophytic fungal communities observed in the first part of survey. This second

part of activity should lead to the identification of the beneficial microbial populations which have a potential beneficial impact on soil suppressiveness.

The last step of this research in WP1 will be combined with findings on organic amendments which will be obtained in WP2, with the aim to find an amendments or cropping practices that is able to induce a shift in soil microbial communities towards a higher abundance of beneficial microbial populations (antagonistic, plant growth promoting, etc).

Findings obtained up to now in WP2 (by DLR and HAID) suggest that:

WP2-A. Organic amendment of different origins can have a variable impact on plant growth in replanted soil.

WP2-B. Amendments such as “green composts” seem to have a negative impact on plant growth as compared to untreated controls, while high quality composts such as Champost seem to have a positive impact on plant growth.

The first screening on commercially available biologically active formulates for reducing replant problems in **apple orchards** showed a variable plant response in bioassays according to the soil samples in which they were performed. In general:

WP3-A. Unsterilized controls never yielded the lowest plant growth among those tested in each experimental site

WP3-B. In all cases, at least four treatments out of the ten under evaluation did not give any growth increase as compared to the untreated control.

WP3-C. MYCOSTOP was the bioformulate more frequently recorded as effective for plant growth followed by other four/five bioformulates based on various mixtures of fungi, mycorrhiza and *Actinomyces* and others

Activity on effectiveness of bioformulates to reduce replant problems in **citrus degraded orchards** is characterized by several constraints not seen in apple crops. These are mostly due to fertility problems deriving from organic matter depletion which naturally occurs in the Mediterranean environment, and are enhanced by tillage and other cropping practices. Therefore, the main target of activity to improve citrus growth is focused on the quantitative and qualitative increase of soil organic matter, without which bioformulates cannot find a suitable environment to survive and establish in. The experimental setting of WP3 on citrus includes both pots as well as full field trials. In both cases the evaluation of growth response requires more time than bioassays on apple (2-3 months). Seven bioformulates mainly represented by mixtures of microorganisms are being tested at IVIA (Spain) and AHRI (Turkey), but no results able to discriminate the effectiveness of treatments are yet available.

The whole activity on effectiveness of innovative cropping practices in inducing changes of soil biological parameters is being evaluated in a degraded citrus orchard in the Valencia region in Spain. Biologically active products, selected according to interest from the organic fruit production sectors in Spain and Turkey, are under evaluation in pot experiments, and are also being evaluated in field experiments at the IVIA experimental citrus orchard.

Open field trials on citrus orchards started in October 2012, however plant growth parameters do not differ up to now. Evaluation of soil microorganism responses to soil treatments, which is performed through microbial counts, needs a set of further evaluations over the whole growing season as well as the evaluation of the plant growth response. The first results of WP4 on citrus will be available in the second part of the project.

1.2 Fulfillment of objectives

BIO-INCRUP is currently achieving its main research objectives, which are listed at the beginning of section *1.1 Summary of main results and conclusions*. The plan of the project activities which are ongoing 18 months after the beginning of the project fully is, as stated in the timetable of the project proposal.

In addition, dissemination activity is guaranteeing an adequate information on principles of the project strategy to increase soil health in fruit tree orchards.

2. Milestones and deliverables status

Milestones:

No ¹	Milestone name	Planned delivery month ²	Actual delivery month ²	Means of verification
M 1.1 (18 months)	Identification of “replant disease” agents on apple and citrus	18	18	Correlation between abundance of pathogenic organisms, and plant growth reductions
M 1.1 (24 months)	Identification of “replant disease” agents on apple and citrus	24	18	Deposition in GenBank nucleotide sequences of pathogenic organisms.
M 1.3	Identification beneficial microorganisms and nematodes	24	18	(1) Deposition in GenBank nucleotide sequences of the organisms; (2) evaluation of biological activity of microorganisms
M.3.2	First evaluation cycle of bio-formulations and bio-pesticides	18	18	Evaluation of effectiveness of treatment by greenhouse bioassay

¹ Please use the numbering convention <WP number>.<number of milestone/deliverable within that WP>. For example, deliverable 4.2 would be the second deliverable from work package 4.

² Measured in months from the project start date (month 1).

Deliverables:

No ¹	Deliverable name and language	Nature ³	Dissemination level ⁴ and link to the document	Planned delivery month ²	Actual delivery month ²
D0.1	Coord-Dissem1	Website	http://www.bio-incrop.org	18	18
D1.1	Apple replant disease (ARD) 1	Report	http://www.orgprints.org/22202/1/D1_1_BIO-INCROP_project_deliverable.pdf	12	12
D1.2	ARD	Tech. bull.	Public*	24	18
D1.3	ARD General	Report	Confidential*	20	18
D3.1	Bio-p1 IVIA	Report	http://www.orgprints.org/22438/1/ES%20Report%202012.pdf	20	18
D4.1	Strategy1	Report	http://www.orgprints.org/22438/1/ES%20Report%202012.pdf	18	18

Additional comments (in case of major changes or deviation from the original list)

Deliverable D1.2 and D1.3 will be inferred from the paper entitled "Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe" submitted to the scientific Journal Applied Soil Ecology D3.1 and D4.1 have been combined in one document

3. Work package description and results:

WP 0	Coordination and Dissemination
Coordinator Luisa M. Manici 1 CRA	
Original description of work:	
The main functions of the coordination team are: i) to guarantee communication among partners for a productive exchange of methodology, tools and techniques; ii) to monitor and report internally the progress of the research activities; iii) to coordinate research activities, within and among the work packages, together with the management committee	

³ Please indicate the nature of the deliverable. For example Report, Paper, Book, Protocol, Prototype, Website, Database, Demonstrator, Meeting, Workshop...

