

IA3 Activity: Human resource exchange

ENDURE - Internal Mobility

Final activity report

- 1- Characterization of Esterase (Enzyme involved in insecticides resistance) in *C. pomonella* in order to find molecular markers to detect resistant insects**
- 2- prospective collaboration on olive fly management**

1. Information about researcher and sending partner

Name and surname:

SIEGWART Myriam

Professional status: (*PhD student, post-doc, junior or senior scientist*)

Junior scientist

Sending partner:

INRA d'Avignon

Institute/Department/Research Unit:

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Supervisor name*:

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Supervisor e-mail*:

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Supervisor phone number*:

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2. Information about hosting partner

Hosting partners:

- University of Modena and Reggio Emilia
- SSSUP of Pisa

Institute/Department/Research Unit:

- Dept. of Agricultural and Food Science
University of Modena and Reggio Emilia
- Land Lab
Scuola Superiore Sant'Anna

Address: *(street, postal code, city)*

- Via Kennedy 17/I 42100 Reggio Emilia Italy
- Piazza Martiri della Libertà 33
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Supervisor name*:

- Stefano CASSANELLI
- Paolo Bàrberi, PhD
Associate Professor in Agronomy & Weed Science
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3. Information about the visit

Duration: *(number of weeks or months)*

1 month

Start date:

17 november 07

End date:

15 dicembre 07

4. Description of the activities and outcomes

Background and context: *maximum 10 lines*

In Reggio Emilia

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is the major insect pest in the orchards of temperate regions. Conventional chemical treatments remain the major mean to maintain low population of the pest. As consequence of these treatments, *C. Pomonella* developed resistances to various groups of synthetic insecticides in Europe. The mechanisms

involved in such forms of resistance include increase in the activity of several metabolic enzymes as cytochrome P450 oxidases, glutathione-S-transferases or Esterases that confer non-specific insecticide resistance. Molecular characterisation of these enzymes could be very useful in the monitoring and the management of insecticides resistance.

In Pisa

Paolo BARBERI's Team is working on functional biodiversity as related to weed/insect/other biota interactions at field and landscape scale, development of DSS and web-based systems for the monitoring and control of the olive fly (*Bactrocera oleae*). In Avignon, we have a similar project on the influence of landscape and of the spatial distribution of culture practices on population dynamic of orchard pests.

Objective: *maximum 10 lines*

In Reggio Emilia

In order to:

- understand enzymatic detoxification of insecticides for a better management of insecticide resistance
- identify molecular markers, allowing a faster detection of resistant insects

We want to characterize carboxyl-esterase gene family, witch is suspected to be involved in insecticide resistance in *C. pomonella*.

In Pisa

Meet Paolo BARBERI's team to present Avignon's projects and try to begin collaboration.

Activities carried out: *maximum 20 lines*

In Reggio Emilia

First, we wanted to know the genetic code of esterase genes. Carboxyl-esterase is a multi-gene family already described in some insect species. That is why we chose to clone a little part of this gene well conserved in the family. It was carried out by using a couple of degenerated primers designed on data from gene banks. Our matrix (RNA) was extracted from midguts of diapausing larvae of 3 laboratory strains, susceptible, selected for resistance to diflubenzuron, and selected for resistance to azinphos methyl. After a reverse transcription, we managed to amplify a piece of DNA (170 pb). We cloned it on a commercial vector, and sequenced it. We found two different sequences witch correspond to esterase after an in silico verification. I started to sequence other different clones after RFLP screening. The objective is to obtain a maximum number of members of this multi gene family.

In an other part, we analyzed the polymorphism of esterases of susceptible and resistant individuals using native gel and iso-electric focusing migrations. We used different concentrations of Acrylamide to optimize the separation. Finally, we saw differences related to strains and tissues (midgut and fat body). In the same time, iso-electric focusing gave us new profiles of esterases. We clearly saw two additional bands in resistant individuals compared to susceptible ones. The same observation was made in proteins from midgut or from fat body. It reinforces the hypothesis of different esterase forms in susceptible and resistant individuals.

In Pisa

We have found a common project: testing insecticide resistance of olive fly samples from Toscana, to evaluate the frequency and the magnitude of resistance of this pest, well known to have current target mutation on Acetylcholine esterase.

Actually, a comparative study of insecticide resistance in French and Greek populations of codling moth is carried out on the occasion of a collaboration (project PLATON) between Dr Benoit Sauphanor and Professor John Tsitsipis's teams. This researcher is specialised in

pesticide resistance in olive fruit fly. This will be the occasion to create a link between these three labs.

5. Links between visit activity and ENDURE

Describe links and relevance of your visit in relation to a specific ENDURE activity(ies) and sub-activity(ies) – maximum 15 lines

Objective of my visit in the University of Reggio Emilia and my visit in the Scuola Superiore Sant'Anna in Pisa exactly correspond to the ENDURE sub-activity:
RA4.1 Pesticide resistance management. More precisely, the goal of these training workshops is detection and management of pesticide resistance.

6. Impact

Added value for the researcher: *maximum 10 lines*

In Reggio Emilia

This training workshop learned me new molecular biology techniques. New interesting approaches on esterases, which are essential enzymes in mechanisms of insecticide resistance will thus be implemented in Avignon. By cloning of the other esterase genes, we could compare the sequences and find specific enzymes involved in pesticide detoxification and define molecular markers for detection of resistance in natural populations of the pest. Starting with known sequences considerably facilitates this work.

In Pisa

Extend relation between our team and other countries. Elaborate new collaborations to get extended pest samples from all over the world. (cf collaboration with Dr John Tsitsipis)

Added value for sending partner and hosting partner: *maximum 10 lines*

In Reggio Emilia

The University of Modena and Reggio Emilia has performed a study on fixed resistant strains of *Cydia pomonella* thanks to this exchange. It has obtained results on esterases on this common project.

In Pisa

Start a new collaboration, estimating the degree of resistance in olive fly population in this region, and add this parameter to their forecasting models.

Date of submission :

20/12/2007



Dr. Maurizio Sattin
IA3 activity leader

Approved