# Project LIFE13 ENV/IT/000461

## “Environmentally friendly biomolecules from agricultural wastes as substitutes of pesticides for plant diseases control”

# “EVERGREEN”

**Start: 01/10/2014**
**End: 31/09/2016**

EVERGREEN 18th month Meeting - II monitoring visit
2016, May 13rd
**ASTRA Innovazione e Sviluppo srl**
**Imola**

Project LIFE13 ENV/IT/461
"Environmentally friendly biomolecules from agricultural wastes as substitutes of pesticides for plant diseases control" (EVERGREEN)

# II MONITORING VISIT AND 18 MONTHS PROGRESS MEETING AGENDA

**Date:** Friday, the 13rd of May 2016
**Place:** Via Emilia Levante 18, Imola - Italy, ASTRA operative premises Mario Neri
**Time:** 9:30 to 18:00
**Invited partners:** All partners are invited to attend this meeting

## AGENDA

**09:30** [ASTRA and DISPAA] Welcome and opening of the meeting

**09:40 TECHNICAL MEETING**

**09:40** [All] EVERGREEN Technical issues: Presentation per Action of Technical activities carried out from October 2014 until the end of April 2016.

**11:00** Coffee break

**11:15** EVERGREEN Technical future issues:
- [DISPAA] Technical activities to be carried out in the last six months of the project
- [All] Open discussion on actions to be carried out in the last six months of the project

**11:30** [DISPAA] Management and dissemination issues, in particular
- [DISPAA] Dissemination activities carried out from October 2014 until end of April 2016
- [DISPAA] Preparation of Final Report

**13:00** Lunch

**14:30 ADMINISTRATIVE MEETING**

**14.30** [EC Monitor team] EVERGREEN administrative issues:
- project consolidate state of costs divided for partner and for claim cost: problem analysis.
- analysis of project costs excel files of each beneficiary
- check of VAT documents and of cost centre print out
- check of the system of recording of working time: Timesheets.
- check of documents of external assistance, contracts, invoices, salary sleeps, proof of payments.
- project consolidate state of costs divided for partner and for claim cost: problem analysis.

**16.30-18.00** Tour of project demonstration sites

EVERGREEN

EVERGREEN kickoff meeting – 2014, October 23rd

Università degli Studi di Firenze,

Polo Scientifico e Tecnologico, Sesto Fiorentino (Firenze)

LIFE13 ENV/IT/000461

LIFE13 ENV/IT/000461 – EVERGREEN
18th month Meeting – II Monitoring visit
2016, May 13rd
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# WHAT ABOUT BENEFICIARIES?

# EVERGREEN

LIFE13 ENV/IT/000461

Home The Project Meet the Partners EU Life+ Programme Documents

UNIVERSITÀ DEGLI STUDI FIRENZE

**DISPAA**
Dipartimento di Scienze
delle Produzioni Agroalimentari
e dell'Ambiente

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

ASTRA
INNOVAZIONE E SVILUPPO
IN AGRICOLTURA

INSTM

Mondo Verde
Casa & Giardino

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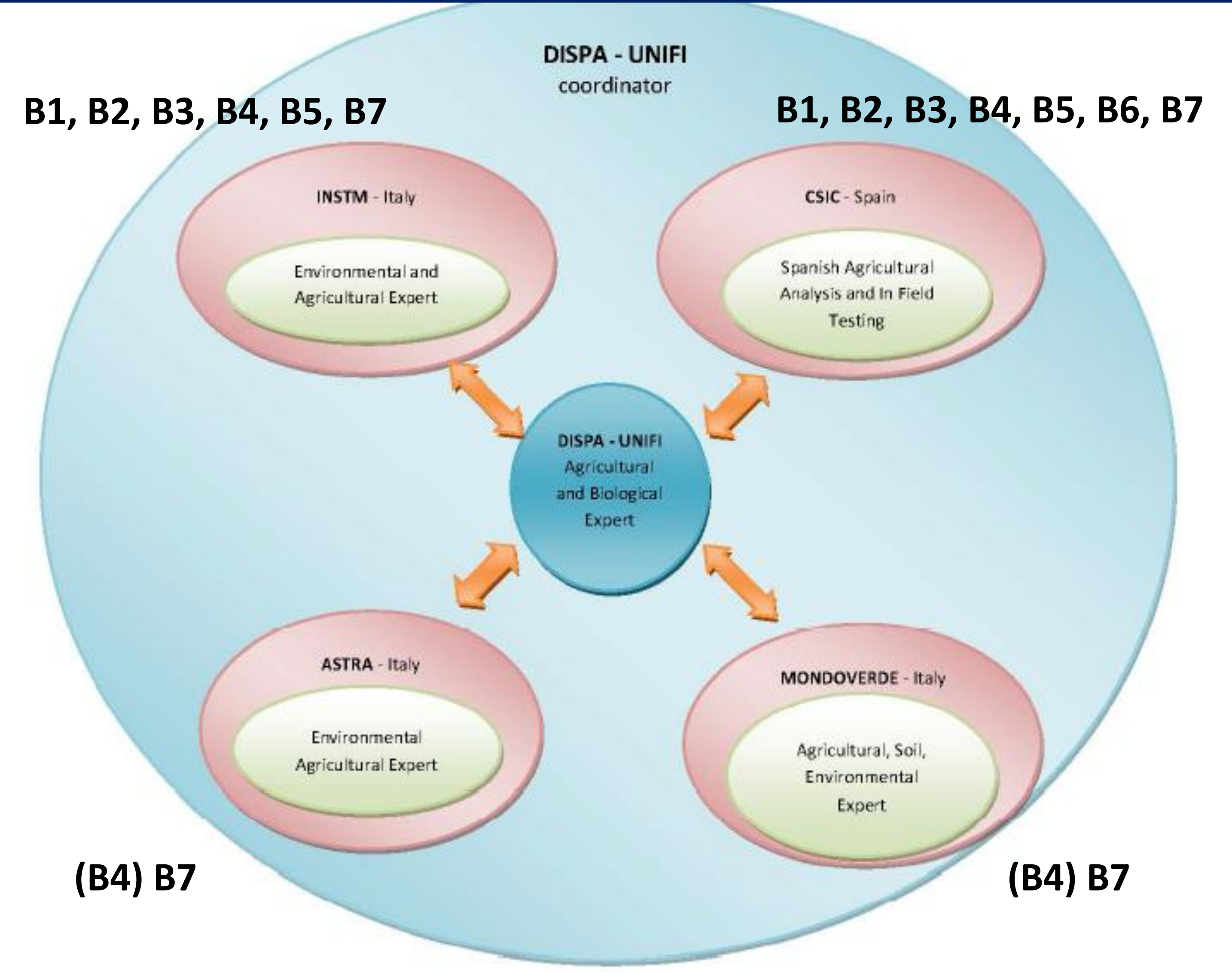

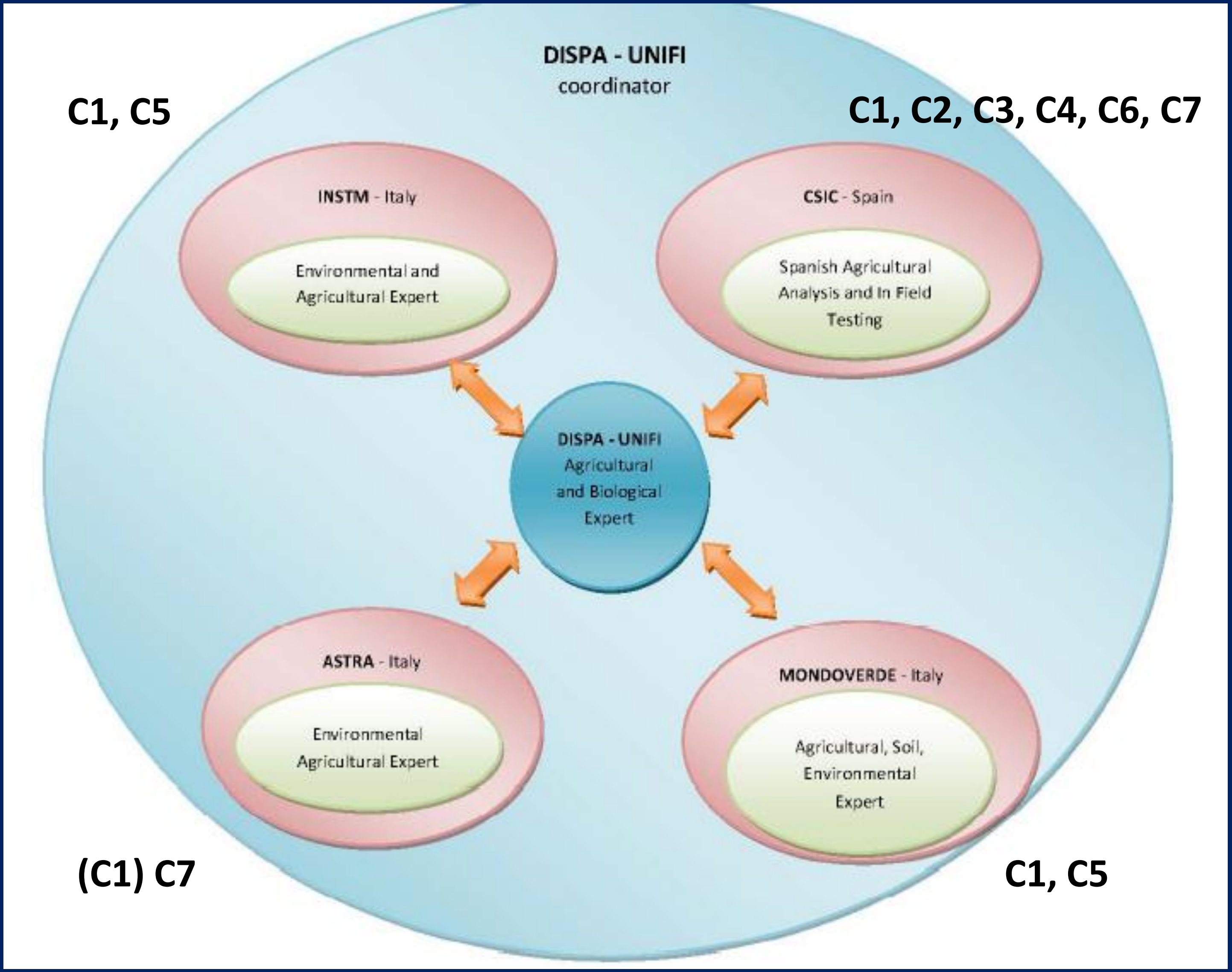

DISPA - UNIFI
coordinator
C1, C5
C1, C2, C3, C4, C6, C7
INSTM - Italy
Environmental and Agricultural Expert
CSIC - Spain
Spanish Agricultural Analysis and In Field Testing
DISPA - UNIFI
Agricultural and Biological Expert
ASTRA - Italy
Environmental Agricultural Expert
MONDOVERDE - Italy
Agricultural, Soil, Environmental Expert
(C1) C7
C1, C5

## TIMETABLE

| Action | | 2014 | | | | 2015 | | | | 2016 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Action numbe | Name of the action | I | II | III | IV | I | II | III | IV | I | II | III | IV |
| **A. Preparatory actions (if needed)** | | | | | | | | | | | | | |
| **B. Implementation actions (obligatory)** | | | | | | | | | | | | | |
| B.1 | Demonstration of the performances of traditional pesticides for the control of bacterial and nematode diseases of plants important for the EU | | | | ■ | ■ | | | | | | | |
| B.2 | Demonstration of the qualitative and quantitative yields of extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste at laboratory scale | | | | | ■ | ■ | ■ | ■ | | | | |
| B.3 | Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and of their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale | | | | | ■ | ■ | ■ | ■ | | | | |
| B.4 | Demonstration of the biological activity of the high quality polyphenolic extracts recovered from not edible biomass and waste, against plant pathogenic bacteria and nematode, in planta | | | | | | ■ | ■ | ■ | | | | |
| B.5 | Demonstration of Kilo-scale extraction of the high quality poly-phenolic bioactive molecules recovered from vegetable not edible biomass and waste | | | | | | | ■ | ■ | ■ | | | |
| B.6 | Demonstration of the null toxicity profile of the high quality poly-phenolic bioactive molecules recovered from vegetable not edible biomass and waste, on model organisms andmicroorganisms. | | | | | | ■ | ■ | ■ | ■ | | | |
| B.7 | Demonstration of the in vivo performances of the high quality poly-phenolic bioactive preparations, recovered from vegetable not edible biomass and waste,at pilot scale level in fieldscreenings. | | | | | | | ■ | ■ | ■ | ■ | ■ | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C. Monitoring of the impact of the project actions (obligatory)** | | | | | | | | | | | | | |
| C.1 | Monitoring on the environmental impact of copper compounds and nematicides for the cropdefence against phytopathogenic bacteria and nematodes | | | | ■ | ■ | | | | | | | |
| C.2 | Monitoring of the absence of side effects for the high quality standardised polyphenolic preparations on common targets of any living organism at laboratory level | | | | | ■ | ■ | ■ | ■ | | | | |
| C.3 | Monitoring of the absence of a direct selection operated by the polyphenolic preparations towards the emergence of bacteria resistant to the polyphenolic molecules themselves, at laboratory level | | | | | | ■ | ■ | ■ | ■ | | | |
| C.4 | Monitoring of the short term environmental benefits from the use of the high quality standardised polyphenolic preparations in plant disease control at pilot scale level in field screenings | | | | | | | ■ | ■ | ■ | ■ | ■ | |
| C.5 | Monitoring of the economic benefits deriving from the recycling of the spent vegetable biomass after the extraction of the high quality standardised polyphenolic molecules at laboratory level | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | |
| C.6 | Monitoring of the absence of a selection on the polyphenolic preparations on the selection of copper and antibiotic resistant bacteria, on plant and in soil, from laboratory to in field screenings. | | | | | | | ■ | ■ | ■ | ■ | ■ | |
| C.7 | Monitoring of technical-socio-economic assessment of the After-Cu project | | | | | | | | | | ■ | ■ | |

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**18th month Meeting – II Monitoring visit**
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| D.1 | Website creation | | | | ■ | | | | | | | | |
| D.2 | Notice boards | | | | ■ | | | | | | | | |
| D.3 | Demonstration workshops in Italy | | | | | | | | ■ | ■ | ■ | ■ | |
| D.4 | Demonstration workshops in Spain | | | | | | | | ■ | ■ | ■ | ■ | |
| D.5 | Diffusion material preparation | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| D.6 | Layman's report | | | | | | | | | | ■ | ■ | |
| D.7 | Articles and press releases | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| D.8 | Networking | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| D.9 | Technical manual | | | | | | | | | ■ | ■ | ■ | |
| D.10 | International conferences and fairs | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| D.11 | Dissemination to institutions and policy makers | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| D.12 | After-LIFE communication plan | | | | | | | | | | | ■ | |
| D.13 | Digital supports for international diffusion | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| **E. Project management and monitoring of the project progress (obligatory)** | | | | | | | | | | | | | |
| E.1 | Project management | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| E.2 | Monitoring | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| E.3 | Audit | | | | | | | | | | | ■ | |

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| Name of the Deliverable | Number of the associated action | Deadline |
|---|---|---|
| EVERGREEN notice boards | D 2 | 12/2014 |
| Report on performances of traditional pesticides | B 1 | 03/2015 |
| Report on the environmental impact of copper salts and nematicides on soil microflora | C 1 | 03/2015 |
| Report on the biological activity of the high quality and standardised polyphenolic molecules | C 2 | 12/2015 |
| Report on the laboratory analysis of the chemical stability of the extracted polyphenolic molecules | B 3 | 12/2015 |
| Report on the laboratory extraction process of high quality polyphenolic molecules from not edible vegetable biomass and waste | B 2 | 12/2015 |
| Report on the planta activity of the high quality and standardised polyphenolic molecules | B 4 | 12/2015 |
| Report on the kilo-scale extraction of high quality polyphenolic molecules | B 5 | 03/2016 |
| Report on the laboratory tests and studies on the monitoring of the selective pressure applied by the treatments with polyphenolic-based preparations | C 3 | 03/2016 |
| Report on the toxicological profile of high quality and standardised polyphenolic molecules on microorganism and organism | B 6 | 03/2016 |

## MILESTONES OF THE PROJECT

| Name of the Milestone | Number of the associated action | Deadline |
|---|---|---|
| 1st Kick off meeting | E 1 | 10/2014 |
| EVERGREEN website | D 1 | 12/2014 |
| Definition of the environmental impact on the composition of bacterial microflora in copper and contaminated soils | C 1 | 03/2015 |
| 2nd coordinating meeting | E 1 | 04/2015 |
| 3rd coordinating meeting | E 1 | 10/2015 |
| Laboratory chemical stability of the extracted polyphenolic molecules | B 3 | 12/2015 |
| Laboratory extraction process of high quality polyphenolic molecules | B 2 | 12/2015 |
| Kilo-scale extraction of high quality polyphenolic molecules | B 5 | 03/2016 |

# FORGET ME NOT…..

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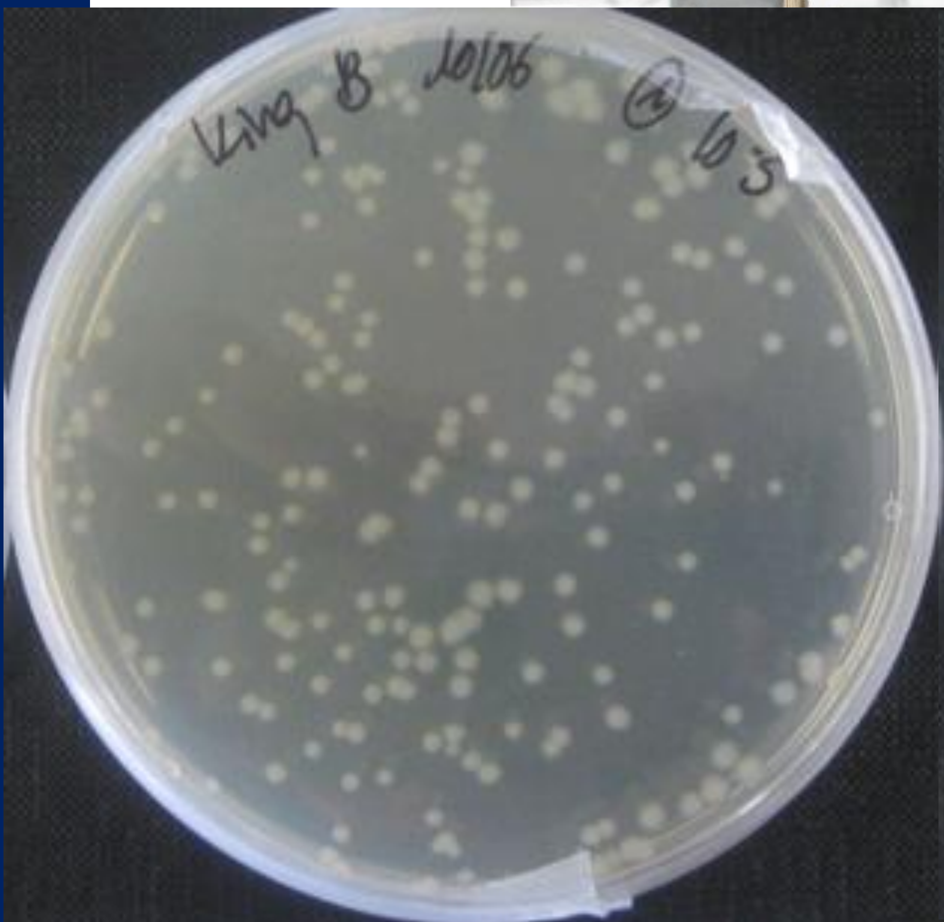
# Action B1