⁴ Please indicate the dissemination level using one of the following codes: PU = Public; INT= Internal (Restricted to other project participants); RE = Restricted to a group specified by the consortium; CO = Confidential, only for members of the consortium.

Report on results obtained and changes to the original plan/WP aims:

Communication among the partners during the first part of the project was guaranteed by following actions.

- Two 2 day- project meetings and one pre-meeting associated to the kick off meeting aiming at agree among the partners who had never met before, on a common plan of activities and criteria to decide the list of biological active products to test in WP3
- Exchange of documents through Dropbox folders
- E-mail messages are used by the coordinator to communicate any information to the partners and is the main means for communicating among partners involved on common activities.

Monitoring of activity and report internally the progress was actually performed through two ways

- Presentation of activities during the second meeting and their discussion followed by adjustments of initial decisions about the experimental setting according to the first results.
- All final reports due within 18 months were submitted to organic e print by the coordinator, but quantity of reports up to now have not required any change or suggestion for their improvement.

Research activity of the project is well described in the Timetable and WP description of the project partners that have followed the work plan without any problem for the coordinator. In some cases (WP2), activities have been anticipated or slightly postponed according to the labor charge of each partner within the WPs. This was discussed with coordinator and the WP leaders for their approval.

A- results obtained:

Dissemination activity is the only on which is possible show results of WP0. Dissemination activity is extensively reported in section 4.

B- comments on deviations from the original plan:

Nothing

WP 1	ELUCIDATION OF SOIL MICROBIAL COMPONENTS INVOLVED IN SOIL-BORNE FUNGAL DISEASES OF ORGANIC FRUIT TREE ORCHARDS IN EUROPE
Responsible partner: 1 CRA, 2 LRC, 3 ACW, 4 HAID, 5 IVIA, 6LFU, 7DLR, WP manager Andreas Naef ACW Agroscope Changins-Wädenswil (Switzerland)	
Original description of work: Setting of diagnostic tools for the biotic component of replant disease, study of microbial communities and nematodes involved in replant disease	
WP 1- Task 1 <i>Evaluation of biotic components involved in "replant disease" etiology.</i>	
- <u>Survey on root endophytic fungal agents</u> of replant disease in apple orchards. This activity will be carried out on the three soil samples (replanted, fallow and sterilized plots) from 9 sites, three per country (Italy -South Tyrol, Germany, and Austria,) using greenhouse bioassay with young rootstock plants. The growing tests will be performed in a common trial at LCR (I). The subcontractor of LRC, UA(I) will perform the evaluation of the root architecture and development on at least 9 samples. CRA-CIN (I) and ACW (CH) will perform this activity by integrating traditional and molecular identification technologies.	
- <u>Survey on biotic components responsible of "replant disorder" in citrus orchards.</u> IVIA (E) will carry out a survey on the biotic components in the degraded citrus orchards object of the activities of the project. The survey on soil biotic component will be done as comparison	

- of some microbial groups within virgin and treated-soils, complemented with pot experiments to elucidate suppressive or disease-induction abilities of microbial groups.
- The role of nematodes in apple replant disease etiology will be investigated by DLR (GE) on the 9 couples of soil samples soil samples conducive for nematodes
 - Pathogenicity test of root endophytic fungal species. The number of pathogens tested depends on the number of strains isolated in previous sub tasks and the growth reduction/root health associated with their abundance on root explants.
- WP 1- Task 2** *Evaluation of bacterial and fungal communities associated with rhizosphere.*
Comparison of rhizosperic bacterial and endophytic fungal communities (diversity and composition) in replant orchards.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

WP 1- Task 1 Evaluation of biotic components involved in “replant disease” etiology.

APPLE

Partners involved in this Task defined the experimental setting during the Kick-off meeting in February 2012 and performed their activity on the same soil samples (9 orchards, three countries)

Three main activities were performed on root endophytic fungal agents of replant disease in apple orchards.

Activity 1.: Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe.

Partners: 1 CRA, 2 LRC, 3 ACW, 4 HAID, 6 LFU, 7 DLR

Soil health was evaluated using a bioassay test with root cuttings of the clonal M9 rootstock, one of the most commonly used rootstocks in Europe. Plant growth response in replant, fallow and gamma ray sterilized soil was evaluated according to the experimental setting described above (three orchards in specialized apple growing areas of Germany, Austria and Italy). Plant growth (parameter of soil health) significantly differed between treatments but not between countries. Root endophytic fungi were confirmed as one of the main components of growth reduction, while endophytic nematodes (*Pratylenchus* sp.) were not. The ecological approach to analysis allowed us to clarify that *Cylindrocarpon*-like fungi (*Ilyonectria* spp. and *Thelonectria* sp.) had a major pathogenic role, while *Pythium* spp. prevailed as a pathogen only in German orchards.

