REPORT of the SHORT TERM SCIENTIFIC MISSION, COST FA1208-ID n°COST-STSM-ECOST-STSM-FA1208-300414-041372

Title: FLIM-FRET training and experiments
Start date: 12/05/2014 **End date:** 23/05/2014

Applicant: JAOUANNET Maëlle Flora

Host:

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Background:

Aphids are among the most devastating sap-feeding insects of plants. Like most plant parasites, aphids require intimate associations with their host plants to gain access to nutrients. Aphid feeding induces responses such as clogging of phloem sieve elements and callose formation, which are suppressed by unknown molecules, probably proteins, in aphid saliva. Therefore, it is likely that aphids, like plant pathogens, deliver proteins (effectors) inside their hosts to modulate host cell processes, suppress plant defenses, and promote infestation.

My current efforts are aimed at characterizing the activities of one aphid effector, MpSecA, that has been previously identified in the secretome of *Myzus persicae*. The functional analysis of MpSecA involves the identification of host proteins targeted by this effector and the elucidation of its molecular action within plant cells.

A yeast two-hybrid screen using a potato cDNA library identified StB as a potential methyltransferase. MpsecA interacting,candidate. Moreover, this protein interaction was additionally confirmed in co-immonoprecipitation assays with *in planta* expressed proteins.

Objectives/ Purpose of the visit:

A short-term scientific stay at the Toulouse Imaging Facility and the Laboratory of Plant-Microbe Interactions in Toulouse allowed me to conduct quantitative non-invasive Fluorescence Lifetime Imaging (FLIM) and monitor the Förster Resonance Energy Transfer (FRET) in order to obtain further proof of the physical interaction between MpSecA and StB in living plant cells. This project is part of collaboration with Dr S. Rivas and Dr L. Deslandes, who have extended experience in microscopy methods and functional analysis of effectors.

Description of the work

FLIM-FRET was measured between the GFP (donor) and RFP (acceptor) molecules fused to StB and MpSecA, respectively. If the two proteins interact, the transfer of energy from the GFP to the RFP decreases the fluorescence lifetime of the GFP fluorophore. In addition, the physical interaction between MpSecA and AtBa and AtBb, the Arabidopsis orthologs of StB, was also investigated. As a specificity control, the fluorescence lifetime of StB-GFP coexpressed with free RFP or with an additional cytoplasmic effector, MpSecB, fused to RFP, have also been measured.

Main results:

Specific reduction of the GFP lifetime of GFP-StB, as well as of its Arabidopsis orthologs AtBa and AtBb fused to GFP, was detected in the presence of RFP-MpSecA thereby confirming the physical interaction between MpSecA its host targets *in vivo*.

Beyond the experimental results obtained during this short-term stay, this training has provided me with the opportunity of testing and getting familiar with two different FLIM-FRET systems. One of these systems is the same available at the James Hutton Institute. The use of FLIM-FRET to study protein-protein interactions has been discussed several times in my laboratory but, due to the lack of appropriate expertise, we have been unable to use it up until now. This training opportunity in Toulouse will not only allow me to implement this technique for my own research project but also to transfer knowledge to other members of my group, and to staff at the James Hutton Institute and University of Dundee.

Projected publications/articles related to or resulting from the STSM:

A publication is in preparation and we hope that the manuscript will be submitted before the end of the year.