## 1st October 2014 - 31st March 2015

**Demonstration of the performances of traditional pesticides for the control of bacterial and nematode diseases of plants important for the EU**

# Action B1

## 1st October 2014 - 31st March 2015



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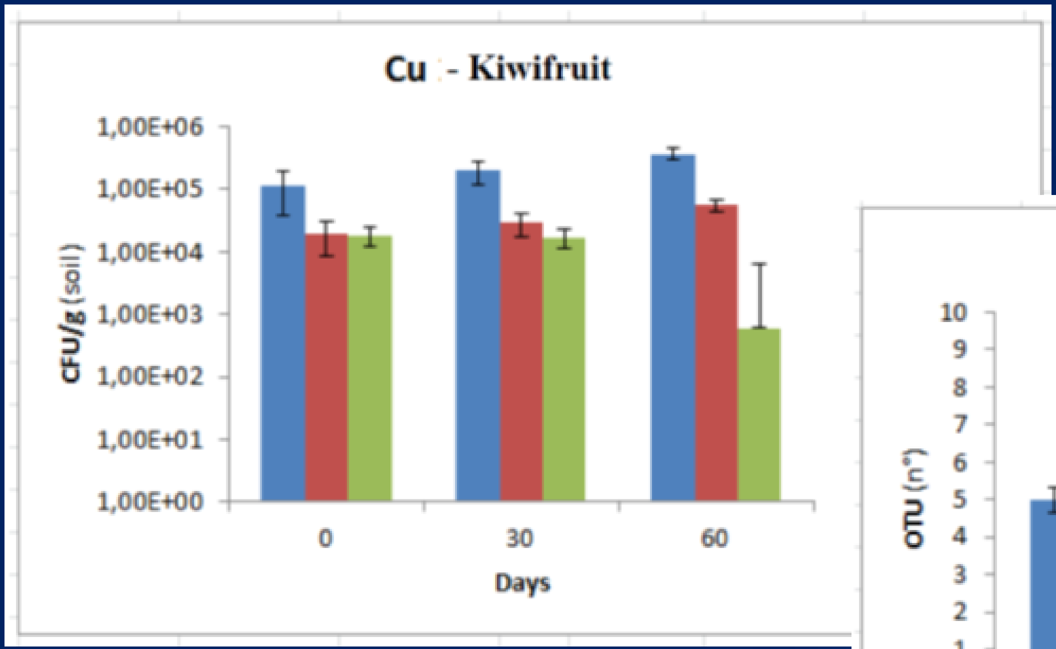
# Action B1

## 1st October 2014 - 31st March 2015



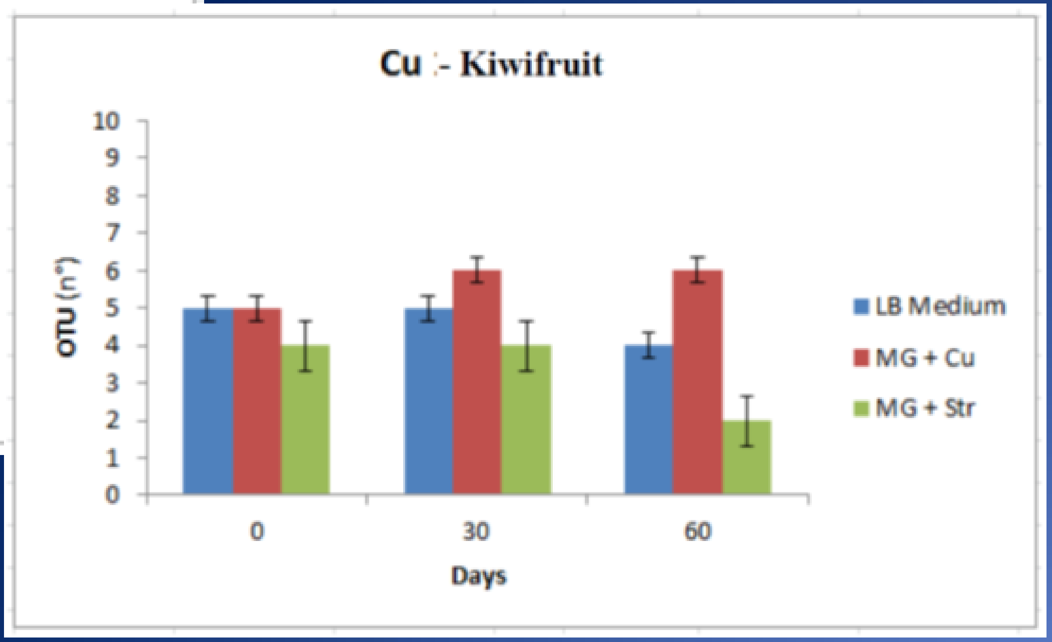




Fig. 7. Variability (as OTU) of *Psa* populations on Kiwifruit potted plants treated with copper spraying, and distribution of copper- and antibiotic-resistant strains.

# Action B1

## 1st October 2014 - 31st March 2015

EVERGREEN (LIFE) B1Act.

Experiment preparation:
Controlled chamber

| N° | Referencia | Dosis |
|---|---|---|
| 1 | Control_1 | 0 |
| 2 | Control_2 | 0 |
| 3 | Control_3 | 0 |

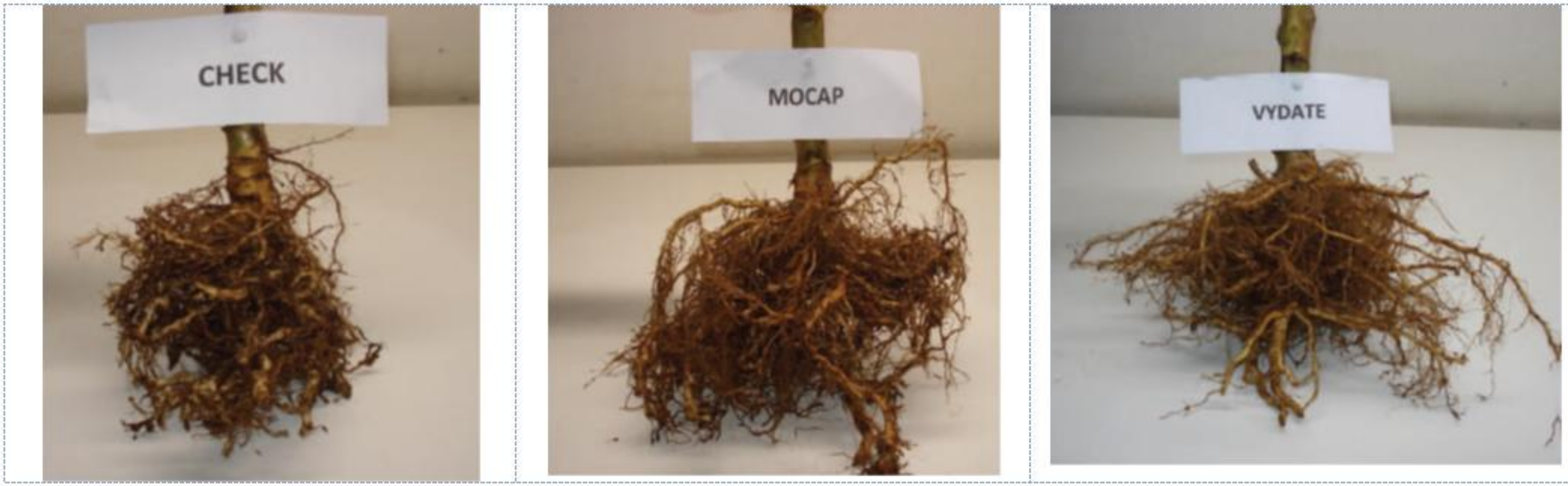



**Fig. 9. Effect on Tobacco roots of *M. incognita* infection following or not several nematicide treatments.**

- Agrochemicals: the early application related to necessity of respecting the 60-day pre-harvest interval is not sufficient to protect plants in a rainy season (2014)
- CHT: act as a biostimulant, with minimal effects on nematode count; however low evidence of nematode feeding on tobacco roots was found.
- Better yield results for the botanicals/biocides than agrochemicals

# Action B1

## 1st October 2014 - 31st March 2015

# Action B2

## 1st January 2015 - 31st December 2015

**Demonstration of the qualitative and quantitative yields of extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste at laboratory scale**

# Action B2

## 1st January 2015 - 31st December 2015

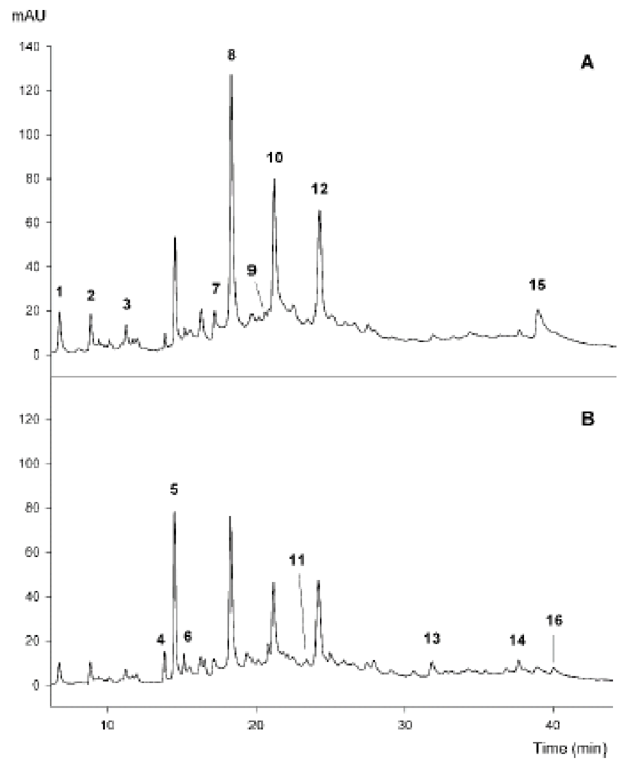



Fig. 4. Chromatographic profile of the liquid fraction of sweet chestnut (fraction 6), registered at 254 nm (A) and 280 nm (B). Peaks: 1. Vescalin; 2. Castalin; 3. Pedunculagin I; 4. Monogalloyl glucose I; 5. Gallic acid; 6. Monogalloyl glucose II; 7. Roburin D; 8. Vescalagin; 9. Dehydrated tergallagic-C-glucoside; 10. Castalagin; 11. Digalloyl glucose; 12. O-galloyl-castalagin isomer; 13. Trigalloyl glucose; 14. Tetragalloyl glucose; 15. Ellagic acid; 16. Pentagalloyl glucose.

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# Action B2

## 1st January 2015 - 31st December 2015



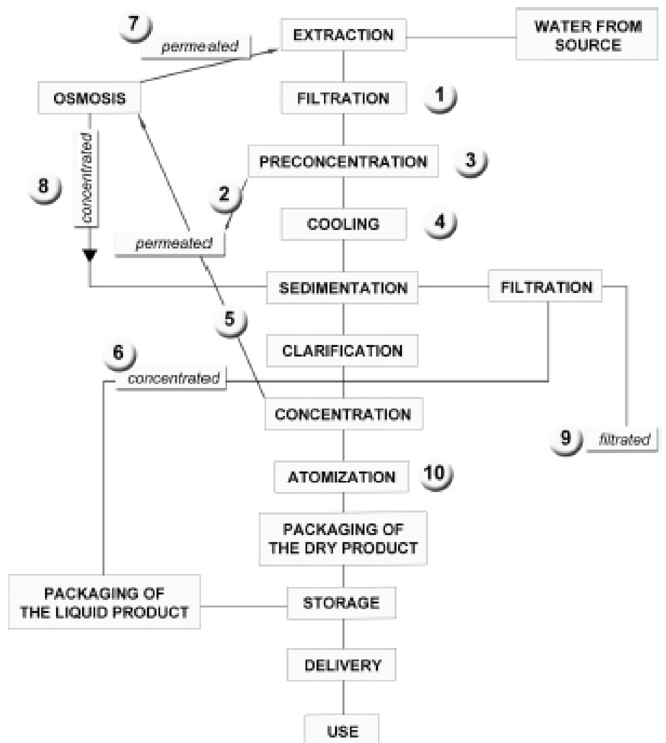


**Figure 2**: Operating diagram of the Gruppo Mauro Saviola extraction and fractionation plant: 1) filtered tannin broths; 2) permeate from nanofiltration step-1; 3) concentrate from nanofiltration step-1; 4) concentrate from nanofiltration step-2; 5) permeate from nanofiltration step-2; 6) concentrate from nanofiltration step-3; 7) osmosis permeate; 8) osmosis concentrate; 9) settled fraction from clarification step; 10) spray-dried obtained from fraction 6.

NPC **Natural Product Communications**

2016
Vol. 11
No. 3
409 - 415

**Hydrolyzable Tannins from Sweet Chestnut Fractions Obtained by a Sustainable and Eco-friendly Industrial Process**

Margherita Campo[1,2]*, Patrizia Pinelli[2] and Annalisa Romani[2]

[1]*Consortium I.N.S.T.M., Via G. Giusti 9, I-50121 Florence, Italy*
[2]*PHYTOLAB (Pharmaceutical, Cosmetic, Food supplement Technology and Analysis) - DiSIA, Department of Statistics, Computer Science, Applications "G. Parenti", University of Florence, Via U. Schiff, 6 – 50019 Sesto Fiorentino, Florence, Italy.*

*margherita.campo@unifi.it*

Received: December 24th, 2014; Accepted: May 3rd, 2015

# Action B2

## 1st January 2015 - 31st December 2015

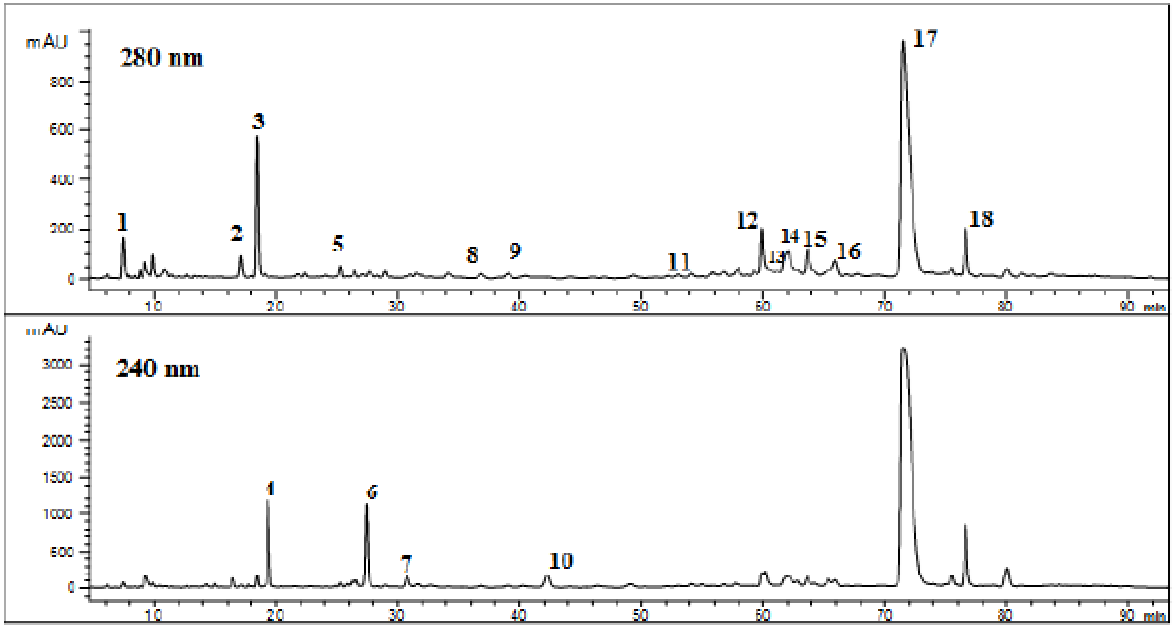


Fig. 5. Chromatograms of PHENOLEA F. Peaks: 1. Hydroxytyrosol derivative; 2. Hydroxytyrosol; 3. Hydroxytyrosol glucoside; 4. Oleoside; 5. Esculin; 6. Demetyl elenolic acid diglucoside; 7. Elenolic acid glucoside; 8. Olivile; 9. Hydroxycinnamic derivative; 10. Elenolic acid glucoside derivative; 11. β-OH-verbascoside; 12. Verbascoside; 13. Luteolin 7-O-glucoside; 14. Pinoresinol; 15. Verbascoside isomer; 16. Acetoxypinoresinol; 17. Oleuropein; 18. Oleuropein isomer.

