Technology transfer and European co-operation: The SEE.ERA-NET PLUS Joint Call project ECOFUN-MICROBIODIV

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SEE-ERA.NET PLUS Joint Call

- **It supports the integration of Western Balkan Countries (WBC)** and its key research communities **into the European Research Area (ERA)**

- **SEE-ERA.NET PLUS** launched a joint call for European Research projects in September 2009

- The call budget was 3.5 million €
- Each approved project can receive up to €150,000
- Duration of projects: 2 years

Thematic areas of the joint call

ICT:

- Software systems for learning process management & support
- ICT for energy efficiency

AgroFood

- Preservation of indigenous species and traditional food products (in SEE/WBC)
- **Interdisciplinary field: Land use impact in agriculture on biodiversity**

Participating countries

- **Western Balkan countries:** Albania, Bosnia and Herzegovina, **Croatia,** FYROM, Montenegro, **Serbia**)
- **EU Member States:** Austria, Bulgaria, **France, Germany**, **Greece,** Romania, Slovenia and
- Turkey as an FP7 Associated Country

- **Minimum conditions for participation:** the consortium has to consist of at least two partners from the WBC and one from other participating countries (2+1 principle)

Partners

Institut National de la Rechreche Agronomique
Lab. of Soil and Environmental Microbiology
Dijon, France
Dr. Fabrice Martin-Laurent

University of Hohenheim
Institute of Soil Science and Land Evaluation,
Section Soil Biology, Germany
Prof. Ellen Kandeler

Rudjer Boskovic Institute
Divison for Marine and Environmental Research
Zagreb, Croatia
Dr. Ines Pertic

University of Novisad
Faculty of Agriculture, Dept. Field & Vegetable Crops
Serbia
Dr. Simonida Djuric

University of Thessaly
Department of Biochemistry & Biotechnology
Larisa, Greece
Dr. Dimitrios Karpouzas

SEE.ERA.NETplus project ECOFUN - MICROBIODIV

Development and implementation of innovative tools to estimate the ecotoxicological impact of low dose pesticide application in agriculture on soil functional microbial diversity

What was the initiative for setting up ECOFUN-MICROBIODIV?

- According to previous (Directive 91/414/EEC) and current legislation (Regulation 2006/388) toxicity of pesticides onto non target soil microorganisms relies solely on simple C and N mineralization tests (OECD 216, 217)

- ***Does not provide a comprehensive assessment of pesticide soil microbial toxicity***

  - Possible impact on microbial diversity and other microbial functions?

What do we need at regulatory level?

- *A toolbox of robust and well tested methodologies which could be used to assess the effects of pesticides at both diversity and functional level*

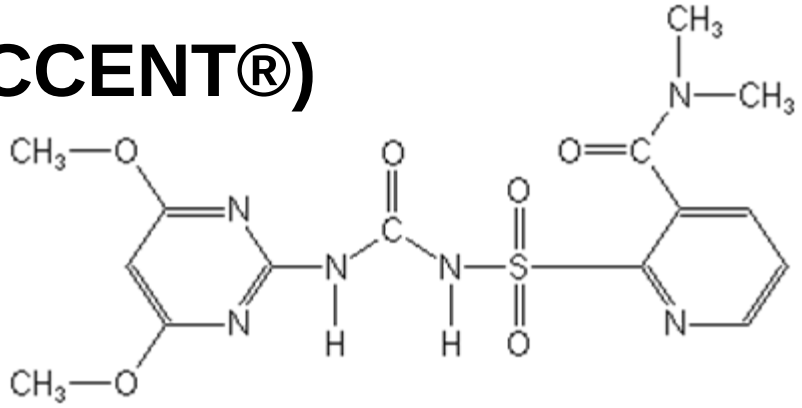
Aims of ECOFUN-MICROBIODIV

To develop and evaluate innovative tools for the robust estimation of the ecotoxicological impact of pesticides used in agriculture on soil functional microbial biodiversity

Sulfonylurea herbicides which are characterized as low-dose high potency pesticides were selected as target pesticide group

Pesticide used as a ‘model’ for the evaluation of the tools?

- **Nicosulfuron (ACCENT®)**



- Selective early- to mid-post emergence control of annual grass and broadleaf weeds and perennial grass weeds like *Sorghum halepense* in maize

- Recommended dose rate: 60 g a.i./ha

Experimental Planning

*A **greenhouse – to – field experimental approach** was followed in order to get a good estimation of the applicability of all our tools at both conditions*

Greenhouse experiment

- Setup in Dijon, France with soil sent by the field site where the field experiment was employed in Serbia
- **Nicosulfuron** applied at **0, x1, x10 and 100x** the recommended dose
- **Five replicate** pots per treatment
- **Pots were seeded with 10 corn seeds and thinned to 4 upon emergence**
- Soil and roots were harvested and sent to partners for analysis

Field Experiment

- Set up in Novi Sad, Serbia in May 2011
- **Nicosulfuron (ACCENT®)** was applied at 4 June 2011 with a knapshak sprayer at **0, x1, x2 and x5** the recommended dose
- Completely Randomized Block design with **four replicate plots** per treatment
- Soil samples are collected before and 2, 7, 14, 28, 56 days after application and at harvest (early October)



Multidisciplinary approach

- **Impact on Crop (WP4)**

  - Maize yield and weed control level (field experiment) 
  - Phytotoxicity on maize (greenhouse experiment)

- **Pesticide dissipation rates (WP5)** 
- **Impact on soil microbial community (WP6)**

  - Microbial Diversity: microbial biomass, PLFAs, ARISA, DGGE on the intraradical community of AM fungi 

  - Microbial Function: qPCR for *amoA* and *catA/pcaH* genes involved in N and C cycling, root colonization by AM fungi involved in P cycling 

  - **Microbial Adaptation** (greenhouse experiment) 

What have we done so far and what is currently running..