Gamma sterilized soil resulted in a more-than-proportional increase of plant growth (42% and 31% respectively vs. replant and fallow) as compared to that observed between fallow and replant soil (18%).

This best performance in gamma-sterilized soil, in addition to the lowest root colonization by *Cylindrocarpon*-like fungi in this treatment, appeared to be due to the different composition of fungal communities as compared to two other treatments (fallow and replant soil), which instead were highly similar to each other. This difference was due to a shift of community composition toward *Fusarium* and binucleate *Rhizoctonia*, which prevailed in sterilized soil.

Findings of this first part of project suggested three main conclusions:

R1 : Sterilized soil gave plant growth significantly higher than that obtained on soil samples taken from orchards. This confirmed that replant disease of apple orchards in Europe has a biotic origin.

R 2 No correlation between plant growth and soil nutritional and biological parameters was found. This suggested that no limiting soil fertility factors involved in growth reduction were evident in this survey on apple orchards.

R3. Only two root endophyte fungal genera over the five most abundant ones isolated from apple rootstock resulted in negative correlations to plant growth (pathogenic). These findings

support the challenges of the BIO-INCROP project: the largest part of endophytes which naturally colonize root can have null effect or beneficial symbiotic relation with apple plants.

Activity 2 Analysis of rhizospheric and bulk soil by molecular tools.

Partner involved: 3. ACW, on material of sampling sites of the survey in WP 1 task 1.

Molecular analysis was performed using specific primers and nested PCR. Preliminary results suggest the following:

- *Cylindrocarpon* was present everywhere, *Pythium* spp. were mostly present in Austria, *Phytophthora* was detected only at trace levels while *Rhizoctonia* numbers were the highest in samples from Germany.

- If a species was detected at a site, the detection frequency in sample repeats was similar in replanted and virgin soil.

Activity 3 Root Architecture.

Partner involved: 2.LRC and University of Ancona on plants taken from bioassay of WP1- task 1.

The activity compares root growth, morphology and architecture of apple seedlings as influenced by 3 different treatments: Replant, Virgin, Sterilized. Root electrolyte leakage was also measured as an indicator of root cells integrity and the level of stress experienced by the plant. Sterilized soil showed increased root growth as compared to Virgin and Replanted samples. Sterilization of soil samples gave a positive effect on lateral initiation equally visible for all root order, thus indicating an early and persistent amelioration of the growth environment. Minor differences were found between Virgin and Replant soil; no further signs of stress were indicated in Replant soils by the electrolyte leakage analysis.

Activity 4 Nematodes survey in apple orchard

Partner involved : 7 DLR, in cooperation with University of Bonn on soil samples from sampling sites of the survey in WP 1 task 1

It was observed that the total nematode count within fallow and replant soil increased within the growing period as could be expected. *Pratylenchus* counts differed between sampling sites. Counts were a lot higher in Italian samples than in any other sampling site. A constant increase or higher count of nematodes especially in the genus of *Pratylenchus* in replant soil could not be observed except in Italy. This suggests that the involvement of nematodes within the replant disease complex may not be prominent but could be seen as an interchangeable component highly depending upon the soil type.

CITRUS

Survey on biotic components responsible of "replant disorder" in citrus orchards.

Partner: 6. IVIA

Given that the Mediterranean agro-environment has as main constraint the natural progressive decline of soil organic matter content, a survey was set up in an open field. A degraded orchard in the area of Valencia was used. Evaluation of the culturable microbial communities (Bacteria, Fungi, Actinomycetes, *Bacillus* sp., Fluorescent pseudomonas, Denitrifying and nitrifying bacteria, Ammonium-oxidation potential) and biological parameters (Microbial biomass carbon and the following enzyme activities: Alkaline phosphomonoesterase, Phosphodiesterase, Urease, Arylsulphatase, β -D-glucosidase, N-acetyl- β -D-glucosaminidase, Dehydrogenase) have been periodically performed since summer 2012 in a 3-year period of conversion to organic Farming. Partial results of soil fertility parameters are available in Deliverable D4.1.

WP 2	SEARCHING FOR INNOVATIVE AGRONOMIC OPTIONS TO INDUCE SOIL SUPPRESSIVENESS IN ORGANIC FRUIT TREE ORCHARDS
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Responsible partner: 1 CRA, 2 LRC, 3 ACW, 4 HAID, 6 LFU, 7 DLR. **WP Manager:** Markus Kelderer, LRC, South Tyrol (Italy)

Original description of work: Selection of a series of naturally available resources (amendments and plants) by early evaluation in greenhouse tests,

WP 2- Task 1 *Selection of compost and organic amendments able to reduce replant disease by increasing microbial diversity and biomass.* Materials are selected according to the origin of the material, composting process, degree of decomposition of the organic matrix and its chemical and physical characteristics. The research centres: LCR (I); ACW (CH), DLR (GE) and HAID (A) in partial cooperation with other experimental centres, perform the evaluation of a defined number of treatments. CRA-CIN (IT) performs the additional evaluation of qualitative changes of microbial communities (Fungi and bacteria) on three treatments selected by LCR (I). Microbial communities of composts will be determined with the COMPOCHIO microarray by LFU (A)

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

WP 2- Task 1 Selection of compost and organic amendments able to reduce replant disease by increasing microbial diversity and biomass.