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# Action B2

## 1st January 2015 - 31st December 2015

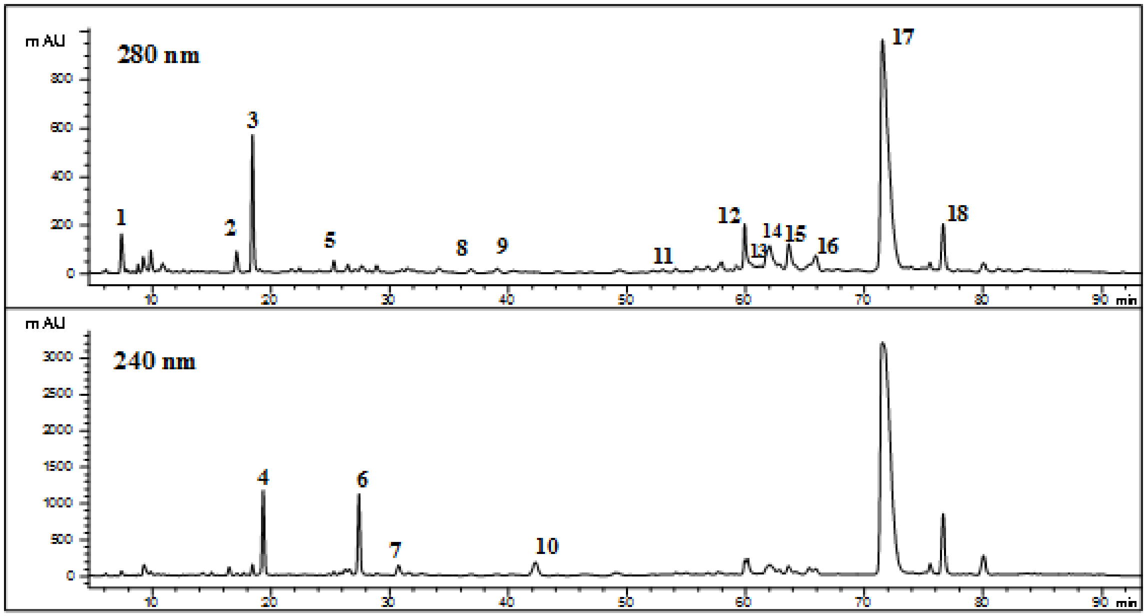


**Figure 2**. Chromatogram of Olea COI GL. *Peaks*: 1. Hydroxytyrosol derivative; 2. Hydroxytyrosol; 3. Hydroxytyrosol glucoside; 4. Oleoside; 5. Esculin; 6. Demetyl elenolic acid diglucoside; 7. Elenolic acid glucoside; 8. Olivile; 9. Hydroxycinnamic derivative; 10. Elenolic acid glucoside derivative; 11. β-OH-verbascoside; 12. Verbascoside; 13. Luteolin 7-*O*-glucoside; 14. Pinoresinol; 15. Verbascoside isomer; 16. Acetoxypinoresinol; 17. Oleuropein; 18. Oleuropein isomer.

# Action B2

## 1st January 2015 - 31st December 2015

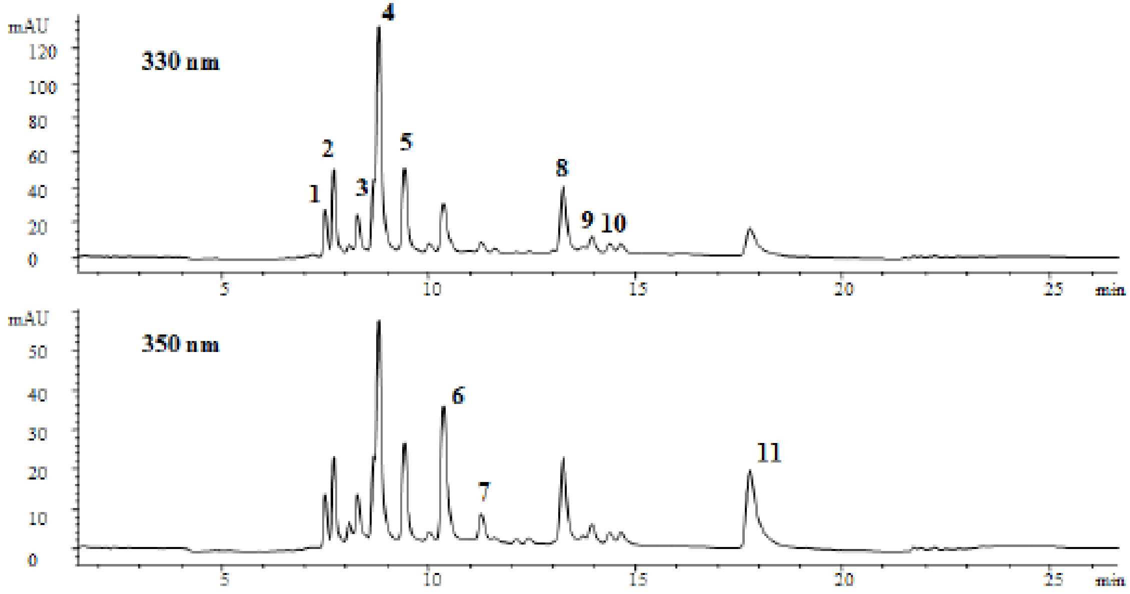


Fig. 6. Chromatograms of CYNARA_SOL fraction. Peaks: 1-O-caffeoylquinic acid; 2. 3-O-caffeoylquinic acid; 3. caffeoylquinic acid; 4. chlorogenic acid; 5 cynarin; 6. luteolin 7-O-rutinoside; 7. luteolin 7-O-glucoside; 8. dicaffeoylquinic acid; 9. dicaffeoylquinic acid; 10. dicaffeoylquinic acid; 11. luteolin.

# Action B2

## 1st January 2015 - 31st December 2015

**Table 2.** HPLC/DAD quantitative analyses of different plant fractions from *Cynara* leaves (CUF and CRO), and two concentrated fractions from CRO: soft extract and spray dried from green leaves. Data are mean values of triplicate analyses (±SD).

| | CUF Cynara GL mg/L | CRO Cynara GL mg/L | Cynara GL Soft extract mg/g | Cynara GL Spray Dried mg/g |
|---|---|---|---|---|
| MCC | 1.07 ± 0.58 | 65.19 ± 13.28 | 6.61± 1.34 | 14.23± 0.48 |
| DCC | 2.81 ± 1.19 | 3.96 ± 5.60 | 7.64± 0.69 | 7.63± 0.20 |
| Chlorogenic acid | 2.04 ± 0.47 | 34.00 ± 7.38 | 11.93± 1.72 | 12.36± 0.03 |
| Cynarin | 0.50 ± 0.43 | 28.94 ± 14.89 | 1.62± 0.01 | 4.41± 0.34 |
| Flavonols | 0.23 ± 0.06 | 10.11 ± 5.39 | 1.09± 0.27 | 3.48± 0.56 |
| **Total Polyphenols** | **6.57 ± 1.92** | **142.21 ± 9.58** | **28.90± 4.02** | **42.10± 0.42** |

CUF = Concentrate of Ultrafiltration; CRO = Concentrate of Reverse Osmosis; GL = Green Leaves.

# Action B2

## 1st January 2015 - 31st December 2015

Table 5: Tannins content and single compounds molecular weights in grape seeds extract.

| | w/w % | MW (Da) |
|---|---|---|
| Gallic acid | traces | 170 |
| Catechin dimer B3 | 0.222 | 578 |
| Catechin | 1.107 | 290 |
| Catechin trimer | 0.321 | 866 |
| Catechin dimer B6 | 0.261 | 578 |
| Catechin dimer B2 | 0.537 | 578 |
| Epicatechin | 1.362 | 290 |
| Catechin trimer | 0.371 | 866 |
| Epicatechin gallate dimer | 0.665 | 730 |
| Epicatechin gallate | 0.610 | 442 |
| Oligomers quantified as tetramers | 5.488 | |
| Epicatechin gallate dimer | 18.06 | 882 |
| ECG oligomers quantified as trimers | 38.30 | |
| ECG oligomers quantified as trimers | 14.97 | |
| **TOTALE** | **82.27** | |

# Action B2

## 1st January 2015 - 31st December 2015

| GRAPE SEEDS | mg/g | mmol/Kg | mg/mL ext | mM ext |
|---|---|---|---|---|
| Gallic acid | 0.042 | 0.246 | 0.008 | 0.049 |
| Catechin dimer B3 | 1.687 | 2.919 | 0.337 | 0.584 |
| Catechin | 0.816 | 2.814 | 0.163 | 0.563 |
| Catechin trimer | 0.000 | 0.000 | 0.000 | 0.000 |
| Catechin dimer B6 | 1.288 | 2.228 | 0.258 | 0.446 |
| Catechin dimer B2 | 0.776 | 1.343 | 0.155 | 0.269 |
| Epicatechin | 0.578 | 1.993 | 0.116 | 0.399 |
| Catechin trimer | 0.487 | 0.562 | 0.097 | 0.112 |
| ECG dimers | 1.649 | 1.870 | 0.330 | 0.374 |
| Catechin oligomers quantified as tetramers | 26.245 | 22.743 | 5.249 | 4.549 |
| ECG dimers | 17.065 | 19.348 | 3.413 | 3.870 |
| catechin/epicatechin trimers digallated | 39.351 | 33.633 | 7.870 | 6.727 |
| catechin/epicatechin trimers digallated | 4.473 | 3.823 | 0.895 | 0.765 |
| **TOTAL** | **94.458** | **93.523** | **18.892** | **18.705** |

**Table 1.** Quali-quantitative analysis of the grape seed hydroalcoholic extract. The results are expressed as mg and mmol of single tannin with respect to the seed weight and volume of extract.

# Action B3

## 1st January 2015 - 31st December 2015

Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and of their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale

# Action B3

## 1st January 2015 - 31st December 2015

## d) EVERGREEN (LIFE)

Project LIFE13 ENV/IT/000461

**CHEMICAL STABILITY**: To know the chemical stability of polyphenols, were prepared solutions at work concentration (0,1 g/l), and these solutions were subjected to :

**FACTORS ASSAYED: pH changes (5-8) and Temperature (Heat) (25,30 and 35ºC)**

| Polyphenol | Tª C | ppm Cs | ppm Ns | pH | ppm Cs | ppm Ns |
|---|---|---|---|---|---|---|
| TC | 25 | 287,74 | 0 | 4 | 254,50 | 0 |
| | 30 | 287,16 | 0 | 5,5 | 233,30 | 0 |
| | 37 | 304,20 | 0 | 7 | 232,20 | 0 |
| | 45 | 300,78 | 0 | 8,5 | 232,55 | 0 |
| | 50 | 299,66 | 0 | 10 | 232,32 | 0 |
| TCO | 25 | 276,25 | 0 | 4 | 288,20 | 0 |
| | 30 | 277,14 | 0 | 5,5 | 292,40 | 0 |
| | 37 | 278,95 | 0 | 7 | 290,30 | 0 |
| | 45 | 287,53 | 0 | 8,5 | 291,98 | 0 |
| | 50 | 294,85 | 0 | 10 | 289,29 | 0 |
| TCC | 25 | 315,49 | 1,48 | 4 | 239,80 | 0,53 |
| | 30 | 336,38 | 1,40 | 5,5 | 240,50 | 0,40 |
| | 37 | 338,65 | 1,36 | 7 | 238,90 | 0,22 |
| | 45 | 347,65 | 1,33 | 8,5 | 239,52 | 0,31 |
| | 50 | 326,63 | 1,39 | 10 | 221,66 | 0,34 |
| TAN | 25 | 307,04 | 0 | 4 | 286,20 | 0 |
| | 30 | 310,77 | 0 | 5,5 | 288,50 | 0 |
| | 37 | 311,25 | 0 | 7 | 286,30 | 0 |
| | 45 | 319,18 | 0 | 8,5 | 289,80 | 0 |
| | 50 | 323,70 | 0 | 10 | 284,17 | 0 |

| Polyphenol | Tª C | ppm Cs | ppm Ns | pH | ppm Cs | ppm Ns |
|---|---|---|---|---|---|---|
| PV | 25 | 289,90 | 0 | 4 | 318,40 | 0 |
| | 30 | 295,19 | 0 | 5,5 | 327,60 | 0 |
| | 37 | 296,33 | 0 | 7 | 338,00 | 0 |
| | 45 | 308,27 | 0 | 8,5 | 337,53 | 0 |
| | 50 | 309,06 | 0 | 10 | 339,69 | 0 |
| EPV | 25 | 372,73 | 2,41 | 4 | 344,50 | 2,24 |
| | 30 | 375,74 | 2,35 | 5,5 | 348,30 | 2,13 |
| | 37 | 357,01 | 2,64 | 7 | 353,80 | 1,79 |
| | 45 | 384,92 | 2,33 | 8,5 | 354,03 | 2,15 |
| | 50 | 385,36 | 2,33 | 10 | 352,74 | 2,26 |
| PFV | 25 | 241,24 | 0 | 4 | 210,90 | 0 |
| | 30 | 239,13 | 0 | 5,5 | 209,60 | 0 |
| | 37 | 238,11 | 0 | 7 | 209,30 | 0 |
| | 45 | 249,01 | 0 | 8,5 | 212,81 | 0 |
| | 50 | 247,41 | 0 | 10 | 213,45 | 0 |

Polyphenols are very stables with the temperature and pH; Water soluble N and C showed only a minor changes with the Tª and pH. It is indicative of the chemical stability of polyphenols.

# Action B3

## 1st January 2015 - 31st December 2015

| | mg/mL GAE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **T0** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T7** |
| TC/O conc | 154.56 | 149.64 | 156.67 | 151.87 | 154.71 | 153.26 | 155.60 | 154.12 |
| TC/O dil | 10.286 | 11.276 | 11.545 | 11.162 | 11.160 | 11.580 | 11.161 | 11.151 |

**Table 9.** Folin-Ciocalteu assay results for the TC/O formulations as such (T0) and during accelerated aging at 40°C (T1-T7). The results are expressed as mg/mL GAE (Gallic Acid Equivalents). Measures: **T0** nov 27, 2015; **T1** dec 4, 2015; **T2** dec 11, 2015; **T3** dec 18, 2015; **T4** dec 23, 2015; **T5** jan 7, 2016; **T6** jan 14, 2016; **T7** feb 15, 2016.

1) TC/O conc (concentrated solution): *Sweet Chestnut liquid fraction* (20%)/*Olea liquid fraction* 3.22% polyphenols (10%) [to dilute to 100 for use].

2) TC/O dil (diluted solution): *Sweet Chestnut liquid fraction* (2%)/*Olea liquid fraction* 3.22% polyphenols (1%) [to dilute to 10 for use].

# Action B3

## 1st January 2015 - 31st December 2015

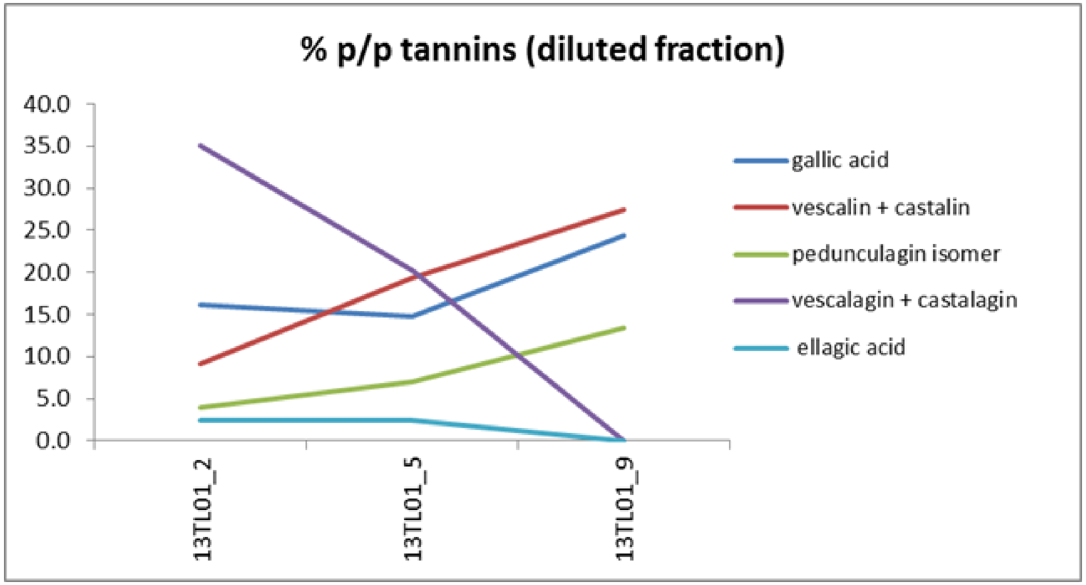


**Figure 1.** Diagram of the polyphenolic composition at 0, 6 and 12 months for the diluted liquid Sweet Chestnut fraction from pilot plant.

| | % p/p | | |
|---|---|---|---|
| | 13TL01_2 | 13TL01_5 | 13TL01_9 |
| gallic acid | 16,1 | 14,8 | 24,3 |
| vescalin + castalin | 9,18 | 19,4 | 27,5 |
| pedunculagin isomer | 3,96 | 7,03 | 13,4 |
| vescalagin + castalagin | 35,0 | 20,3 | 0,00 |
| ellagic acid | 2,36 | 2,45 | 0,00 |

**Table 1.** Polyphenolic composition at 0, 6 and 12 months for the diluted liquid Sweet Chestnut fraction from pilot plant (results expressed in %p/p of compounds).

# Action B3

## 1st January 2015 - 31st December 2015

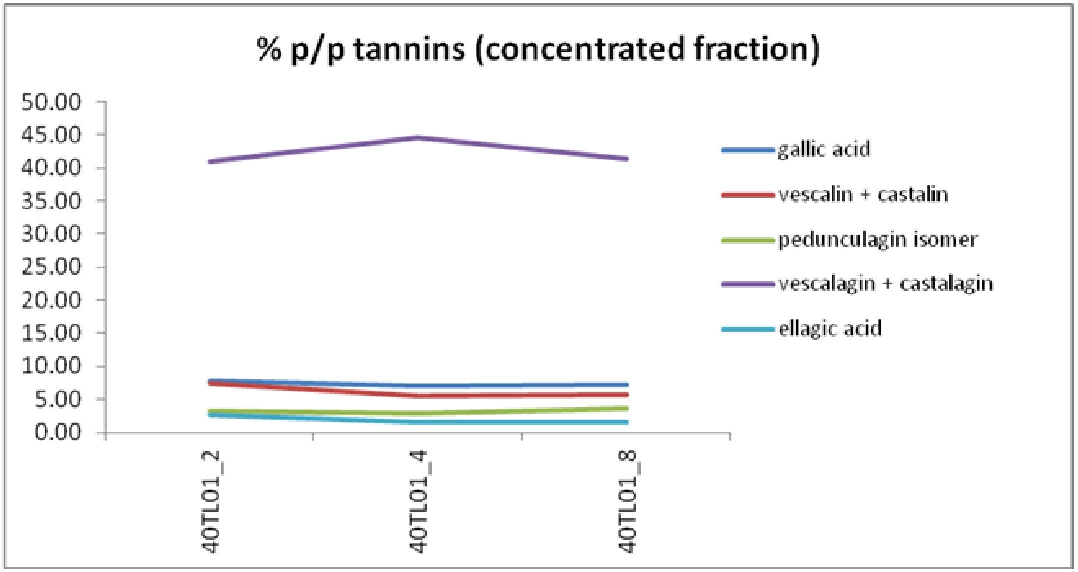


**Figure 2.** Diagram of the polyphenolic composition at 0, 6 and 12 months for the concentrated liquid Sweet Chestnut fraction from pilot plant.

| | % p/p | | |
|---|---|---|---|
| | 40TL01_2 | 40TL01_4 | 40TL01_8 |
| gallic acid | 7,75 | 7,07 | 7,18 |
| vescalin + castalin | 7,43 | 5,54 | 5,73 |
| pedunculagin isomer | 3,32 | 2,88 | 3,60 |
| vescalagin + castalagin | 40,9 | 44,6 | 41,4 |
| ellagic acid | 2,63 | 1,53 | 1,53 |

**Table 2.** Polyphenolic composition at 0, 6 and 12 months for the concentrated liquid Sweet Chestnut fraction from pilot plant (results expressed in %p/p of compounds).