We have set up two web-sites

- http://www4.inra.fr/ecofun_microbiodiv
- http://www.wix.com/fabricemarti8/ecofun-microbiodiv

151 visits from 35 different countries …Good visibility of the project

Greenhouse experiment – Agronomic measurements

Clear inhibition of growth at x10 and x100 the recommended dose of nicosulfuron

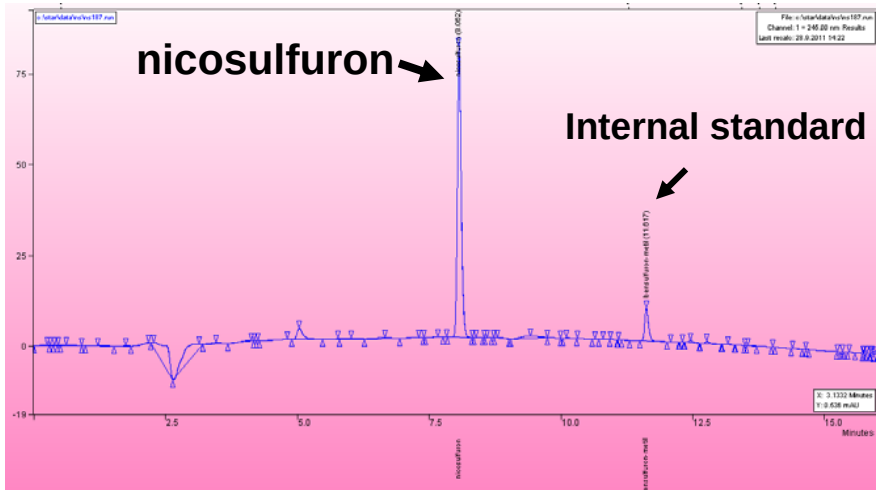
On going work

- *Impact on symbiotic AM fungi (colonization and diversity)*
- *Impact on other non-target soil micro-organisms*

Greenhouse experiment – Pesticide Dissipation

- A new HPLC-DAD method for the extraction and quantification of nicosulfuron residues in soil was developed and tested with satisfactory recoveries (>80%)

- ***Measurements on the dissipation of nicosulfuron are <u>on-going</u>***



Greenhouse experiment – Impact on soil microbes

The following measurements are on-going:

- *Root colonization by AM fungi* 
- *Intraradical diversity of AM fungi by DGGE* 
- *Application of molecular and biochemical tools to identify the impact of nicosulfuron on the diversity and function of soil microbes* 
- *Microbial adaptation to nicosulfuron* 

Field experiment – Agronomic measurements

- Major weeds in the field were identified

Sorghum halepense

Chenopodium album

Amaranthus retroflexus

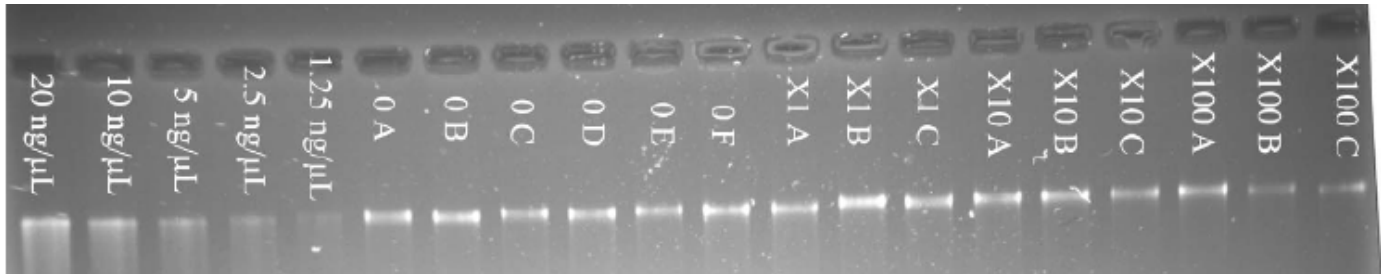
Weed control efficiency and corn yield are currently measured since the field experiment is still running

Field experiment – Pesticide Dissipation

- *The extraction and HPLC method developed is used in on going measurements for the determination of the dissipation of nicosulfuron at field scale* 

Field experiment – Impact on soil microorganisms

DNA extraction from soil samples **from the first two sampling dates** were completed by RBI using the ISO 11063 method developed by INRA in collaboration with other EU partners



On going work

Soil and root samples from the final sampling dates will be collected and sent to the partners for further analysis

a. PLFAs, enzymatic activities

b. A-RISA, catA/pcaH genes qPCR

c: DGGE AM fungi, Root Colonization, amoA gene qPCR

Are we in line with the aim of the SEE.ERA.NET call?

- *Technology transfer from research groups from MS like Germany, France, Greece to research groups in Croatia and Serbia (WBC) which have shown good research activity and this project will help them to be integrated in the research network of EU and unravel their full potential*

- *Deals with an important scientific issue whose resolution will advance our risk assessment procedures for pesticide soil microbial toxicity*

The project will be completed in October 2012 with a closing meeting in Greece