This task focuses on APPLE, while the effect of organic amendments on citrus are evaluated in open field trials (WP4). According to the original plan, WP2 activity should have begun in the second year of the project; however German and Austrian Partners, which were not directly involved in the bioassay test of WP1 performed at LRC, anticipated the first evaluation on organic amendment.

Partners involved in this Task defined the qualitative group of amendments and the experimental setting during the Kick-off meeting in February 2012. Bioassay trials were performed with M9 rootstocks using soil samples from one orchard affected by a replant problem. Each of the partners involved tested at least seven composts selected as follows:

- o one reference compost
- o at least 6 others composts. Which should be selected within the four following classes:
 - green waste i.e. grape compost
 - manure derived compost (i.e. poultry manure, cow compost, horse manure)
 - selected urban waste
 - other compost (i.e. Germany proposed to include brown carbon mixed with organic materials Compost)

Partner 6. LFU Microbial Ecology (A)

WP 2- Task 1: Physical and chemical parameters of composts, as well as microbial biomass measurements on composts used by ACW (CH), DLR (GE) and HAID (A) were determined. The same parameters were measured on compost amended soils after evaluation of plant growth response in bioassay tests.

Results indicated that the spent mushroom composts (CHAMPIHUN from Switzerland and CHAMPOST from Germany) had the highest basal respiration and microbial biomass values. In addition, these composts had the highest amount of microbial carbon as a percentage of organic carbon.

In order to evaluate the composition of microbial communities in composts, the COMPOCHIP microarray is being used. It is expected that all arrays will be analysed before October, 2013.

Partner 7. DLR (GE)

Seven composts and organic amendments were selected according to EU-organic farming guidelines.

Chosen organic amendments were: a traditional Green-cuttings-Compost (compost from regional green waste), CHAMPOST (the composted substrate from mushroom farming), APPLE

POMACE-COMPOST (composted apple pomace mixed with horse manure), VERMICOMPOST (worm composted horse manure), GRAPE-POMACE-COMPOST (composted grape pomace), PALATERRA 2 (substrate according to TERRA PRETA PRINCIPLE (fermentation with lactic acid bacteria with addition of charcoal) and OLIVE POMACE-COMPOST (composted olive pomace).

Trial should be repeated, as CHAMPOST and APPLE POMACE composts showed significant increase in growth as compared to untreated and reference compost, similar to that of the pasteurized variant.

Partner 4. HAID (A)

Replant soil from a replanted orchard of the research station Haidegg was selected to collect soil samples for a bioassay on effectiveness of commercial bioformulates. After three months, plant growth significantly differed among treatments.

STABLE MANURE, FRUIT WASTE and URBAN WASTE gave plant growth (shoot length) significantly higher than the following treatments: control, SLUDGE, TERRA PRETA (fermentation with lactic acid bacteria with addition of charcoal), GREEN MANURE and RAINWORM HUMUS.

Comments on deviations from the original plan:

Trials performed up to now gave a significant difference among treatments, but not all treatments showed a significant plant growth increase as compared to the untreated control. Nevertheless, as the first evaluation on the effectiveness of composts for increasing plant growth in replant soil is not yet concluded by all the partner, we have to wait for whole data of this first part of WP2 to be able to formulate any conclusion. Afterwards, amendments will be clustered on the basis of organic material of origin and original microbial composition which will be evaluated with the COMPOCHIP microarray by partner 6 LFU Microbial Ecology (A).

WP 3 | SURVEY ON THE AVAILABLE LOW INPUT TOOLS FOR CONTROLLING REPLANT DISEASE

Responsible partner: 2 LRC, 3 ACW, 4 HAID, 5 IVIA, 7 DLR, 8 GDAR (EHRI & AHRI). **WP manager:** Thomas Rühmer, HAID Graz-Haidegg, (Austria)"

Original description of work: Activities: of Germany (DLR) and Austria (HAID) on apple crops and the one of Spain (IVIA) on citrus, will be based on greenhouse tests using naturally infected soil. Turkey (GDAR) will also add artificially inoculated treatments. The investigated low input tools for controlling replant problems are

WP 3-Task 1 *Evaluation of biological active formulates and strains for the biological control of replant disease.*

Commercially available bioformulates (Micorrhyza, *Pythium oligandrum*, *Trichoderma* spp.), and strains available from other research centres, formulations of organic amendments commercialised or simply promoted as active in reducing replant disease in horticulture will be subjected to a comparative evaluation. Any available compound admitted in organic farming and microorganisms which has not yet been evaluated for controlling replant disease, or has given contrasting results will be included in this first screening performed by UDS (TR); DLR (GE) HAID (A), LRC (I) and IVIA (E). The steps of this activity are:

Early comparative evaluation of a number of bio-pesticides/formulates varying from 10 to 15 treatments for each Centre, by EHRI & AHRI and DLR (GE) and IVIA (E). LRC (I) and HAID (A) will perform this activity on selected number of treatments of interest, which could differ from those inserted in the main comparative evaluation. The result should represent a critical overview of the bio-compounds available for controlling replant disease in apple and citrus. The list of treatments will be partly shared and partly defined by the participants together with the WP leader according to the target pathogens.

