European Network for the Durable Exploitation of Crop Protection Strategies

IA3 activity: human resource exchange
SA3.2 sub-activity: foster the participation of research teams from INCO target countries

ENDURE Grants for INCO scientists

Final activity report

(The form has to be filled in and sent to the activity leader – message should be sent to his p.a. federica.piccolo@ibaf.cnr.it – within 15 days after the end of the visit)

1. Information about researcher and sending partner

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2. Information about hosting partner

Hosting partner: National Research Council (CNR)
Institute/Department/Research Unit: Institute of Sciences of Food Production (ISPA)
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3. Information about the visit

Duration: 3 months
Starting date: 3 February 2010
Ending date: 28 April 2010

4. Description of the activities and outcomes

Background and context:

Weed biological control is an alternative or additive use of natural enemies of weeds in different pest management systems. Invasive and parasitic weeds, and those species, which are difficult to eradicate or control with conventional methods (e.g. weeds insensitive to common herbicides), are usually targeted for biocontrol. Herbivory insects and plant pathogens, especially, fungi have been explored as biocontrol agents. The latter organisms can be produced on artificial media, formulated and used as bioherbicides that could explain extended interest to them. Moreover, some plant pathogenic organisms are capable of producing phytotoxic metabolites that can be utilized in different ways to combat weeds.

Two species of phytopathogenic fungi, namely *Stagonospora cirsii* and *Ascochyta tussilaginis*, proved to have high potential as mycoherbicides for biological control of *Cirsium arvense* and *Sonchus arvensis*, respectively. These two plant species from the *Asteraceae* family are the most important perennial weeds worldwide. Both fungi were found to be producers of phytotoxic compounds that could be used as natural herbicides. However, additional research is needed for the practical application of these findings.

For the development of an effective mycoherbicide it is important to elucidate the exact taxonomical position of biocontrol agents and the relationships with other organisms. It is important at least for two reasons: 1) to predict their properties (host range, metabolite production) and 2) to find more easily suitable molecular markers to track the biocontrol organisms after its release in the environment.

Most of the mycoherbicidal agents are hemi- or necrotrophic organisms producing various toxic metabolites that can affect the infection process, influence the virulence level in plant tissues, or help to combat other antagonistic microorganisms. Highly phytotoxic metabolites can be used as 1) natural herbicides, 2) templates for build up of new herbicides, and 3) markers for the selection of the most effective strains.

Objective:

The overall objective of my stay at ISPA was to learn and use different molecular and toxicological techniques in order to better characterize potential mycoherbicides, in particular, *Stagonospora cirsii* and *Ascochyta tussilaginis*. This included several main tasks: 1) to elucidate the taxonomical position of the selected strains by analyzing ITS and β-tubulin DNA sequences with comparison to GenBank data; 2) to compare *S. cirsii* strains isolated from *C. arvense* and *S. arvensis* using AFLP analysis and virulence tests; 3) to compare metabolic and toxicity profiles of different strains of *S. cirsii* and *A. tussilaginis*; 4) to purify phytotoxic extracts obtained from cultures of *S. cirsii* and *A. tussilaginis*.

Activities carried out:

1) Investigation of morphological clues and strain variability of *A. tussilaginis* and *S. cirsii* grown on 3 diagnostic agar media;

2) Production and lyophilisation of mycelium for 40 strains of *A. tussilaginis*, *S. cirsii* and relative species, extraction of DNA, sequencing taxonomically valued genes and phylogenetic data analysis;
3) Amplified length polymorphism (AFLP) analysis of 30 strains of *S. cirsii* strains isolated from *C. arvense* and *S. arvensis*.
4) Growing cultures of 12 strains of *S. cirsii* and 11 strains of *S. cirsii* both on liquid and solid media, production of ethyl acetate extracts from culture liquid and mycelia, respectively. Further evaluation of yields of extractive matter and comparison of their metabolite profiles using high performance thin layer chromatography (HPTLC) with different solvent systems.
5) Assays of biological activities (phytotoxic, antibiotic and zootoxic) of the obtained extracts.
6) Isolation and purification of phytotoxic metabolites produced by *A. tussilaginis* and *S. cirsii* using liquid column chromatography and preparative TLC with simultaneous monitoring of bioactivity of chromatography fractions.
7) Preparation of a manuscript on the molecular and phenotypic variability of *S. cirsii*;
8) Discussions on current and possible future research.