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2016, May 13rd
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# Action B3

## 1st January 2015 - 31st December 2015

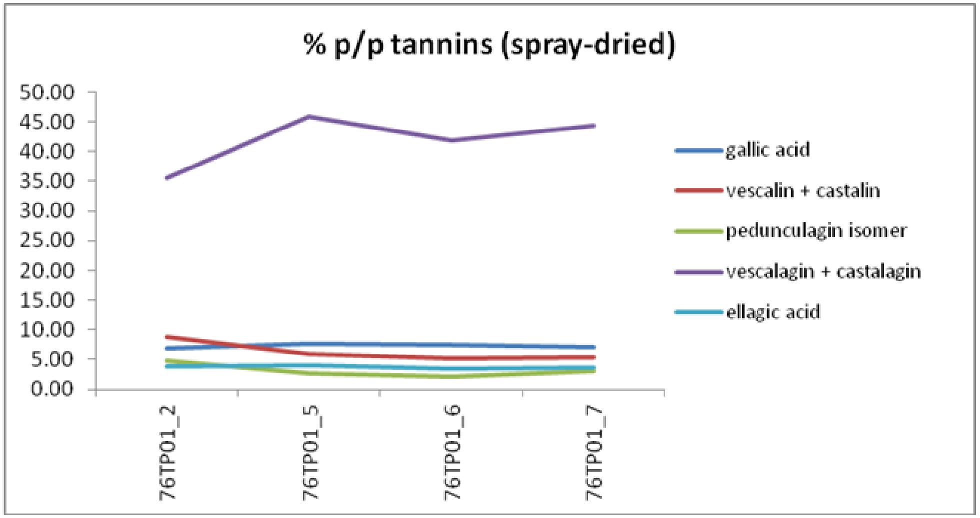


**Figure 3.** Diagram of the polyphenolic composition at 0, 4, 8 and 12 months for the spray dried Sweet Chestnut fraction from pilot plant.

| | % p/p | | | |
|---|---|---|---|---|
| | 76TP01_2 | 76TP01_5 | 76TP01_6 | 76TP01_7 |
| gallic acid | 6,81 | 7,65 | 7,50 | 7,06 |
| vescalin + castalin | 8,86 | 5,88 | 5,14 | 5,35 |
| pedunculagin isomer | 4,82 | 2,66 | 2,04 | 3,18 |
| vescalagin + castalagin | 35,5 | 45,9 | 41,8 | 44,4 |
| ellagic acid | 3,77 | 4,04 | 3,38 | 3,69 |

**Table 3.** Polyphenolic composition at 0, 4, 8 and 12 months for the spray dried Sweet Chestnut fraction from pilot plant (results expressed in %p/p of compounds).

# Action B3

## 1st January 2015 - 31st December 2015

| Total polyphenols content (mg/g) | Phenolea FF | Phenolea FS | Phenolea OH-Tyr |
|---|---|---|---|
| T0 | 248.07 | 60.75 | 303.14 |
| T12 | 240.23 | 54.50 | 276.70 |

**Table 4.** Stability of Olea fractions at time 0 (T0) and at 12 months (T12) at room temperature.

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# Action B3

## 1st January 2015 - 31st December 2015

Fig. 1. Infinite M200 Pro (Tecan) multimode reader.

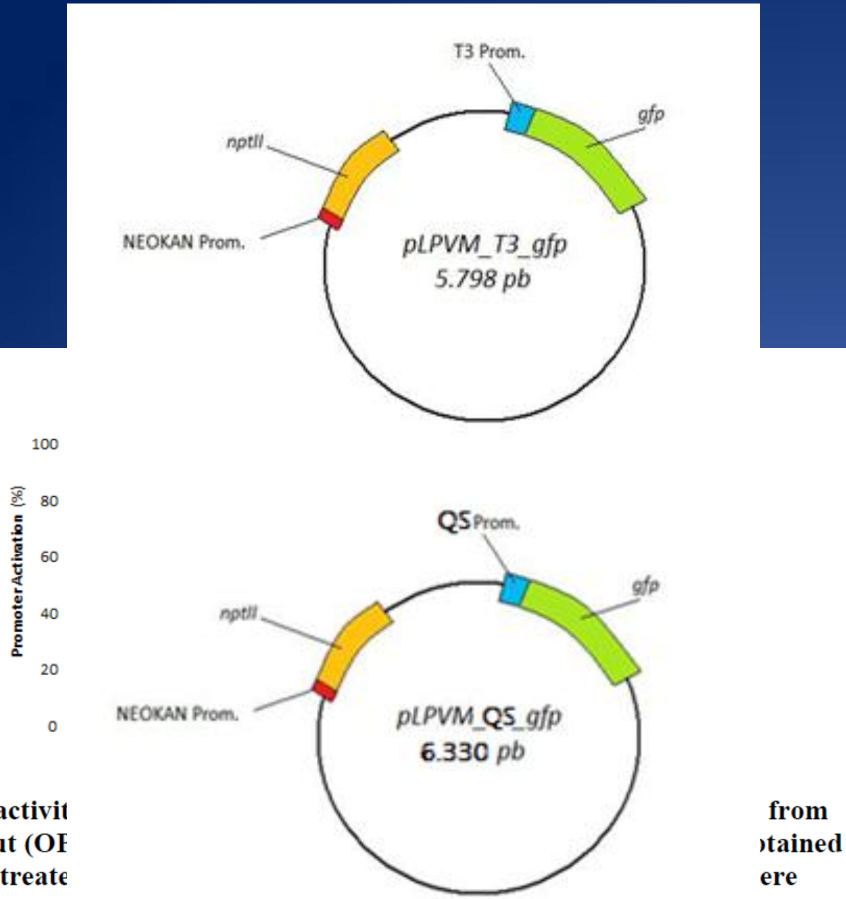
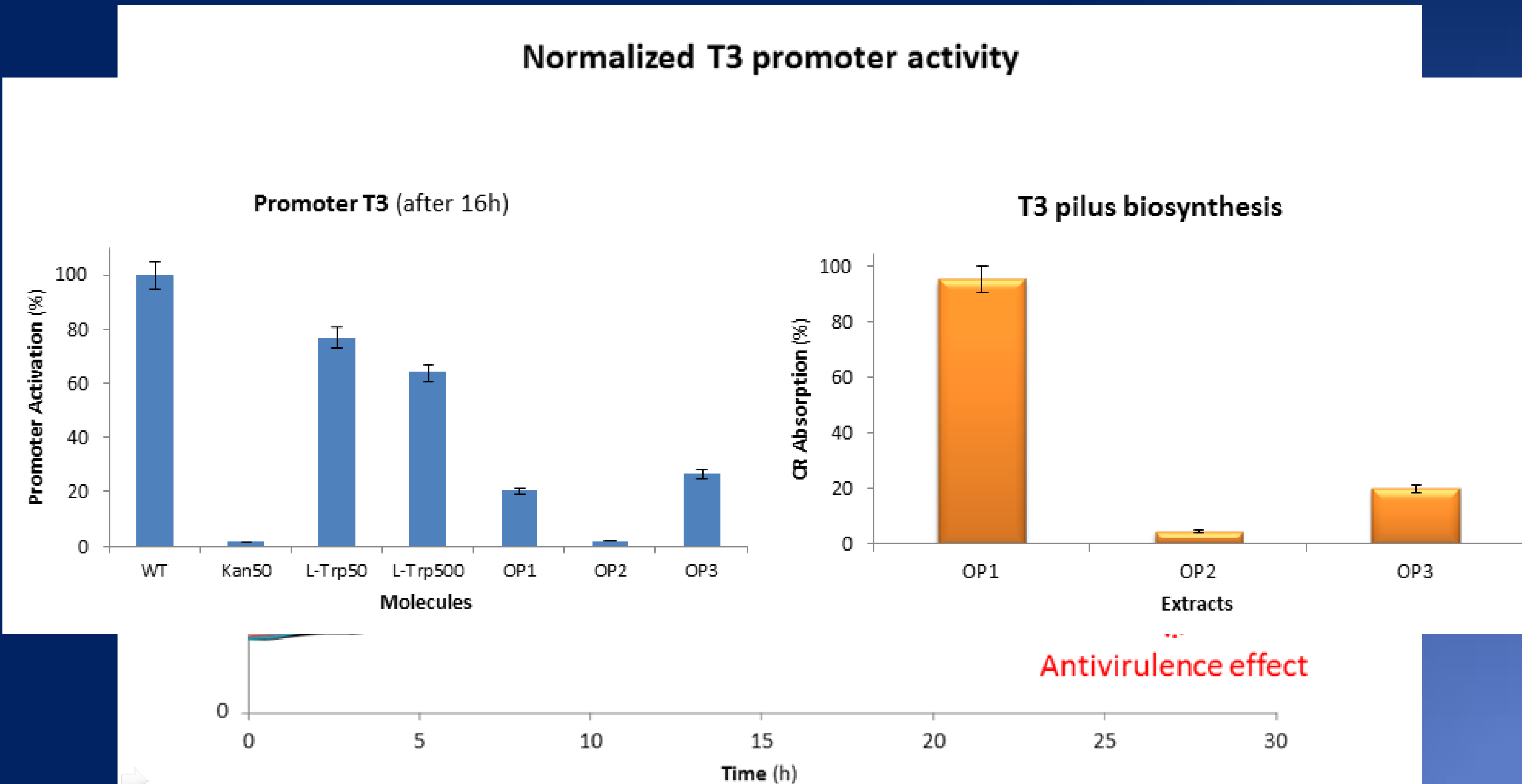



Fig. 5. pT3 activit ... from
sweet chestnut (OF ... otained
on the untreate ... ere
examin

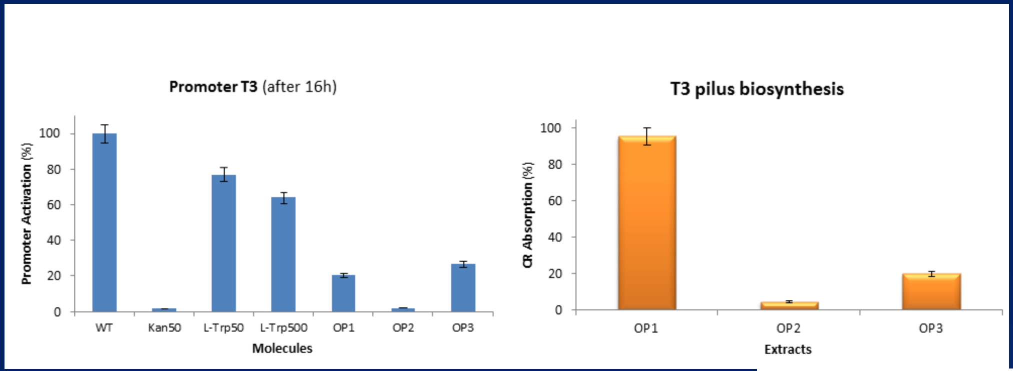
# Action B3

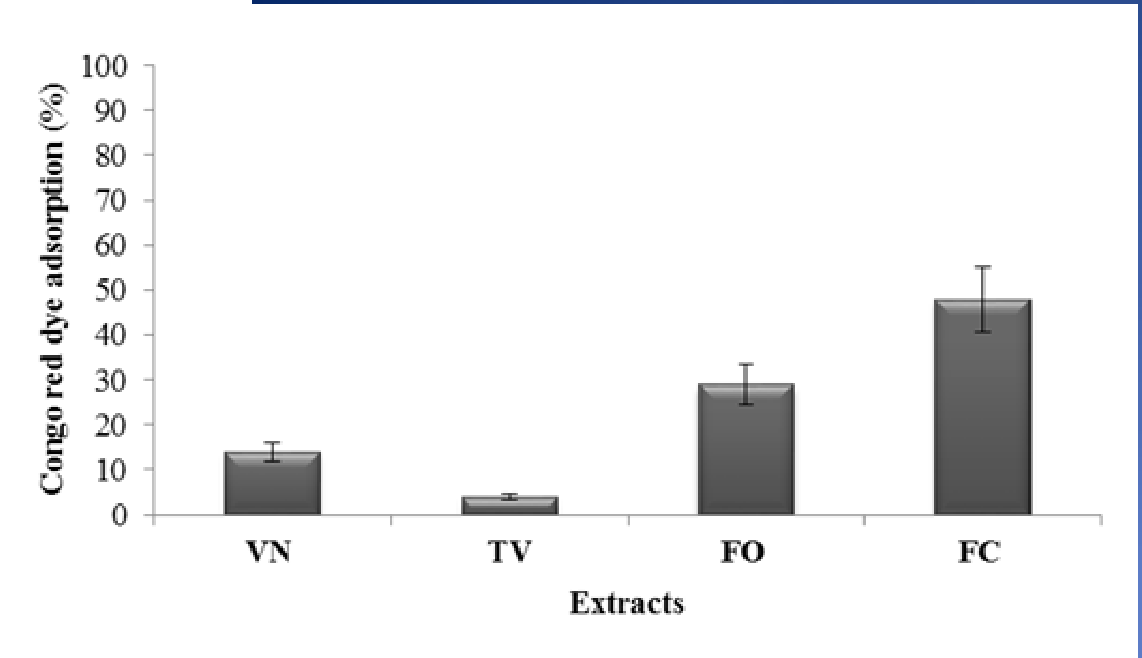
## 1st January 2015 - 31st December 2015



# Action B3

## 1st January 2015 - 31st December 2015




Biancalani *et al.*, 2016
PLoS ONE submitted

# Action B4

## 1st April 2015 - 31st December 2015

Demonstration of the biological activity of the high quality polyphenolic extracts recovered from not edible biomass and waste, against plant pathogenic bacteria and nematode, in planta

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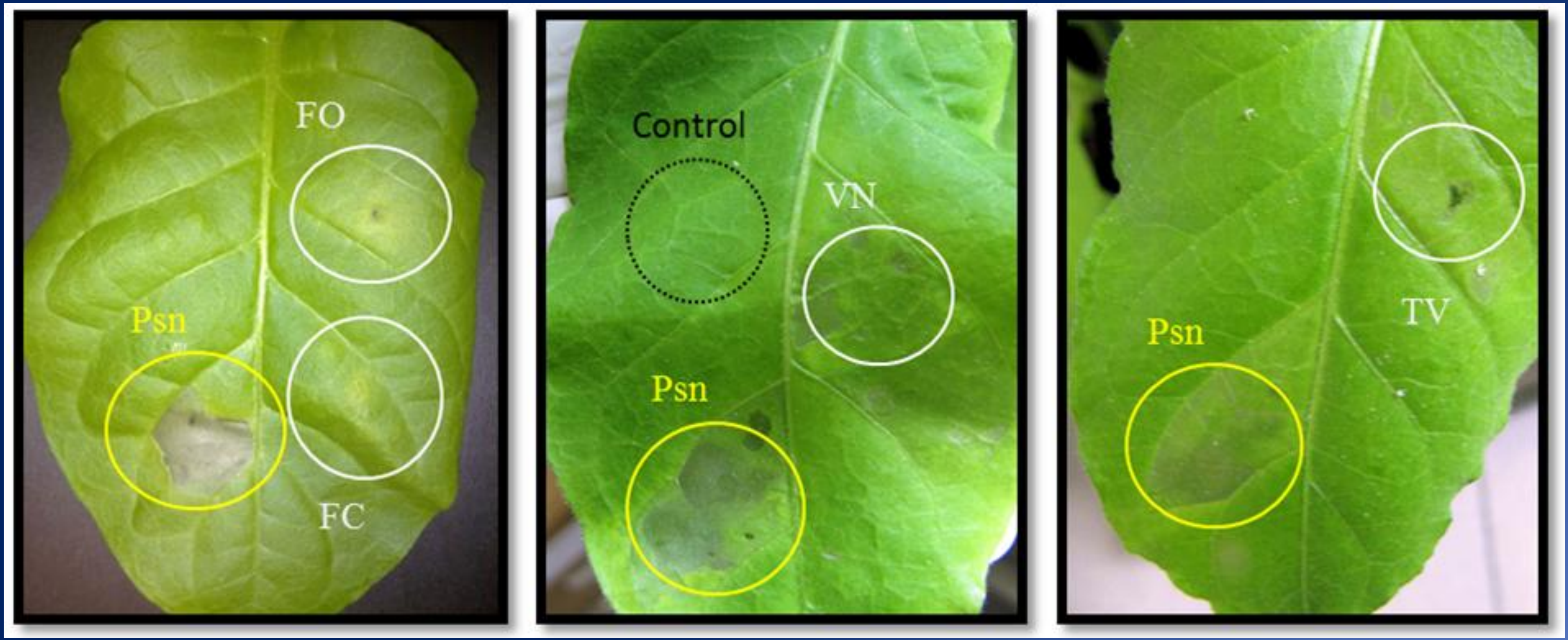
# Action B4