Report on results obtained and changes to the original plan/WP aims:

(A- results obtained:

WP 3-Task 1 Evaluation of biological active formulates and strains for the biological control of replant disease.

General comment Application rates and methodologies used for application were those recommended by manufacturers or resellers

APPLE

A list of commercial available biological products for controlling apple replant disease have been subjected to a first round of evaluation by 7. DLR (GE), 4. HAID (A), 2. LRC (I) and 8. GDAR (TR) in a bioassay test on natural soil from replanted apple orchards.

The control in all these trials was represented by an untreated control; however some partners inserted an additional pasteurized control to estimate an additional parameter called the “growth potential”.

The common list of products under testing (see ANNEX 1) includes several commercial formulates decided according common criteria during kick off meeting:

Result of the first cycle gave a variable response:

Partner 4. HAID Over 14 products and one control subjected to bioassay test for plant growth, the Austrian partner did not observe any significant differences of among the treatments with bioformulates and the untreated control. However, untreated control showed a better performance than 5 bioformulates (among them RHIZOVITAL® and EKOPROP®, EKOPROP NEMAX®), while it showed a worst performance than other nine. Indeed, the highest plant growth was obtained with the following bioformulates: e OZOR®, MYCOSTOP® and CONDOR®.

Partner 7 DLR (GE) Observed a significant difference of plant growth among treatments. In this case, the positive reference pasteurized replant soil, inserted as an additional control, allowed an estimation of also the growth potential. MICOSAT F®, MYCOSTOP®, EKOPROP NEMAX®, TIFI® and KOBA FIT® showed significant increase in growth, similar to that of the pasteurized variant. Particularly interesting was the effect of the applied products on nitrate availability induced in soil.

Partner 2. LRC (I) Observed a significant difference of plant growth among treatments. NUTRILIFE based bio-formulates gave a plant growth significantly higher than control. In any case, the control did not significantly differ in shoot length and dry matter, from five of ten bioformulates tested. This trial will be repeated because of the finding in the previous bioassay, that shoot length and dry matter production showed low correlation to each other

Partner 8 EHRI (TR) This partner has been performing WP3 trials with natural soil artificially infected with *Phytophthora*. The bioassay is performed for a longer period as compared to the other partners working on Apple. However plant growth parameters are periodically evaluated. According to the first evaluations, differences between tree heights were found to statistically differ. The most effective formulates were determined as CROPSET®, GREEN MIRACLE® (ACTINOVATE®), ALEXIN 95PS®, while the least effective treatment was TRICHO PLUS®, which gave a growth reduction compared to the artificially inoculated control.

WP 3-Task 1 Evaluation of biologically active formulates and strains for the biological control of replant disease.

CITRUS

Partner 5. IVIA (E)

A wide array of biologically active commercial products, together with an abiotic control, are being currently tested in two different assays: an open-air pot experiment and a field trial. The evaluated products and its main characteristics are displayed in ANNEX 2 – Spain.

In the open-air pot experiment, all the products are being tested on 1-year old clementine trees (*Citrus clementina* var. *Clemenules*), grafted on Citrange Carrizo rootstock (*C. sinensis* x *Poncirus trifoliata*). Together with a constant monitoring of the appearance of any symptom of disease, two growth parameters are being measured to evaluate the effect of the treatments: plant height and width of the trunk base, estimated as the average of the values taken in both sides of the grafting point. A second measurement after the one taken at the start of the experiment has already been made, showing no statistically-significant differences for any treatment. No indications of disease have been detected on the trees at the moment.

The same products are also being evaluated in a field trial set in the citrus degraded orchard already mentioned in WP1. Result of this open field trial are described in WP4.

B- comments on deviations from the original plan:

The activity of Partner 8 GDAR has been slightly modified respect the original proposal because the partner changed. Anyway, EHRI & AHRI agree to the common protocol of WP3 program, with some adjustments concerning methodology (they perform artificial inoculation of *Phytophthora cactorum*) and target pathogens.

WP 4 | INNOVATIVE MANAGEMENT OPTIONS FOR INCREASING FUNCTIONAL SOIL BIOLOGY IN ORGANIC FRUIT TREE CROPS IN MEDITERRANEAN AND TEMPERATE GROWING AREAS IN EUROPE

Responsible partner: 5 IVIA and 2 LRC,3 ACW, 4 HAID, 7 DLR. WP manager: Rodolfo Canet, IVIA, Valencia (Spain)"

Original description of work: Activities and methodologies WP 4 is the only WP with planned field activities, and WP 4 is divided in two tasks:

T1: Several activities of IVIA (E) aimed to investigate the management strategies for controlling replant problems in degraded citrus orchards. The Spanish colleagues' activity is focused on strategies aimed to contrast losses of microbial biomass and diversity, a huge problem of agricultural soils of southern Europe.