5. Links between visit activity and ENDURE

The research work carried out at ISPA was focused on advanced study on biological control agents and natural products that can be used for the development of ecologically friendly Integrated Pest Management systems. Hence, the program met the aim and the general purposes of ENDURE. Specifically, the work was directed to the development of biological control and natural herbicides against troublesome perennial weeds such as *Cirsium arvense* and *Sonchus arvensis* that are difficult to eradicate with common agricultural practice especially in organic cropping systems. From the scientific point of view, it was linked maximally with ENDURE sub-activity RA4.3 "Exploitation of natural biological processes" within Activity RA4. "Improving the basic understanding of the biology of the crop-pest systems".

Our study on taxonomy of *S. cirsii* strains showed that this fungus should be re-classified as *Stagonosporopsis* or *Phoma* species. This fungus, as well as *A. tussilaginis,* seems to be distant from other well-known pathogens of *Asteraceae* plants such as *Phoma liguicola* (syn. *Ascochyta chrysanthemi*) and *P. exigua*. Ethyl acetate extracts from cultures of both fungi showed high phytotoxic activity and low or none antibiotic and zootoxic activity (Fig. 1-2, Supplement). These data demonstrated good level of safety of these potential bioherbicides. Our work showed high variability in strains of *S. cirsii* and *A. tussilaginis* by molecular and phenotypic markers (Fig. 3-6, Supplement) that could allow the selection of better biocontrol strains and producers of bioactive metabolites.

6. Impact

Added value for the researcher:

- Increased skills in toxicology and molecular genetics
- Huge amount of valuable experimental data obtained within the relatively short period
- Possibility of a continuative collaboration with ISPA
- Exchange of scientific material (data and fungal strains) for the future collaborative research

- Preparation of a draft manuscript (partners from both institutes will be co-authors) for an international peer-reviewed journal
- Experience about organization of research work in the advanced labs of ISPA
- Beautiful memories about Italian life and new people met

Added value for sending partner and hosting partner:

For the sending partner

- The increased scientific level of the research can be planned
- The increased experience of a senior scientists leading some national projects
- It has started to be in touch with European scientific network

For the hosting partner

- Participation in the development of new natural products for weed biocontrol
- Joint manuscripts will be prepared for international peer-reviewed journals.

All of these will promote the establishment of a collaboration between Italian and Russian institutions.