## 1st April 2015 - 31st December 2015




HR inhibition on Tobacco challenged by *Pseudomonas savastanoi*

Biancalani *et al.*, 2016 – PLoS ONE submitted

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2016, May 13rd
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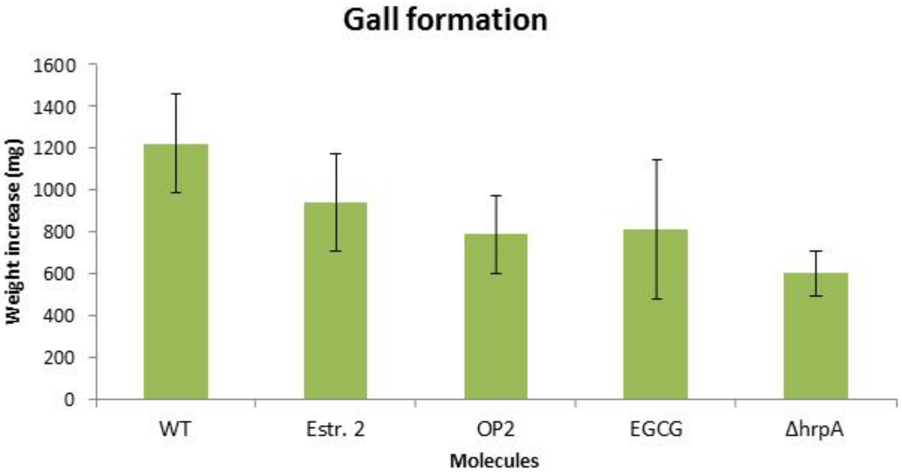
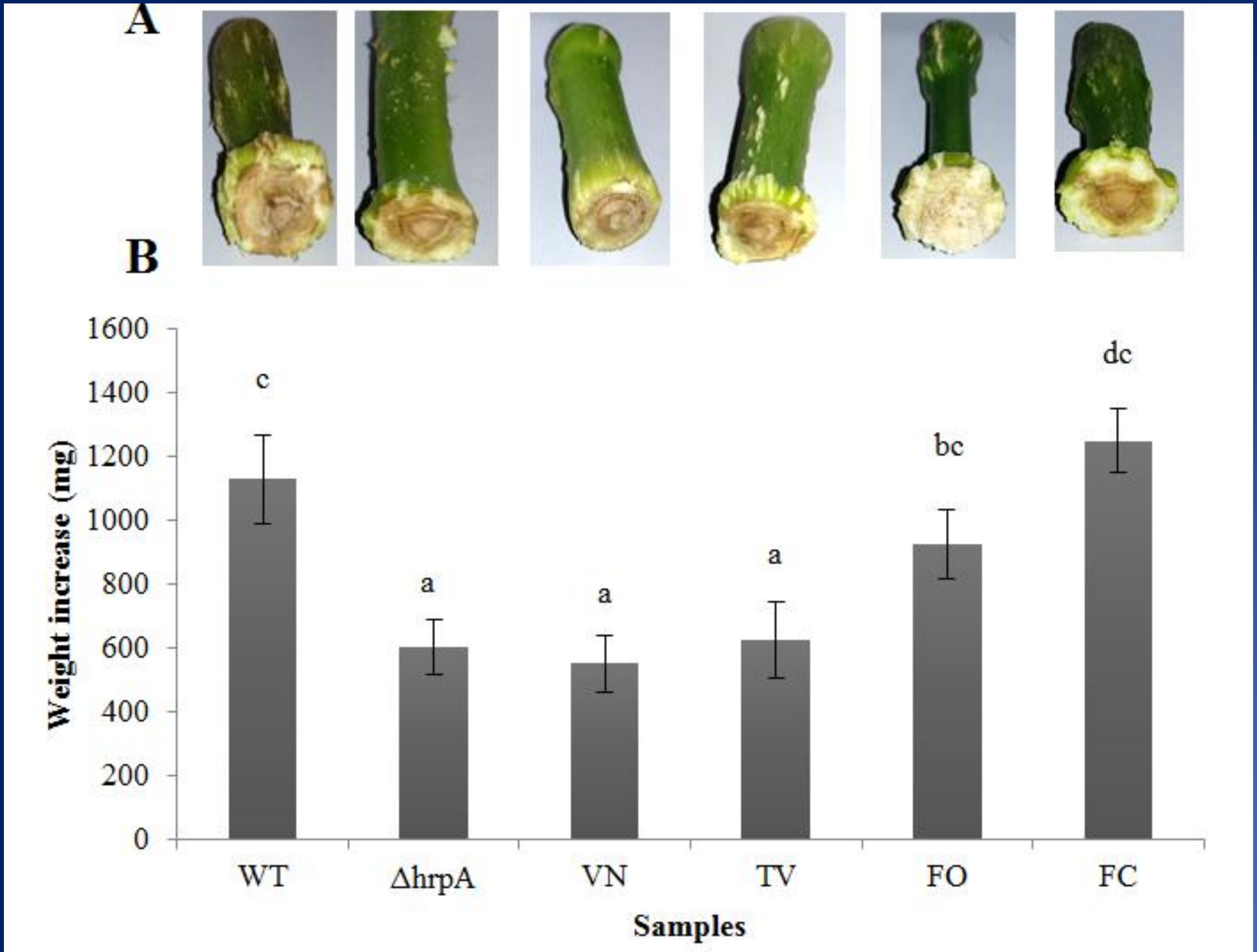
# Action B4

## 1st April 2015 - 31st December 2015



# Action B4

## 1st April 2015 - 31st December 2015




Biancalani *et al.*, 2016 – PLoS ONE submitted

# Action B4

1st April 2015 - 31st December 2015

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

## EVERGREEN (LIFE) B4 Act.

Project LIFE13 ENV/IT/000461

## B4 ACTIVITY (KIWI, OLIVE AND TOBACCO)

Demostration of the biological activity of polyphenols DONE!!! (From April 2015 to January 2016)

## Effects ON PLANTS: SOME INITIAL EXPERIMENTS

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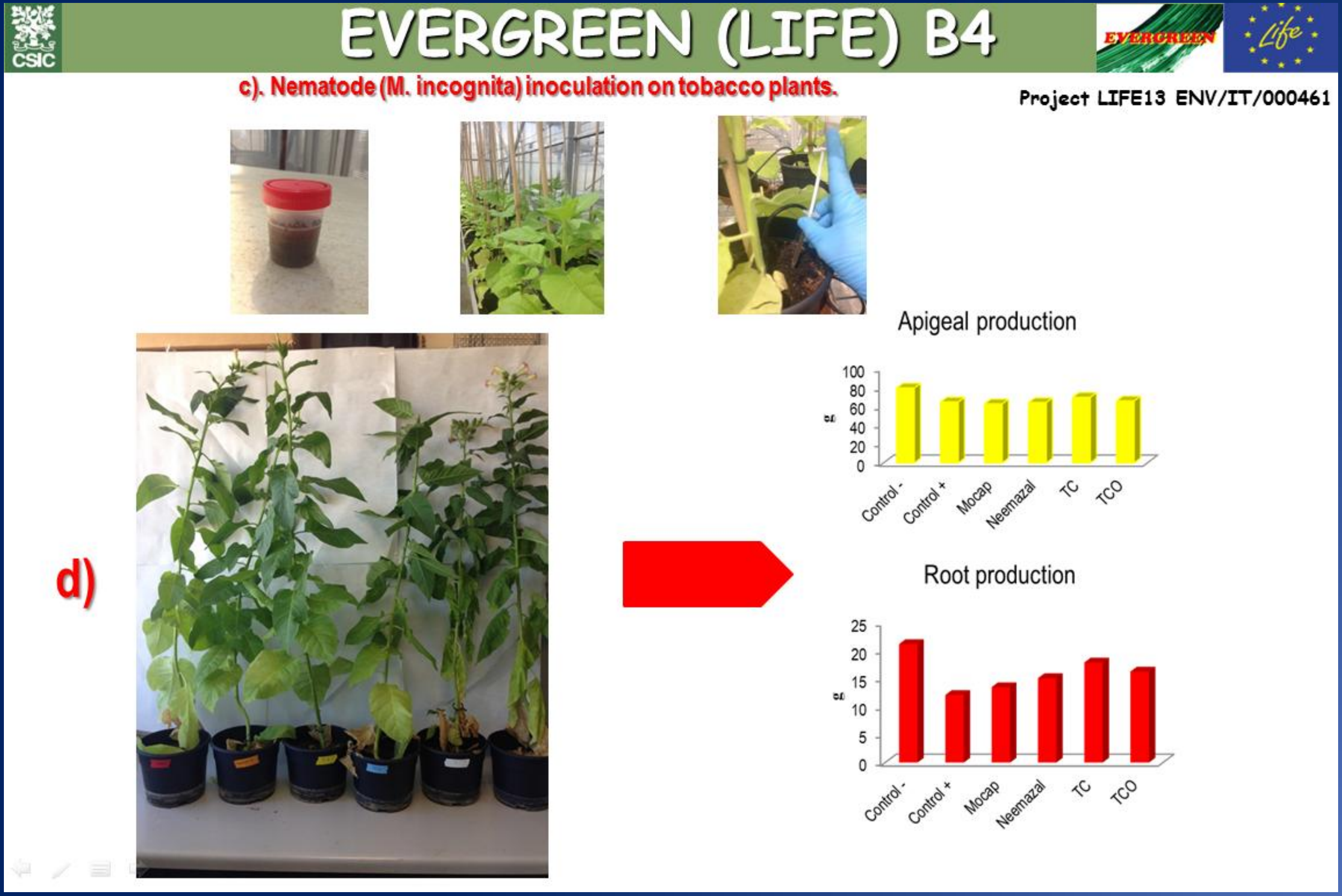
# Action B4

## 1st April 2015 - 31st December 2015



# Action B4

## 1st April 2015 - 31st December 2015

**Table 1**. Nematode pot experiment 2015. Results of nematode root gall index and plant epigeal DM determinations at 20 and 44 DAT. Soil for nematode count, Tissue Mass Density of roots (TMDr), and Fine (<0.5 mm ø) Roots percentage was sampled at 58 DAT.

| Treatm.No. | 2015 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nematode count No/200 $cm^3$ | TMDr ($g/cm^3$) | Fine roots (%) | Barker grading[a] | | DM epigeal yield (g/pot) | |
| | 58 DAT | 58 DAT | 58 DAT | 20 DAT | 58 DAT | 20 DAT | 58 DAT |
| Non infested control | NI | 0.22 b | 70.5 a | NI | NI | 9.3 ab | 19.7 b |
| Infested control | 634 a | 0.09 d | 48.5 b | 3.1 a | 4.4 a | 4.1 c | 7.4 d |
| Etoprofos | 51 c | 0.18 c | 68.0 a | 0.3 c | 1.2 b | 8.5 b | 16.3 c |
| CHT-MM | 503 b | 0.25 a | 74.0 a | 0.5 b | 1.1 b | 9.8 a | 22.5 a |
| CHT | 427 b | 0.24 ab | 73.0 a | 0.5 b | 1.2 b | 10.2 a | 21.8 ab |

[a] 0=0-10%; 1=11-20%; 2=21-50%; 3=51-80%; 4=81-90%; 5=91-100% [7]

Means within a column followed by the same letters are not significantly different (P = 0.05).

# Action B5

## 1st July 2015 - 31st March 2016

Demonstration of Kilo-scale extraction of the high quality polyphenolic bioactive molecules recovered from vegetable not edible biomass and waste

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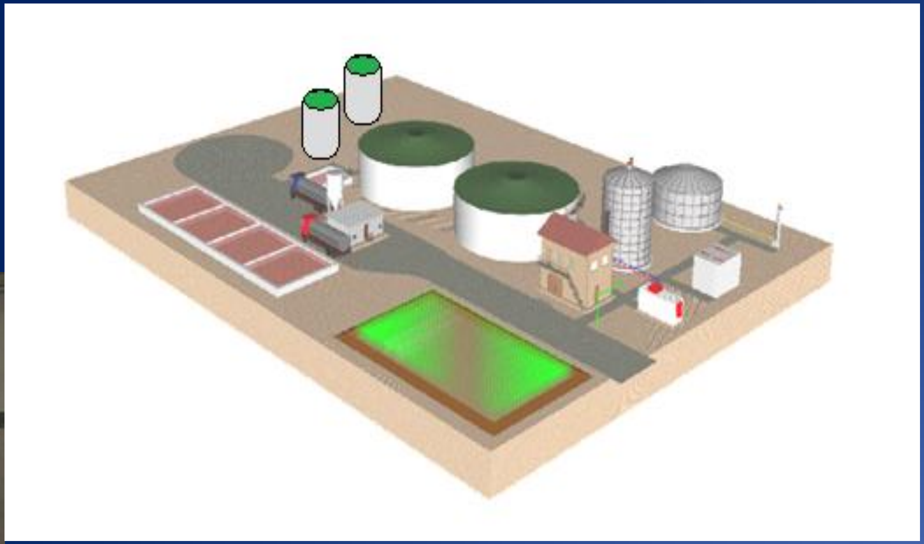
# Action B5

## $1^{st}$ July 2015 - $31^{st}$ March 2016



Polyfunctional platform for the production of antioxidant extracts and biogas from byproducts of *Olea europaea* L.

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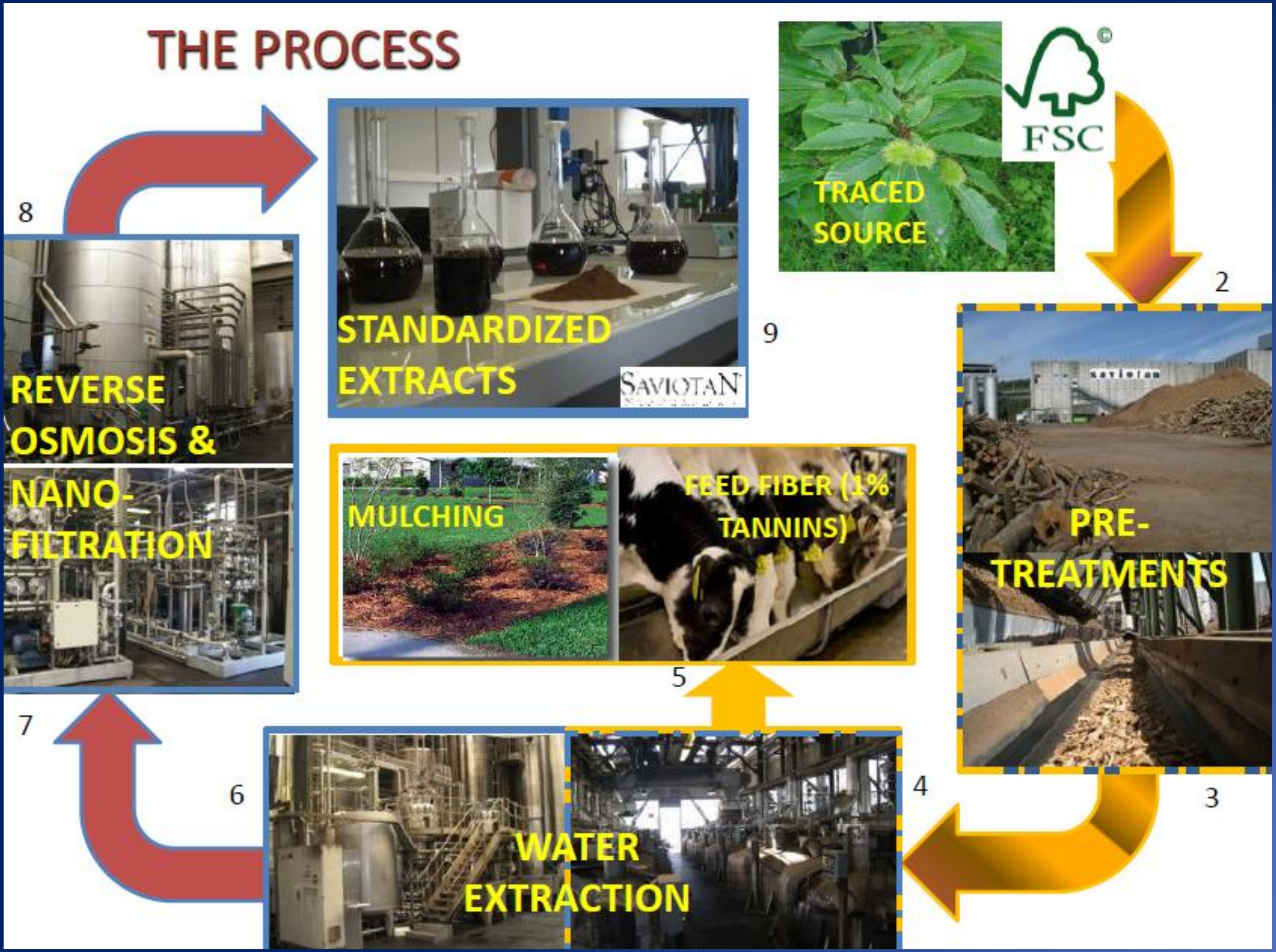
ASTRA srl

# Action B5

## 1st July 2015 - 31st March 2016



# Action B6

## 1st April 2015 - 31st March 2016

Demonstration of the null toxicity profile of the high quality polyphenolic bioactive molecules recovered from vegetable not edible biomass and waste, on model organisms and microorganisms

# Action B6

## 1st April 2015 - 31st March 2016

Test No. 202: *Daphnia magna*
**Acute Immobilization Test**
OECD Guidelines for the Testing of Chemicals
Retrieved July 4, 2013

Genus *Artemia* in **ecotoxicity testing**
*Environmental Pollution* 144 (2006) 453-462

# Action B6

## 1st April 2015 - 31st March 2016

**Table 1.** ***Daphnia magna*** **acute toxicity** ***in vitro*** **test, with EGCG and OP2 tannins.**

| Extract | EC50 24h (µM) | EC50 48h (µM) |
|---|---|---|
| Chestnut tannins OP2 | 25.6 | 25.6 |
| EGCG | 25.6 | 25.6 |
| [a]$K_2Cr_2O_7$ | 4,55 | 4,55 |

(a) Toxicity positive control

# Action B6

## 1st April 2015 - 31st March 2016

Table 2. *A. salina* acute toxicity *in vitro* test with EGCG and OP2 tannins.

| Extract | EC50 96h (µM) |
|---|---|
| Chestnut tannins OP2 | 26.2 |
| EGCG | 26.2 |

# Action B6

## 1st April 2015 - 31st March 2016

## c) EVERGREEN-ECOTX

| Reference | Ecotoxicity (mg/l) |
|---|---|
| TC 1g/l | 351110 |
| | 292600 |
| TC 0,1 g/l | 6990000 |
| | 5120000 |
| TCO 1g/l | 343350 |
| | 269280 |
| TCO 0,1g/l | 4720000 |
| | 5140000 |
| TCC 1g/l | 431120 |
| | 624830 |
| TCC 0,1g/l | 3790000 |
| | 5820000 |
| TAN 1g/l | 366810 |
| | 349200 |
| TAN 0,1g/l | 5290000 |
| | 6010000 |
| PV 1g/l | 239570 |
| | 249100 |
| PV 0,1g/l | 447948110 |
| | 316232270 |
| EPV 1g/l | 83990 |
| | 80680 |
| EPV 0,1g/l | 295670 |
| | 406140 |
| PFV 1g/l | 5190000 |
| | 3800000 |
| PFV 0,1g/l | 7480000 |
| | 8380000 |

Project LIFE13 ENV/IT/000461

### ECOTOXICITY ASSAY

A toxicity test was carried out using luminescent bacteria (Microtox), in which the inhibition of the luminescence of Photobacterium phosphoreum was measured using a luminometer (Kapanen and Itävaara, 2001) after adding extracts of the samples. This assay uses a suspension of luminescent bacteria (Photobacterium phosphoreum) as bioassay organism for measuring acute toxicity in aqueous extracts (Bulich, 1979; Matthews and Hastings, 1987). Lyophilized bacteria were used after rehydration in the commercial solution. All assays were carried out at 15 ºC with 15 min and 30 min contact periods between 0.5 ml of bacterial suspension and compost suspension. Compost suspension was prepared by mixing 1g sample with 10ml of 2 % NaCl (w/w) solution

### DATA RELEVANCE

Ecotoxicity assays were carried out with all polyphenols at two different concentrations (1g/L and 0.1 g/L).