T2 . Ongoing trials in open fields already performed by Regional Research stations of Austria, Germany and Italy on practices, amendments and bio-formulates whose results are of interest for organic farming (Tasks 2 and 3)

WP 4-Task 1 *Cropping practices for the improvement of soil biological fertility and health in degraded citrus orchards.*

A total surface of 0.85 hectares (corresponding to ten sub-plots of 850 m²) in a large citrus farm affected by yield decline (whose biotic and nutritional components will be a preliminary verified) will be replanted and managed under strict organic farming techniques. Species composition/management of vegetative cover and organic amendments are the main options investigated, together with solarisation alone or combined with biofumigation. The five treatments are:

- VC. Vegetative cover: permanent vegetative cover set to increase soil diversity, soil protection by erosion and in the respect of climatic condition of the growing area.
- OA. Organic amendments: an amount of compost calculated on basis of the prefixed results, will be applied at soil preparation.
- FS Bio-fumigation combined with solarisation in pre-soil preparation.
- S Solarisation in pre-soil preparation.
- C Control, the soil is not subjected to any special treatment.

WP 4-Task 2 *Soil management and pre-plant combined treatments to reduce apple replant disease in apple orchards.*

- Compost and bio-pesticides in pre-plant will be evaluated by HAID (A) in cooperation with LFU (A) for microbial biomass, which is one of the main parameters of soil suppressiveness.
- Effect of soil management on root development and architecture in apple orchards. An ongoing study of the effect of four different soil managements (tillage + compost, mixture of

cover crop, mono-specie cover crop, tillage, control) will be continued by LRC (I) in cooperation with the sub-contractor UA (I) with specific expertise (on going activity with project: Radici: Source of funding: MIPAAF)

- Combined options in pre-plant of new orchards. Fungal and bacteria antagonistic isolates combined with soil activator substances and selected rootstocks to enhance plant growth in replanted apple orchards DLR (GE) in cooperation with AWC (CH).

Report on results obtained and changes to the original plan/WP aims:

(max 1 page, Arial, size 11)

A- results obtained:

WP 4-Task 1 Cropping practices for the improvement of soil biological fertility and health in degraded citrus orchards. (IVIA, E) CITRUS

In the main area of the 1.2 ha orchard available at Gandía, a field trial has been set up to evaluate different practices to fight against soil-borne pathogens based on disinfection and/or enhancement of soil microbial activity and diversity. Treatments are the following: - Solarization (S); - Biodisinfectant vegetal cover (VC), mixture of three *Brassica* and *Sinapis alba*); - Organic amendment (OA, ovine manure); - Solarization + organic amendment (S+OA); - Untreated control (U).

All treatments were evaluated in triplicated experimental subplots, each consisting of eight 1-year old orange trees (*C. sinensis* var. *Salustiana*) grafted on Citrange Carrizo rootstock (*C. sinensis* x *Poncirus trifoliata*), planted at 6x4 m.

Work on the field trial started previously to the project, when in autumn 2011, the farm's proprietors decided to remove the old plantation of the orchard lent later for research. Treatments were applied in Spring 2012. According to first round of evaluation concluded by the end of 2012, clear effects of the treatments on the microbial populations in soil were also found, although the statistical significance of the results were negatively affected by the usually high variability of biological parameters in agricultural soils, most particularly in those recently subjected to labours. Solarization was again the main promoter of changes, although in this case the concurrent use of manure seemed to have a stronger influence than it did in biochemical parameters.

Sub-task WP 3-Task 1 Evaluation of biological active formulates and strains for the biological control of replant disease. Performed in open field trials in in Gandia citrus orchards of WP4.

In this case, the treatments have been applied in sets of eight individual trees selected at random within the area of the replanted orchard not used for the WP1-WP4 assay. The applications started in October 2012, just after plantation, and similar growth measurements (plant height and trunk base width) were made at the start and will be made again during March 2013. No significant effects are expected given the short time between measurements and the stop in activity citrus have in winter.

WP 4-Task 2 Soil management and pre-plant combined treatments to reduce apple replant disease in apple orchards 7. DLR (GE), 4. HAID (A), 2. LRC (I) APPLE

Partner 2. LCR (IT) The research centre of Laimburg, after having observed results of the survey on root associated microorganism done with bioassay in WP1-Tak1, started a new study on an ongoing 7 year- trial on different soil managements in replanted apple orchards. The aim of this trial is to compare the impact of three organic amendments combined with bioformulates on replant disease apparently caused by *Pythium* spp.

Partners 4. HAID (A). The field trial in the fruit-growing region of Styria was started in 2009 and was evaluated in autumn 2012. The aim of this field trial was to improve vegetative growth of apple trees on a third generation replanted apple orchards. This trial was included in BIO-INCROP to continue the evaluation of the application of compost combined with two different biological formulates in preplant. TRICHOSTAR® (*Trichoderma harzianum* T58) and SYMBIVIT® (*Glomus* spp.), gave an increased shoot growth as compared to the respective single

treatments (Compost, TRICHOSTAR, SYMBIVIT and untreated control. However conclusion on field trials can be inferred only from multi-annual data (i.e. 3-year of BIO-INCROP Project).