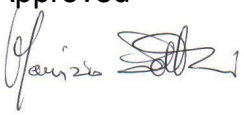
Date of submission

12 May 2010

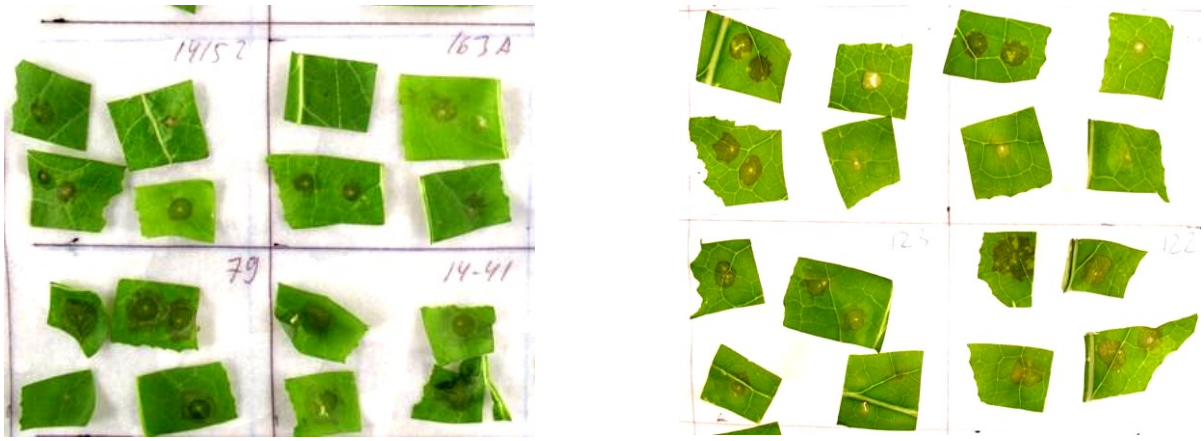
endure
diversifying crop protection

Dr. Maurizio Sattin
IA3 activity leader

Approved

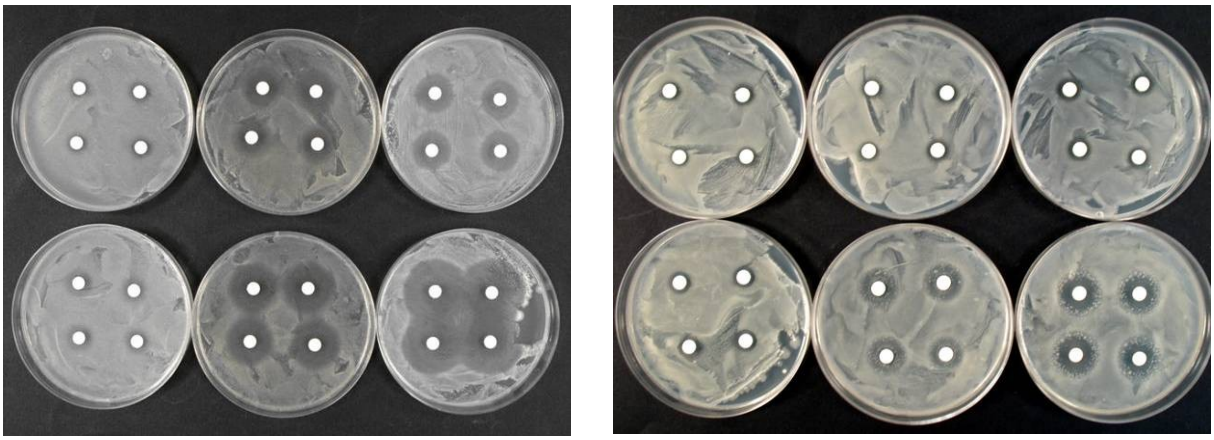


SUPPLEMENT



A **B**

Figure 1. Phytotoxic activity of crude extracts obtained from solid cultures of *Stagonospora cirsii* (A) and *Ascochyta tussilaginis* (B)



A B

Figure 2. Variability of antibiotic activity of crude extracts obtained from solid (upper line) and liquid cultures (lower line) of *Stagonospora cirsii* (A) and *Ascochyta tussilaginis* (B)

A B

Figure 3. Variability in morphology of selected *Stagonospora cirsii* (A) and *Ascochyta tussilaginis* (B) strains on oatmeal agar

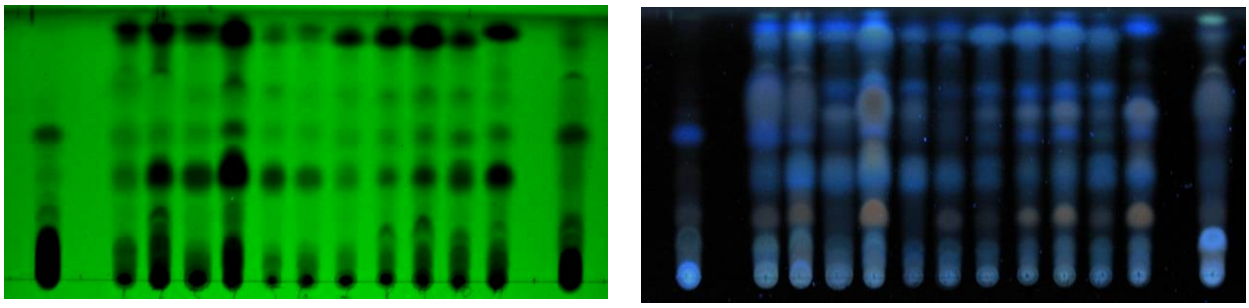


Figure 4. Metabolic profile of the extracts produced from liquid (lanes 2-12) and solid culture (lanes 1 and 13) of different *Stagonospora cirsii* strains visualized by different techniques

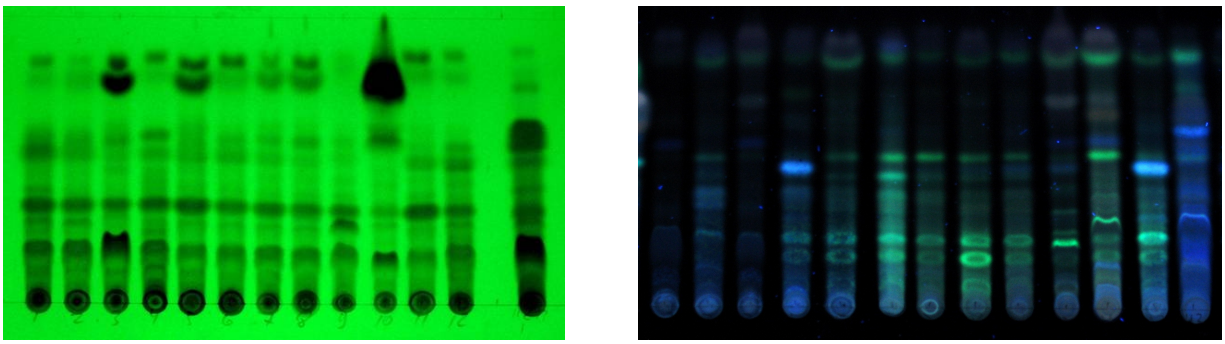


Figure 5. Metabolic profile of the extracts produced from liquid (lanes 1-12) and solid culture (lane13) of different *Ascochyta tussilaginis* strains visualized by different techniques