Ecotoxicity assay were done with the seven Evergreen polyphenols at two concentrations 1 g/l and 0,1 g/l (polyphenol concentration used in plants assays) (Table 3).

According to our results and with Spanish low (BOE 10 de noviembre de 1989, número 270/1989) it is considered that a substance is toxic when its leached has an EC50 (15 minutes, 15ºC) less or equal than **3000 mg/l**. The EC50 obtained in polyphenols samples demonstrated that none of them are toxic for soil organisms.

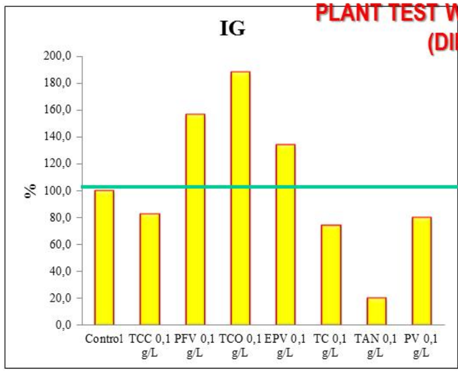
# Action B6

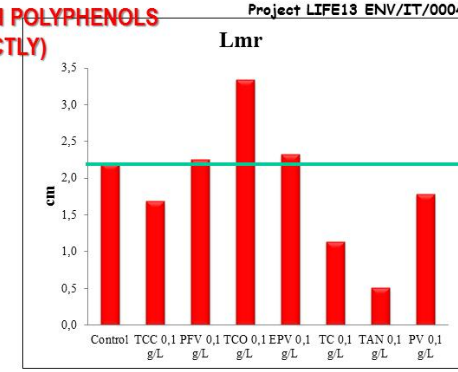
## 1st April 2015 - 31st March 2016

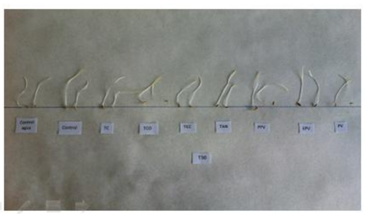
### b) EVERGREEN-PHYTOTOXICITY

**PLANT TEST WITH POLYPHENOLS (DIRECTLY)**

Project LIFE13 ENV/IT/000461



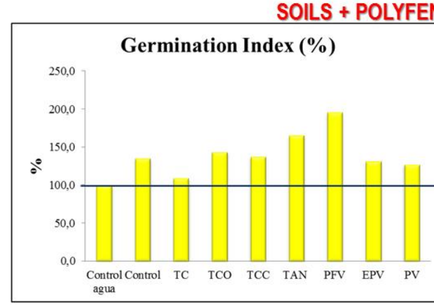



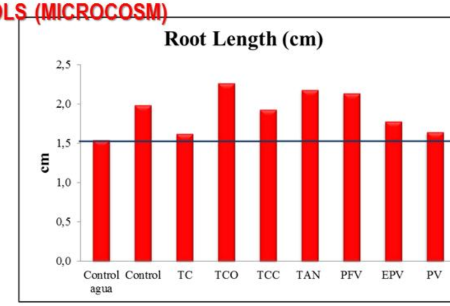
Plants test were carried out using directly polyphenols.
Some inhibition was showed with some polyphenols.
A bio-stimulant effect was also showed by TCO polyphenol

### b) EVERGREEN (LIFE)

**PLANT TEST WITH EXTRACTS OF SOILS + POLYFENOLS (MICROCOSM)**

Project LIFE13 ENV/IT/000461




Plants test were carried out using extracts of microcosms
Experiment at the beginning of the microcosm:
-- No inhibition is showed for any polyphenols,
-- A possible bio-stimulant effect was appreciated by all studied polyphenols

# Action B7

## 1st July 2015 – 30th September 2016

**Demonstration of the *in vivo* performances of the high quality polyphenolic bioactive preparations, recovered from vegetable not edible biomass and waste,at pilot scale level in field screenings**

LIFE13 ENV/IT/000461 – EVERGREEN
18th month Meeting – II Monitoring visit
2016, May 13rd
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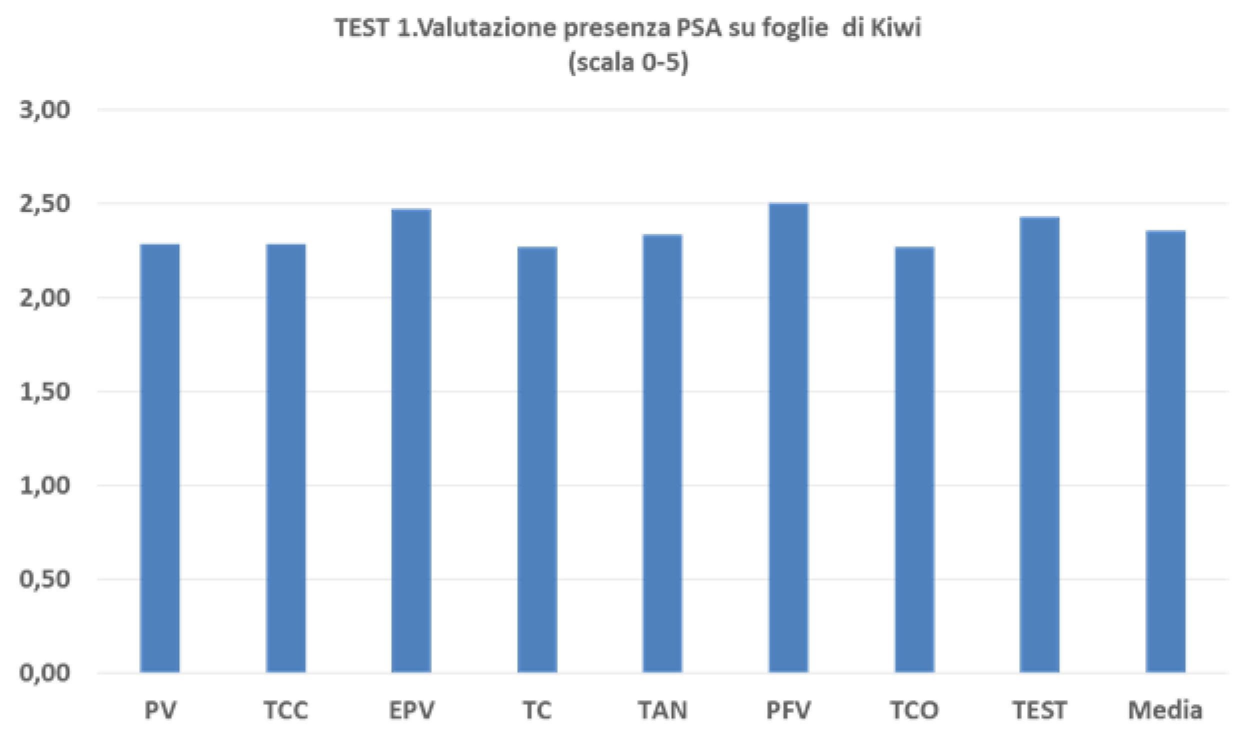
# Action B7

## 1st July 2015 – 30th September 2016



# Action B7

## 1st July 2015 – 30th September 2016

### MATERIALS AND METHODS

**Plants**

Kiwi (Actinida chinensis
Olive (Olea europea var. Arbequina)
Tobacco ( var., Waltai…)

**Pathogens**

*Pseudomona syringae actinidiae*
*Pseudomona savastanoi nerii.*
Nematodo: *Meloydogine incognita*

**Treatments**

Polyphenols (Photo 1):
Form 1 (liquid): TC 2%, O 1% in water (1:10)
Form 2 (liquid): TC 1,5%, O 1%, and V 0,3% in water (1:10)
Form 3 (gel): TC 0,2% and O 0,1% in water
Form 4 (gel): TC 0,15%, O 0,1% and V 0,03% in water
$CuSO_4$ (6 k $Cu^{++}$/Ha/año)

**Nematicide (Photo 2):**

Mocap
Neemazal

Project LIFE13 ENV/IT/000461



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# Action B7

## $1^{st}$ July 2015 – $30^{th}$ September 2016

Tomato plants has been inoculated with *pseudomonas tomato* and treated with tannins


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18th month Meeting – II Monitoring visit
2016, May 13rd
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# Action B7

## 1st July 2015 – 30th September 2016

Comparison between healthy plants and plants damaged by nematodes

# Action C1

## 1st October 2014 - 31st March 2015

Monitoring on the environmental impact of copper compounds and nematicides for the crop defence against phytopathogenic bacteria and nematodes

LIFE13 ENV/IT/000461 – EVERGREEN
18th month Meeting – II Monitoring visit
2016, May 13rd
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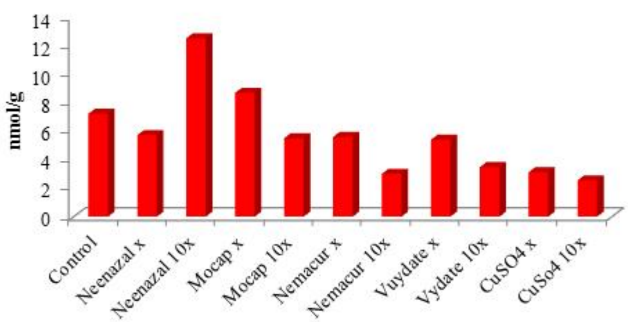
# Action C1

## 1st October 2014 - 31st March 2015

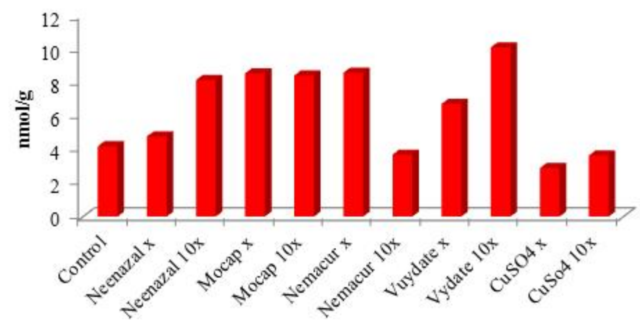
## EVERGREEN (LIFE) C1

Project LIFE13 ENV/IT/000461

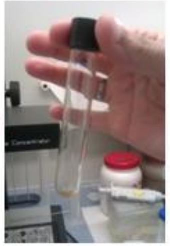
### Total PLFA T0




### Total PLFA T60



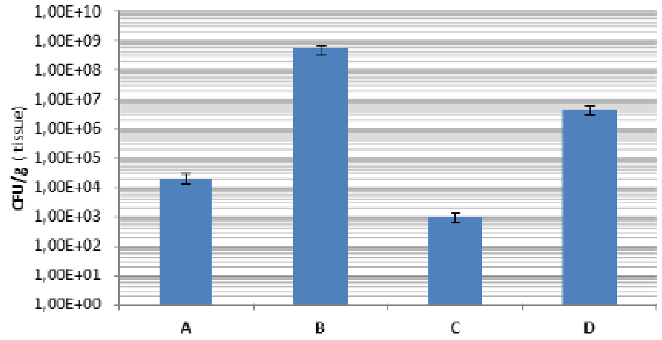




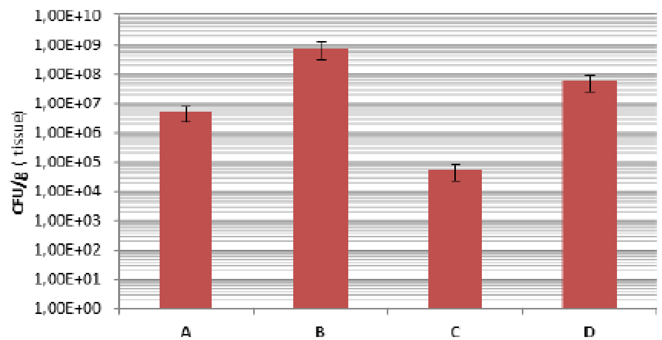
INFLUENCE OF Cu Salt AND NEMATICIDES ON SOIL BIODIVERSITY

# Action C1

## 1st October 2014 - 31st March 2015




Psa on kiwifruit plant




(A) Epiphytic Psv/Psa on Cu untreated plants; (B) Endophytic Psv/Psa on Cu untreated plants; (C) Epiphytic Psv/Psa on Cu treated plants; (D) Endophytic Psv/Psa on Cu treated plants.

# Action C2

## 1st January 2015 - 31st December 2015

Monitoring of the absence of side effects for the high quality standardised polyphenolic preparations on common targets of any living organism at laboratory level

# Action C2

## 1st January 2015 - 31st December 2015

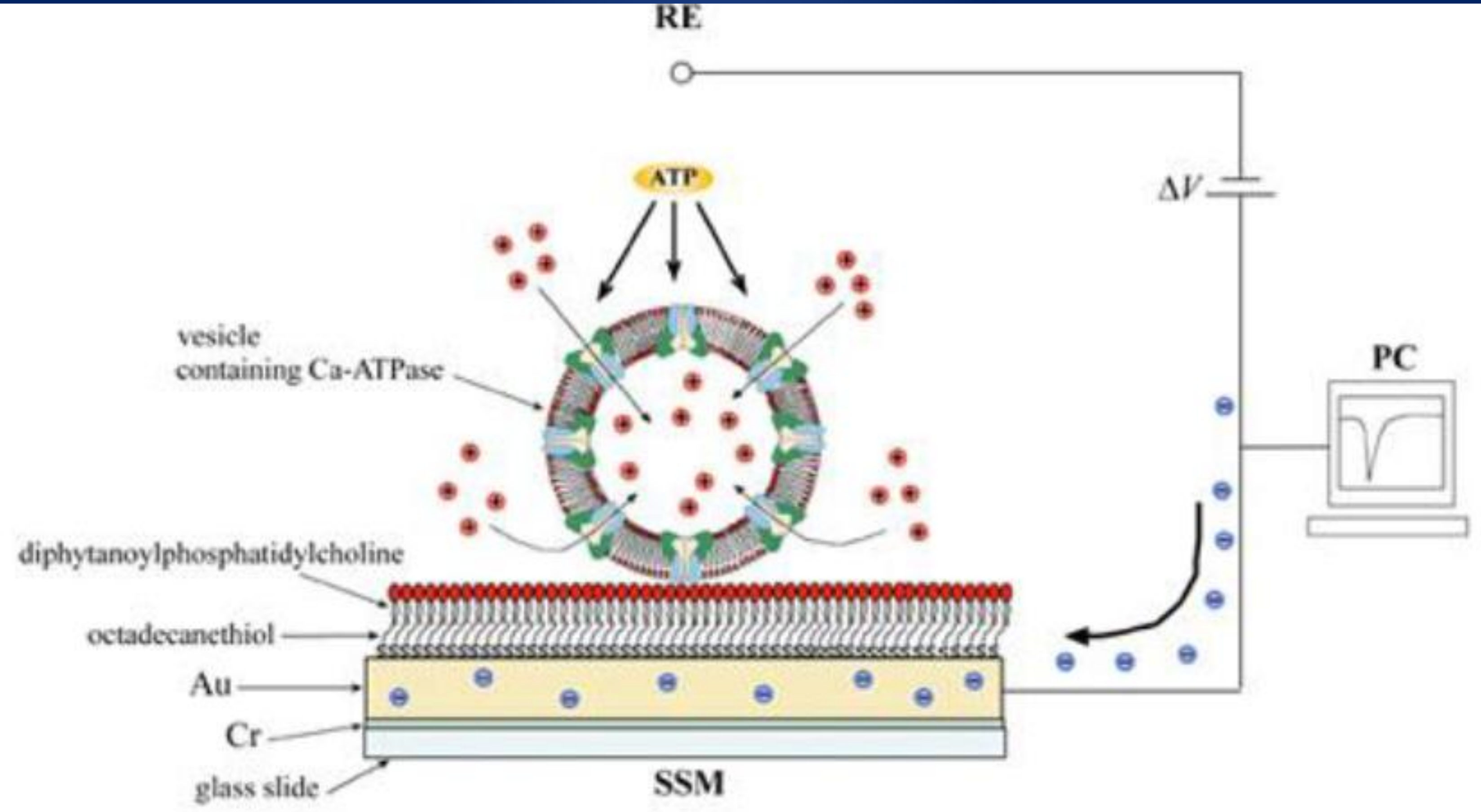



Fig. 1. Solid Supported Membrane (SSM) experimental set up.