Partner 7. DLR (GE) After having observed interesting results in the first round of WP3 trials on bioformulates to reduce apple replant disease obtained at Rheinpfalz Center of Competence (DLR), a field trial has been planted in June 2013 aiming at verifying effectiveness of some bioformulates. The best variants CHAMPOST with and without EKOPROP NEMAX®, MICOSAT F®, MYCOSTOP® and TIFI® were combined in a field trial with a replication of 4 x 6 with reference to untreated conditions. To validate the good results obtained with CHAMPOST on soil suppressiveness, CHAMPOST was also applied pasteurized in an additional trial. Results are yet to be obtained.

B- comments on deviations from the original plan:

WP 4 Task 2 APPLE rootstocks

An additional field trial was conducted by DLR to screen the properties of different rootstocks under replant conditions. This trial was not planned in the original proposal. Results are still to be obtained.

4. Publications and dissemination activities

4.1 List extracted from Organic Eprints

(Publications affiliated to European Union > CORE Organic II > “project acronym”, grouped by EPrint type, with date of extraction)

The list can have these headers:

<http://orgprints.org/view/type/>

Journal paper

Manici, L.; Donatelli, M.; Fumagalli, D.; Lazzari, A. Bregaglio, S.(2012) **Potential Response of Soil-Borne Fungal Pathogens Affecting Crops to a Scenario of Climate Change in Europe.** *iEMSS 2012 Proceedings*, VI, 8 pp.

Manici, L.; Kelderer, Markus; Franke-Whittle, I-H; 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*, unpubl, pp. 1-10. [Submitted]

Newspaper or magazine article

Canet, Rodolfo (2012) **Técnicas de cultivo innovadoras para incrementar la salud del suelo en el cultivo ecológico de frutales: el proyecto BIO-INCROP.** *AE. Agricultura y ganadería ecológica. Revista de Divulgación Técnica*, April 2012, 2012 (7), p. 30.

Kelderer, M.; Casera, C.; Lardschneider, E., Gramm, D.(2012) **Neues aus dem Versuchswesen im ökologischen Obst- und Weinbau.** *Obst- und Weinbau*, November 2012, 49 (11), pp. 374-377.

Rühmer, T. (2012). Neues Projekt zur Bekämpfung von Nachbauproblemen. *Besseres Obst* 6/2012, pp.7-8.

Laimburg , Research Centre- Department , Fruit Tree production (2012) **BIOINCROP: CAUSES OF REPLANT DISEASE. BIOINCROP: URSACHEN MÜDER BÖDEN BIOINCROP: OORZAKEN BODEMMOEHEID.**] *European Fruit Magazine*, 31 July 2012, 2012 (7), p. 31.

Naef, A.(2013) **Nachbauprobleme im Apfelanbau in der Schweiz.** *Innofrutta - Obstbau*, July 2013 (7), pp. 18-19. [In Press]

Working paper

Canet, Rodolfo (2013) **Deliverable Strategy1 No D3.1 - D4.1. First Report on Activities in Spain.** Working paper. [Unpublished]

Manici, L.; Kelderer, M., Naef, A. (2013) **D1.1 BIO-INCROP Report Disseminated at internal level. Apple replant disease (ARD) 1.** BIO-INCROP deliverable, no. D1_1, Consiglio nazionale per la ricerca in Agricoltura (IT), Centro di Sperimentazione Agraria e Forestale (IT); Agroscope Changins-Wädenswil Research Station ACW (CH) . [Submitted]

Conference paper, poster, etc.

Manici, L.(2011) **BIO-INCROP.** CORE Organic Research Seminar, Paris, France, 29 November 2011. [Submitted]

Manici, L.M.; Baab, G.;Canet, R.; Kaymak, S.; Kelderer, M.;Insam, H.; Naef, A.; Pinar, H.; Rühmer, T., Whittle, I.(2012) **Exploitation of natural resources to increase soil health: BIO-INCROP, a project on organic fruit tree cropping systems.** In: *ESA12 Abstracts*, University of Helsinki, Department of Agricultural Science publication series , Helsinki, Finland, Vol 14, pp. 24-25.

Naef, A.; Hilber-Bodmer, M., Sartori, N. (2013) **Nachbauproblematik bei Apfelbäumen.** Poster at: Agroscope | Tage der offenen Türe, Wädenswil, 7-8 June 2013.

Project description

{Project} Bio-Incrop: ***Innovative cropping technique to increase soil health in organic fruit tree crops.*** Runs 2012 - 2014. Project Leader(s): Manici, Luisa Maria.

{Project} CORE II: ***Innovative Kulturmaßnahmen zur Förderung der Bodengesundheit im ökologischen Obstbau "BIO INCROP".*** [Innovative cultural measures for the support(promotion) of the ground health in the ecological fruit growing "BIOLOGY INCROP".] Runs 2012 - 2014. Project Leader(s):Baab, Gerhard, Dienstleistungszentrum Ländlicher Raum Rheinpfalz - Kompetenzzentrum Gartenbau, D-Rheinbach.