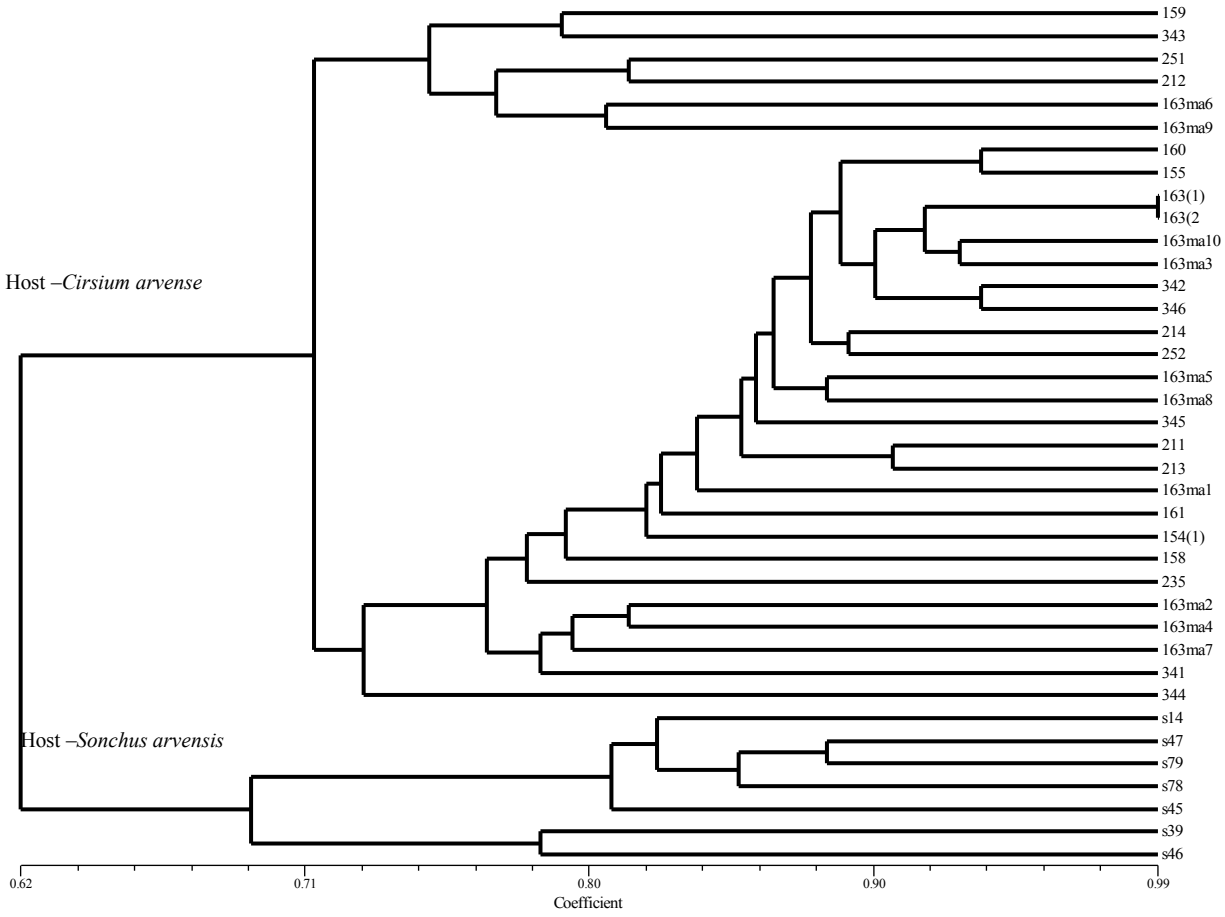


Figure 6. Dendrogram built by NTSYS software for the 22 monoconidial and 8 ascospore isolates of *Stagonospora cirsii* obtained by the unweighted pair group method of AFLP analysis