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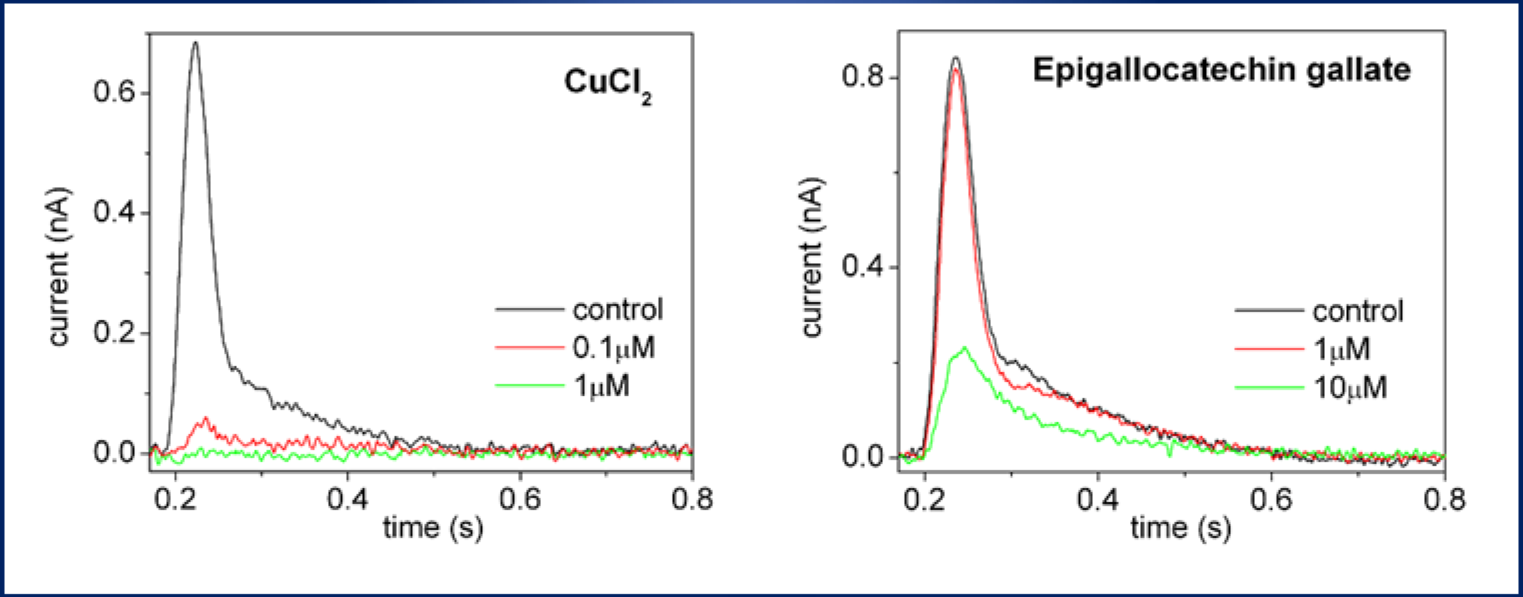
# Action C2

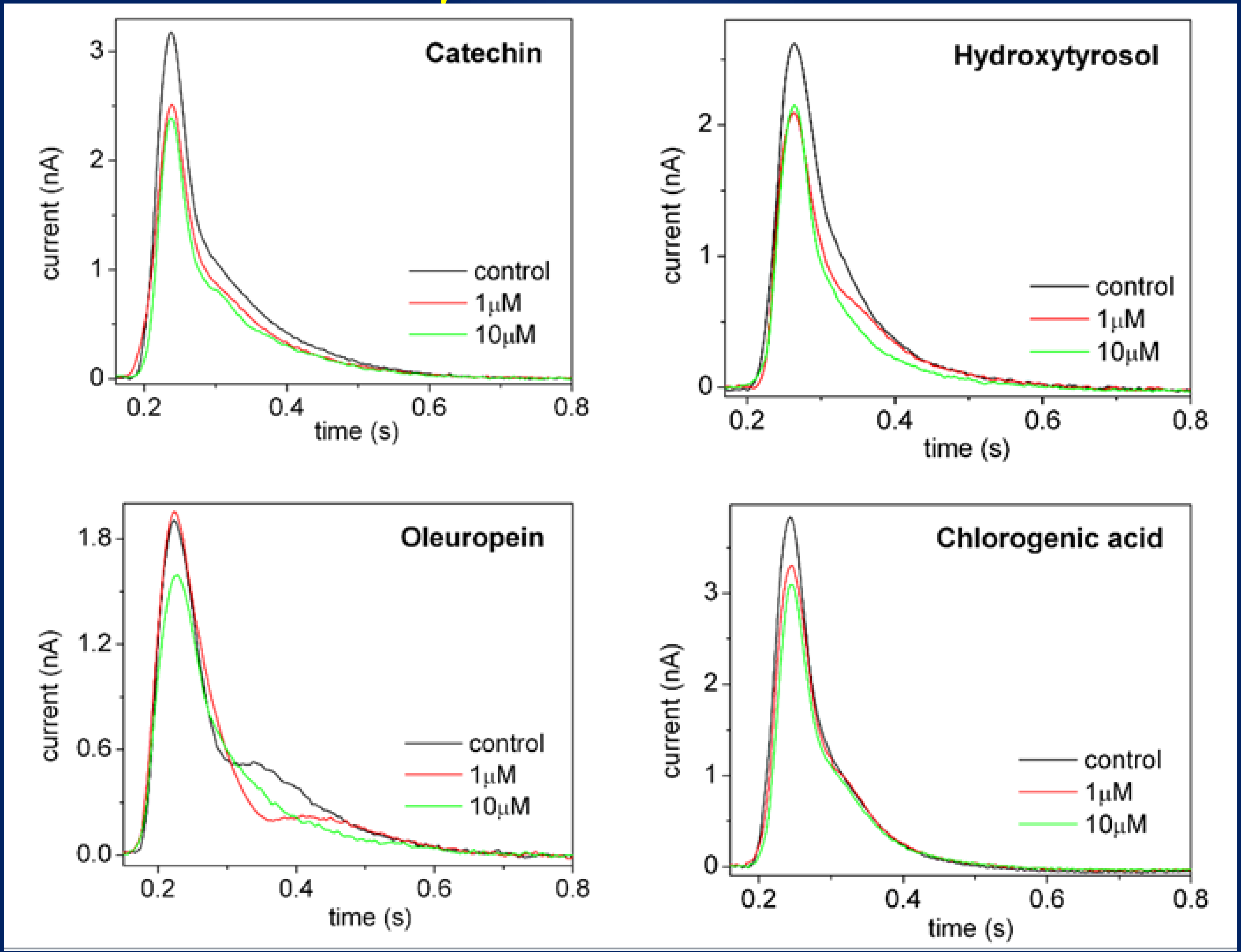
## 1st January 2015 - 31st December 2015




Biancalani *et al.*, 2016 – PLoS ONE submitted

# Action C2

## 1st January 2015 - 31st December 2015



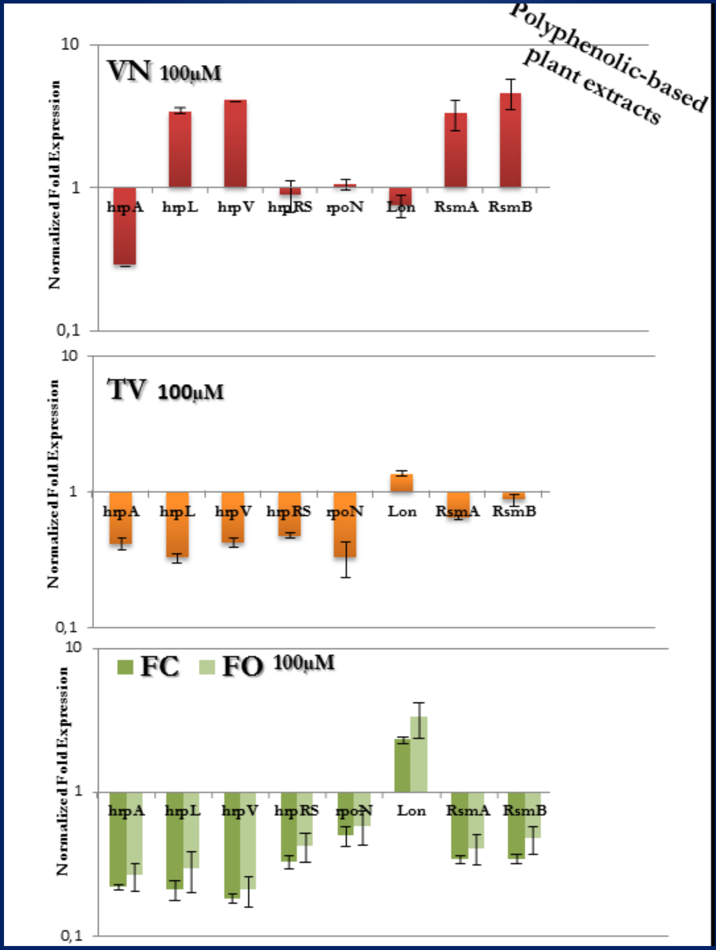
# Action C3

## 1st April 2015 - 31st March 2016

Monitoring of the absence of a direct selection operated by the polyphenolic preparations towards the emergence of bacteria resistant to the polyphenolic molecules themselves, at laboratory level

# Action C3

## 1st April 2015 - 31st March 2016



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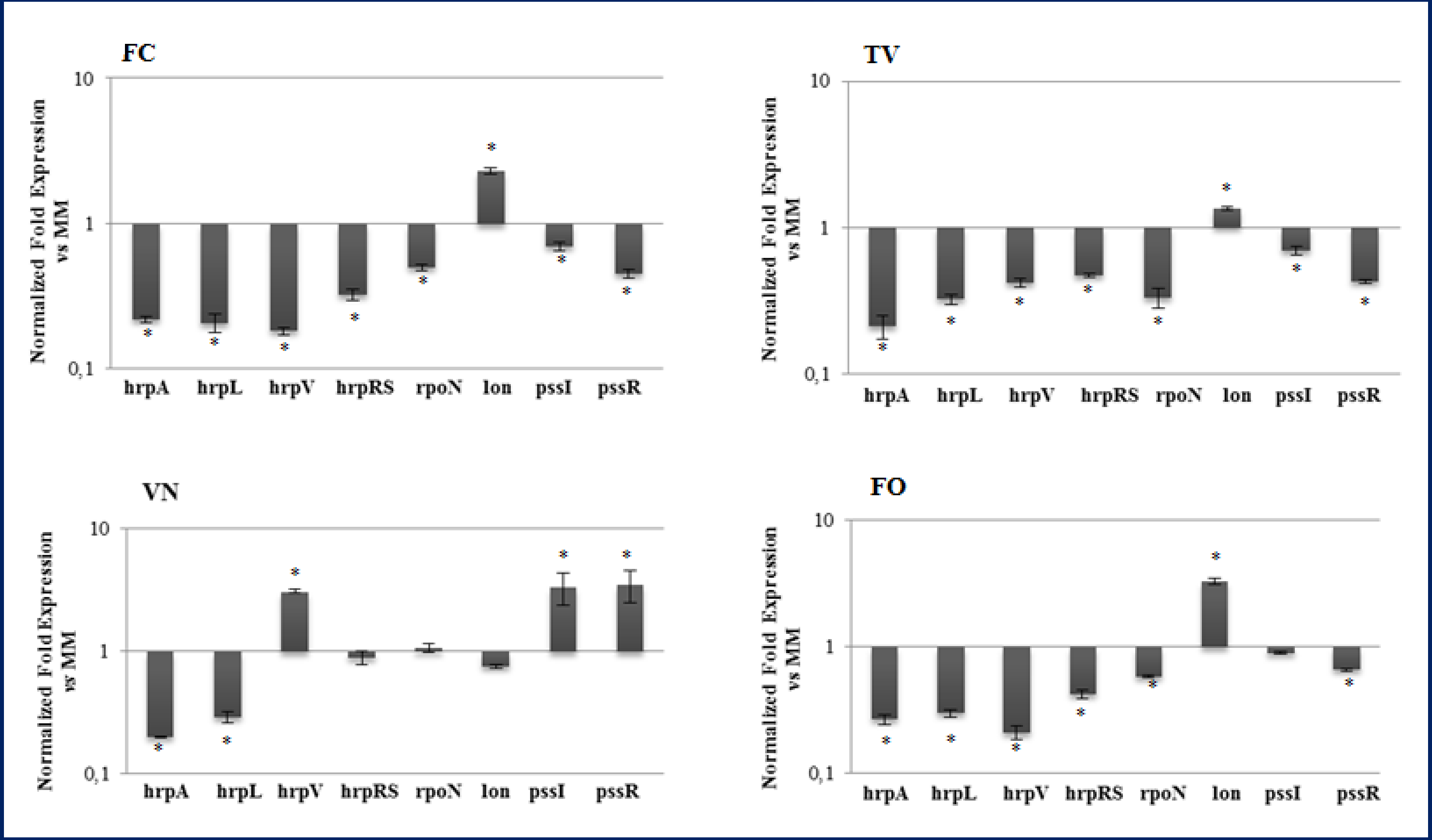
# Action C3

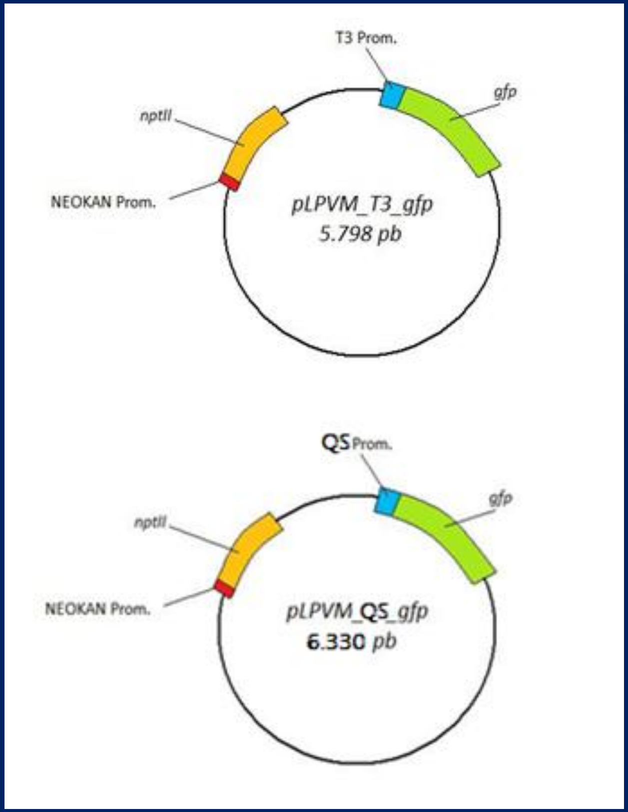
## 1st April 2015 - 31st March 2016




Biancalani *et al.*, 2016 – PLoS ONE submitted

# Action C3

## 1st April 2015 - 31st March 2016




Verified at 20, 60 and 80 generation treated

| Code | Extract/ Molecule | *hrpa* promoter Effect* | QS promoter Effect* |
|---|---|---|---|
| VN | Grape seed | 0.52 ± 0.011 | 1.30 ± 0.007 |
| TV | Green tea leaf | 0.46 ± 0.013 | 0.89 ± 0.004 |
| FO | Olive leaf | 0.75 ± 0.008 | 0.68 ± 0.025 |
| FC | Artichoke leaf | 0.47 ± 0.017 | 0.44 ± 0.031 |
| Kan | Kanamycin | 0.21 ± 0.018 | 0.23 ± 0.022 |
| PCA | p-Coumaric acid | 1.03 ± 0.022 | 0.98 ± 0.006 |

(*) Normalized fold versus WT condition (without treatment, only MM medium)

# Action C4

## 1st July 2015 – 30th September 2016

Monitoring of the short term environmental benefits from the use of the high quality standardised polyphenolic preparations in plant disease control at pilot scale level in field screenings

# Action C4

## 1st July 2015 – 30th September 2016

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

### B7/C4 ACTIVITY

EVERGREEN Life
Project LIFE13 ENV/IT/000461

**KIWI**

**Treatments:**

1. Control-: –bacteria – treatment
2. Control +: + bacteria – treatment
3. Control $CuSO_4$: - bacteria + $CuSO_4$
4. $CuSO_4$: + bacteria + $CuSO_4$

a) Spraying 24h before bacterial inoculation. b.) Spraying 24h after bacterial inoculation.

5. Form 1 (liquid): + bacteria + treatment Form 1. Spray
6. Form 2 (liquid): + bacteria + treatment Form 2

Supply polyphenol (form 1/ form 2) or $CuSO_4$ (100 c.c.) on soil next to the roots. Let it be absorbed during a week.
Spraying polyphenol or $CuSO_4$ solution on aerial part of the plant. Let it be absorbed (24 h).

**PFLAs as a measure of the microbiological variability of the soil**

In our study, total PLFAs decreased in the soil when nematicides and copper wer added. This phenomenon could be due to the negative effect of these agrochemicals o soil-life, that was particularly evident at 60 days with copper and Nemacur.

| PLFAs (nmol g-1) T_0 | Control | NeemAzal 1x | NeemAzal 10x | Mocap 1x | Mocap 10x | Nemacur 1x | Nemacur 10x | Vydate 1x | Vydate 10x | CuSO4 1x | CuSO4 10x |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | 3.34 d | 2.31 bc | 3.42 d | 3.70 d | 1.46 ab | 1.89 ab | 1.68 ab | 2.87 cd | 1.97 ab | 1.44 ab | 1.19 a |
| Gram + | 2.00 ab | 1.94 ab | 5.00 c | 2.28 b | 1.69 ab | 1.80 ab | 1.02 ab | 1.46 ab | 0.73 a | 0.97 ab | 0.83 ab |
| Gram - | 0.11 a | 0.12 a | 0.50 b | 0.07 a | 0.07 a | 0.00 a | 0.03 a | 0.05 a | 0.03 a | 0.05 a | 0.00 a |
| Fungi | 5.35 cd | 4.25 bcd | 8.43 e | 5.99 d | 3.16 ab | 3.70 abc | 2.70 ab | 4.33 bcd | 2.70 ab | 2.41 ab | 2.02 a |
| Saturated PLFAs | 5.98 cd | 4.43 bc | 7.85 e | 7.27 de | 4.52 bc | 4.52 bc | 2.43 a | 4.64 bc | 2.97 ab | 2.47 a | 2.16 a |
| Monosaturated PLFAs | 1.13 a | 1.18 a | 4.17 b | 1.33 a | 0.91 a | 1.08 a | 0.54 a | 0.70 a | 0.47 a | 0.60 a | 0.38 a |
| Actinobacteria | 0.57 ab | 0.16 bcd | 0.23 d | 0.20 cd | 0.16 bcd | 0.123 bcd | 0.00 a | 0.15 bcd | 0.05 ab | 0.09 abc | 0.09 abc |

Bacterial. fungal. $Gram^+$. $Gram^-$. satured and monosatured PLFAs concentration in microcosm soils T0

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# Action C4

## 1st July 2015 – 30th September 2016

Photo 1. Microcosm containers with different treatments al the beginning of the assay,

- Control (soil)
- TC (chesnut A)
- TCO (chesnut+olive)
- TCC (chesnut+artichoke)
- TAN (chesnut B)
- PFV (red vine leaves)
- EPV (grapefruit seeds)
- PV (orujo grape poder)

**MICROCOSM** →

TIMES: 0,1 AND 2 MONTHS

- CE
- Macro and microelements
- Ct, Nt, COT
- Water Soluble carbon and nitrogen
- B- Glucosidase
- Fosfatase
- Des-hidrogenase
- Urease
- Microbial respiration
- PLFAs