{Project} ***BIO-INCROP PROJECT.*** Runs 2012 - 2016. Project Leader(s): Manici , Dr. L.M., Consiglio Nazionale per la Ricerca e la Sperimentazione in Agricoltura .

Other

Kelderer, M (2012) **Tätigkeit am VZ-Laimburg zum Ökologischen kologischen Anbau 2012/ Presentazione delle prove in AGRICOLTURA BIOLOGICA svolte presso il Centro**

Sperimentale nel 2012. Land- und forstwirtschaftliches Versuchszentrum Laimburg, Centro di Sperimentazione Agraria e Forestale, Sektion Obstbau, sezione Frutticoltura. [Completed]

This list was generated on **Thu Jun 27 13:07:59 2013 CEST**.

4.2 Additional dissemination activities

- INTERPOMA. International exhibition on pome fruit held in Bolzano 15-17th November 2012. BIO-INCROP project was presented with poster, A4 leaflet, and research material such as M9 rootstock plantlets used for bioassay test, pure colonies of filamentous fungi isolated from roots.
- Communication by several partners of BIO-INCROP at the Congress on Replant Disease organized within INTERPOMA.

4.3 Further possible actions for dissemination

- List publications/deliverables arising from your project that Funding Bodies should consider disseminating (e.g. to reach a broader audience)
- Indicate publications/deliverables that could usefully be translated (if this has not been done, and indicate target language)

4.4 Specific questions regarding dissemination and publications

- Is the project website up-to-date? YES
- List the categories of end-users/main users of the research results and how they have been addressed/will be addressed by dissemination activities. (1) Farmers and techniques of organic fruit production (Project web site, technical publications and field days at Laimburg Research Centre (IT) and Agroscope-ACW Wädenswil (CH) (2) Researchers (Congress and publication on ISI journals) (3)

5. Added value of the transnational cooperation in relation to the subject

(max 1 page, please describe the main advantages of the transnational research cooperation compared to a national research project approach in regard to the subject of the project. You may in particular expand on new research ideas raised by the project, research cooperation established during the project, research funding obtained etc.)

Adoption of two deeply different fruit three crops (apple and citrus) and transnational research cooperation, which combined expertise in crop science referring to differing agro-environments, proved to be a great means for searching innovative techniques in organic fruit tree production for the common aim of increasing soil health. The main positive effects are:

- More complete experimental setting; in fact, experiments were decided on basis of multiple experiences and needs of different agro-environments.
- Methodology overall improved, thanks to the preliminary exchanges among partners made to set common methodologies.
- Reduction of weaknesses in research activity, thanks to a faster adjustment of the methodologies and the short-term objectives.
- It is easier to motivate partners thank to common objectives of research activity.

NNEX 1: CHANGES IN WORK PLAN AND PROBLEMS ENCOUNTERED

Changes in consortium and work plan

Original Consortium of BIO-INCROP was changed before the beginning of the project due to administrative problems for which the Turkish partner on University of Isparta could not directly receive funds from the Turkish representative body (GDAR) of COII.. Therefore a new partner of Egirdir Horticulture Research Institute of Isparta (working on apple), with a cooperation of a partner at Alata Horticultural Research Institute (working on citrus) were inserted. This change required only small changes to the original research plan of the Turkish partner. Colleagues of EHRI and AHRI are now involved in WP3.

Problems encountered, delays and corrective actions planned or taken, if any:

(Arial, size 11)

ANNEX 2: COST OVERVIEW AND DEVIATIONS FROM BUDGET

Project budget and costs in € (if in National currencies, please indicate):

Partner no.	1	2	3	4	5	6	7	8
TOTAL BUDGET	110.000	86.000	50.000	41.215	134.543	49.960	117.615	50.000
Spent at Mid term	42.000	15.000	25.900	21.511	63.900	12.723	53.000	20.500
<i>Spent in 2nd period</i>				-				0
TOTAL SPENT	42.000	15.000	25.900	21,511	63.900	12.723	53.000	20.500
DEVIATION	68.000	71.000	24.100	19.704	70.643	37.237	64.615	29.500

Person months (PM) spent on the project:

Partner no.	1	2	3	4	5	6	7	8
TOTAL PM budgeted	20.000	69.000	22.600	25.153	25.390	20.000	86.036	20.000
Spent at Mid term	20.000	5.000	12.200	11.528	10.850	1.925	33.888	9.280
<i>PM spent in 2nd period</i>								
TOTAL PM SPENT	20.000	5.000	12.200	11.528	10.850	1.925	33.888	9.280
DEVIATION	0	64.000	10.400	13.625	14.540	18.075	52.148	10.720

Reasons for major deviations in spending compared to original budget:

(Arial, size 11)

**ANNEX 3: RECOMMENDATIONS TO THE CORE ORGANIC CONSORTIUM IN
RELATION TO LAUNCHING AND MONITORING OF FUTURE TRANSNATIONALLY
FUNDED RESEARCH PROJECTS**

(max ½ page)