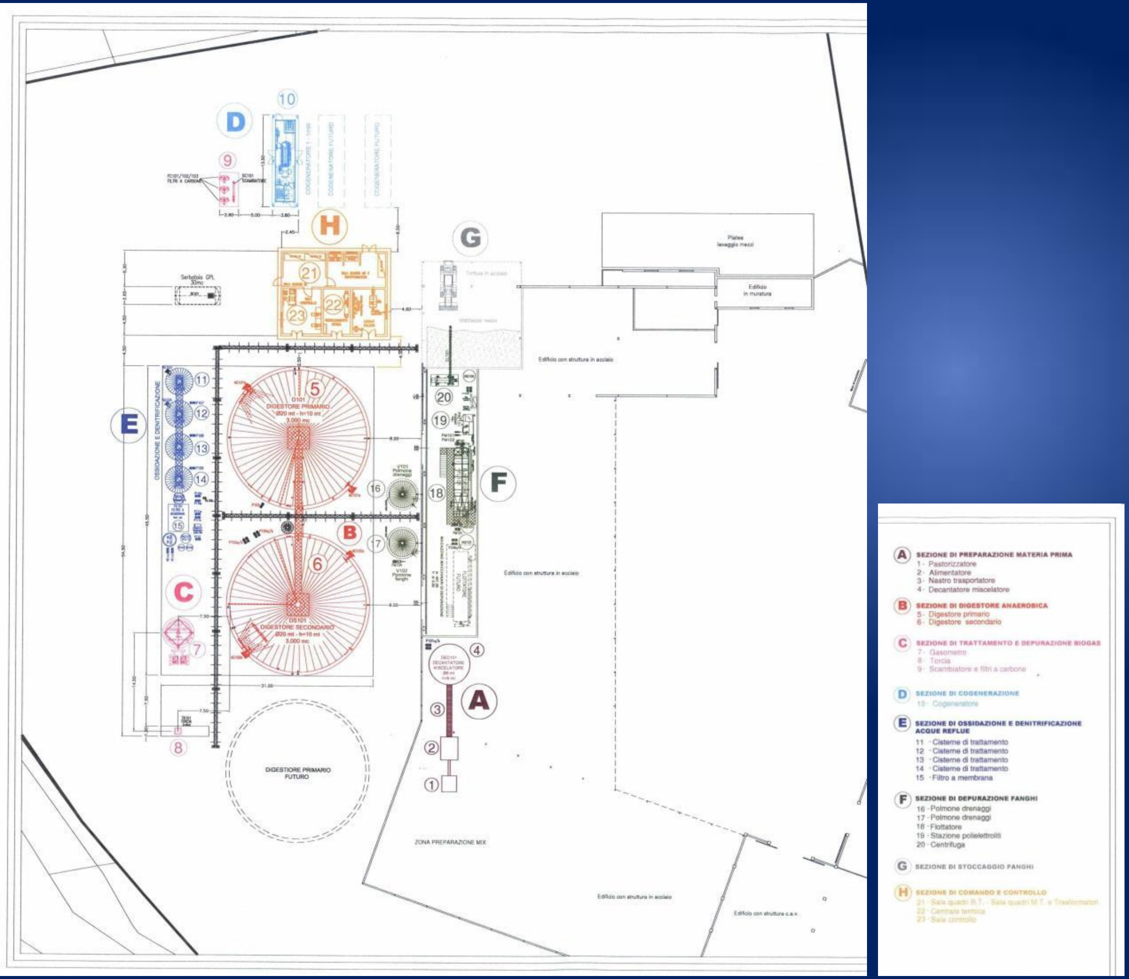
# Action C5

## 1st April 2015 – 30th September 2016

**Monitoring of the economic benefits deriving from the recycling of the spent vegetable biomass after the extraction of the high quality standardised polyphenolic molecules at laboratory level**

# Action C5

## 1st April 2015 – 30th September 2016




PCT/IT2009000246, filing date 05/06/09,A. Romani; D. Pangia; G. Marchinni. "Integrated process for recovery of a polyphenol fraction and anaerobic digestion of olive mill wastes";

PCT/IT2008/000135, filing date 01/04/08. Pizzichini M., Romani A., Pizzichini D., Russo C., Pinelli P. "Process for producing refined nutraceutic extracts from artichoke waste and from other plants of the Cynara genus"

**"This demonstration is delegated to INSTM and Mondo Verde"**

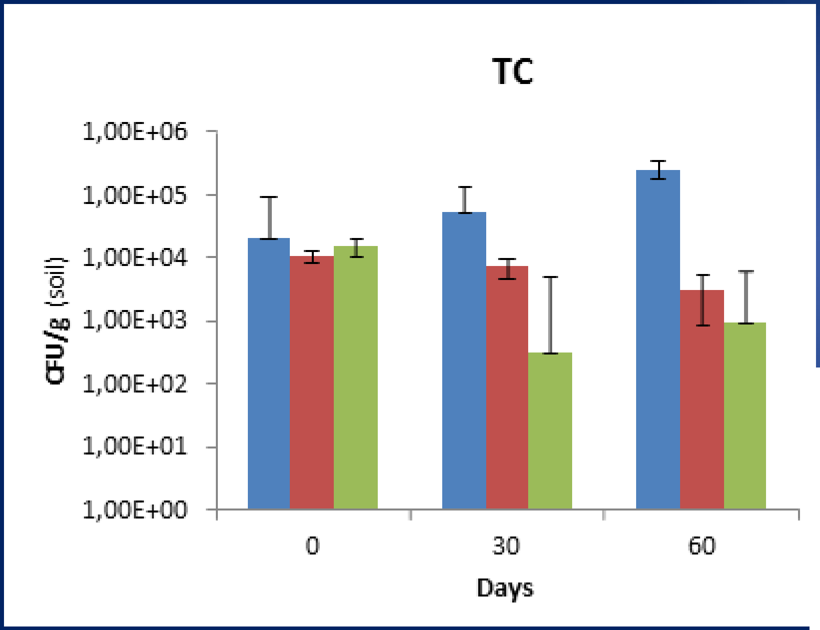
# Action C6

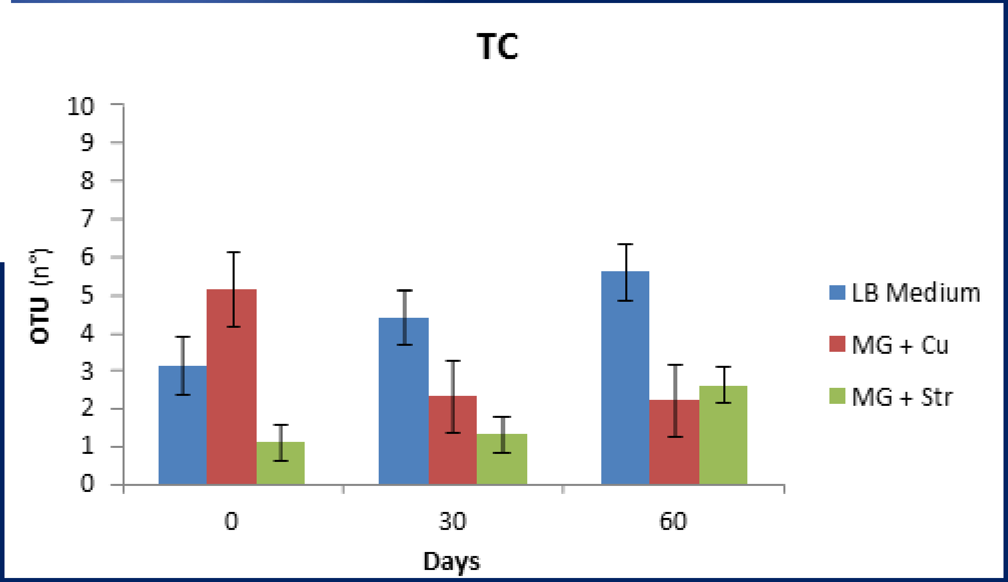
## 1st July 2015 – 30th September 2016

Monitoring of the absence of a selection on the polyphenolic preparations on copper and antibiotic resistant bacteria, on plant and in soil, from laboratory to in field screenings

# Action C6

## 1st July 2015 – 30th September 2016



# Action C7

## 1st April 2015 – 30th September 2016

## Monitoring of technical-socio-economic assessment of the EVERGREEN project

# Action C7

## 1st April 2015 – 30th September 2016

# Action C7

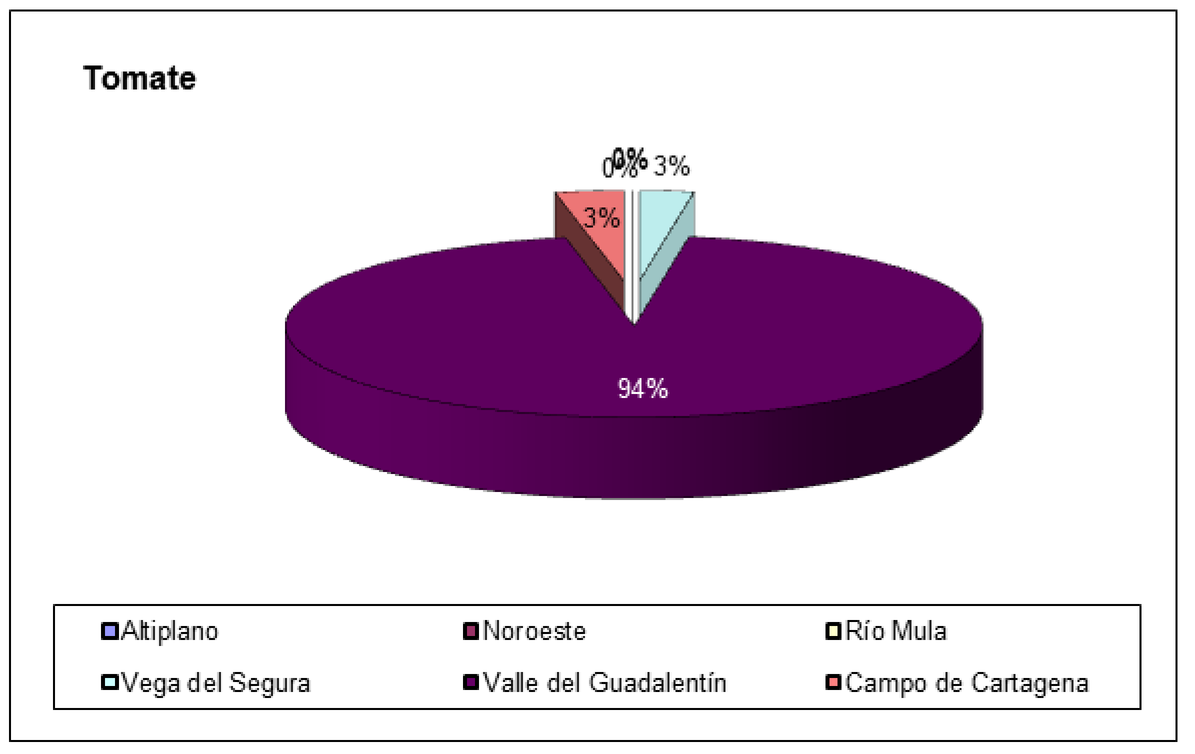
## 1st April 2015 – 30th September 2016


"DISPAA, CEBAS, and ASTRA will be involved in…"

**DATA FROM ALL BENEFICIARIES HAVE TO BE FREELY AVAILABLE**
**for analysis and evaluation**

# Action C7

## 1st April 2015 – 30th September 2016



# TECHNICAL ACTIVITIES CARRIED OUT

## B. Implementation actions

**B2** **Demonstration of the qualitative and quantitative yields of extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste at laboratory scale**

**B3** **Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and of their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale**

**B4** **Demonstration of the biological activity of the high quality polyphenolic extracts recovered from not edible biomass and waste, against plant pathogenic bacteria and nematode, *in planta***

**B5** **Demonstration of Kilo-scale extraction of the high quality poly-phenolic bioactive molecules recovered from vegetable not edible biomass and waste**

**B6** **Demonstration of the null toxicity profile of the high quality poly-phenolic bioactive molecules recovered from vegetable not edible biomass and waste, on model organisms and microorganisms.**

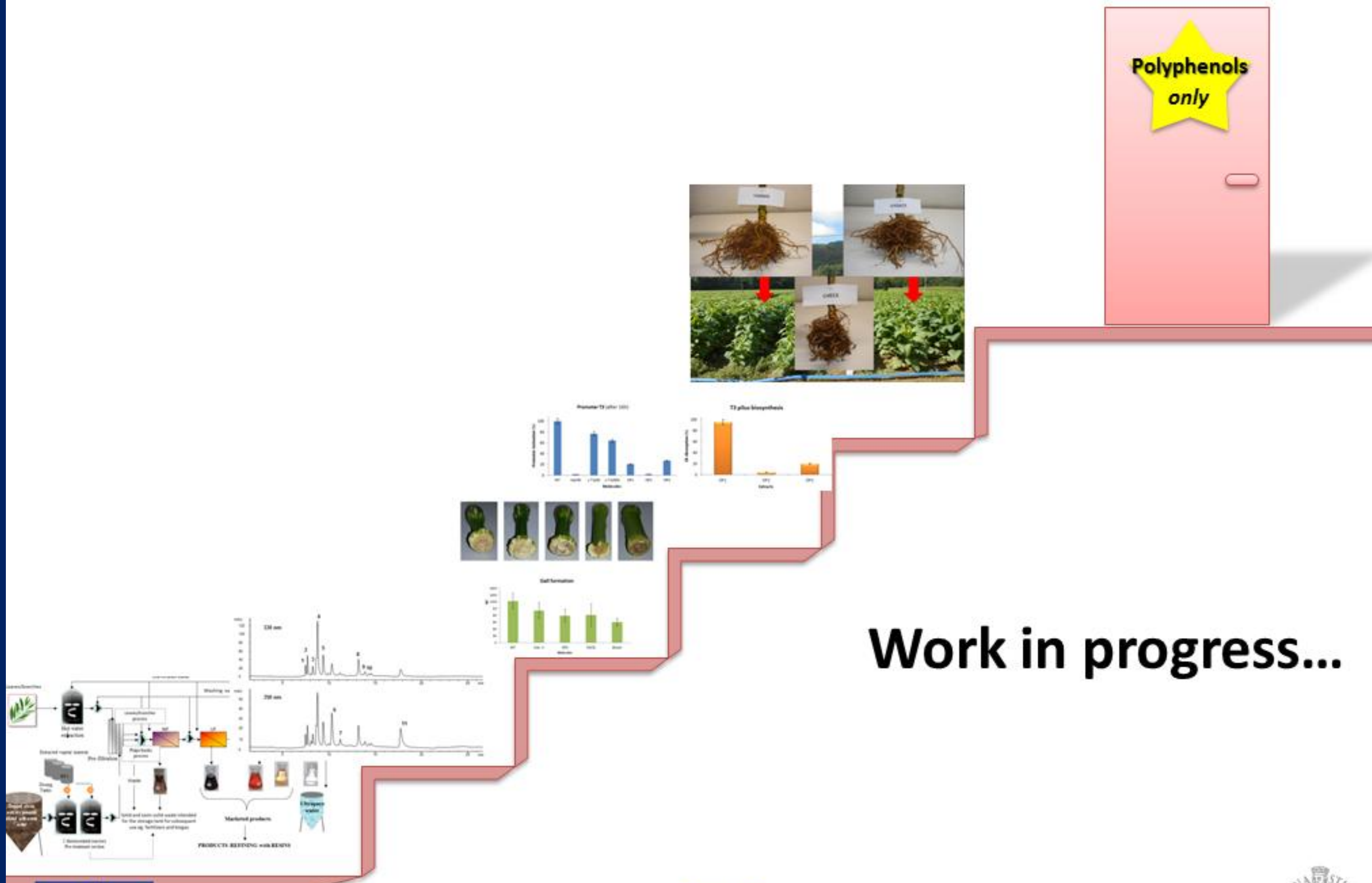
# TECHNICAL ACTIVITIES CARRIED OUT

## C. Monitoring of the impact of the project actions

**C2** Monitoring of the absence of side effects for the high quality standardised polyphenolic preparations on common targets of any living organism at laboratory level

**C3** Monitoring of the absence of a direct selection operated by the polyphenolic preparations towards the emergence of bacteria resistant to the polyphenolic molecules themselves, at laboratory level

**C6** Monitoring of the absence of a selection on the polyphenolic preparations on the selection of copper and antibiotic resistant bacteria, on plant and in soil, from laboratory to in field screenings.




**Work in progress...**

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Dept. of Statistics

Dept. of Biology

Genexpress

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Molecular Plant Pathology

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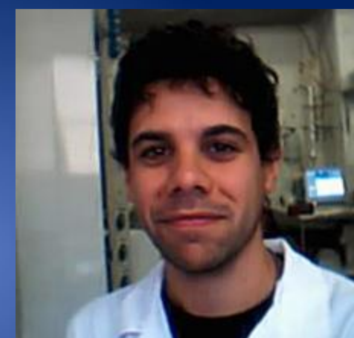

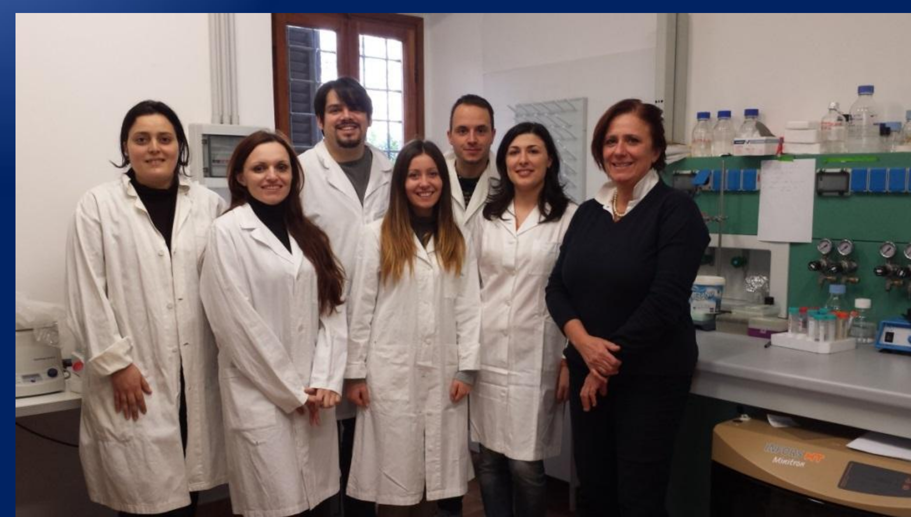

# And now, associated beneficiaries

# and then Dissemination and Financial issues

Dr. Silvia Borselli
Dr. Costantino Raspi