REPORT of the SHORT TERM SCIENTIFIC MISSION, COST FA1208-ID n° COST-STSM-ECOST-STSM-FA1208-170916-078885

Title: Sustainable resistance to downy mildew in Sunflower crop: Localization and action of downy mildew conserved RXLR effectors in sunflower cells

Start date: 2016-09-17 **End date:** 2016-10-02

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Host: Dr. Tolga Bozkurt, Imperial College, London (UK)

Background:

Sunflower downy mildew is caused by the pathogen *Plasmopara halstedii*, an obligate biotrophic oomycete belonging to the Peronosporales and has a great economic impact on sunflower crop (Gascuel *et al.*, 2015). Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers, impair strongly sunflower seed yield. *P. halstedii* is representative of the group of downy mildews in which genomic data is limited to a few species such as *Hyaloperonospora arabidopsidis*, and *Peronospora tabacina*, respectively pathogens of model plants *Arabidopsis thaliana* and *Nicotiana benthamiana* (Baxter *et al.*, 2010; Derevnina *et al.*, 2015). A *P. halstedii* genomic sequence was published recently (Sharma *et al.*, 2015).

P. halstedii is probably a highly adaptable species as indicated by a high level of inter-pathotype polymorphism observed among its predicted effectors (Gascuel *et al.*, 2016) and also by the recent species history leading to the emergence of numerous virulent pathotypes (Ahmed *et al.*, 2012; Pecrix *et al.*, unpublished data). The rapid generation of genetic diversity among effectors is probably linked to the frequent resistance gene breakdown observed in field conditions.

In model oomycetes, two classes of effectors have been shown to be translocated into the host plant, RXLRs and CRNs, but oomycete avirulence genes described so far are RXLRs. For this reason, we chose to concentrate our efforts on the selection of the conserved RXLR effectors among the 16 pathotypes recorded till now in France (at least 6 of which have been recorded worldwide) and to use them in the screening of various resistant sunflower germplasms. The

sunflower resistances recognizing these conserved effectors are at least wide range and should have a better sustainability since directed against conserved effectors still present during the recent evolution of the pathogen and therefore, likely essential to the pathogen. We started to perform the subcellular localization of *P.halstedii* RXLR conserved effectors in sunflower and in *N. benthamiana* cells; these results should help in deciphering their mode of action in sunflower sustainable resistance.

Objectives/ Purpose of the visit:

The objectives of this short term mission are (i) to precisely determine *Pl. halstedii* effector localizations using established subcellular markers in the model plant *N. benthamiana* and benefiting of expertise and tools available in Tolga Bozkurt's group, (ii) to search for similar conserved effectors in another close downy mildew pathogen, *P. tabacina*, and (iii) to discuss opportunities for collaboration with Tolga's group.

Description of the work:

1. Fine tuning subcellular localization in *N.benthamiana* of *Plasmopara halstedii* RXLR effectors

- Co-localizations of 6 different *P.halstedii* YFP-effector fusions putatively localized to cell plasma membrane or in cell vesicles, in non –infected *N.benthamiana* cells and in haustoriated cells infected by *Peronospora tabacina*, with appropriate Ds-Red markers.

2. Expertise in localization analyses based on our previous experiments in sunflower

3. Search for conserved effectors between the two downy mildew agents *Pl. halstedii* and *P. tabacina*: by blast analyses, with files provided by Tolga Bozkurt.

Main results:

1. The first experiment was done with 4 *P.halstedii* YFP-effector fusions that we putatively localized to the plasma membrane of sunflower and in *Nicotiana benthamiana* cells. To confirm or not this localization, we used the RFP-StRem1.3 fusion, that labels plasma membrane of the *N. benthamiana* plant cell, and in particular the plasma membrane that surrounds the oomycete haustoria, allowing localization of haustoria in infected cells (Bozkurt *et al*., 2014). We performed Agroinfiltration of our 4 constructs carrying YFP-effector fusions (C3, C9, C11, and C30) together with the RFP-StRem1.3 fusion in *N. benthamiana* plants, and after 2 hours we infected the agroinfiltrated leaves with spores of *P.tabacina* collected from inoculated *N.benthamiana* plants. Three days post infection we could localize YFP fluorescence corresponding to the *P.halstedii* effector (yellow) and RFP fluorescence (red) corresponding to the appropriate marker used (**Fig 1, A-D**).

We also did another experiment with the C6 and C26 *P.halstedii* RXLR YFP fusions, together with a RFP-Golgi marker and the RFP-StRem1.3, respectively (**Fig1, E-F**).

Using the Leica Confocal imaging facilities of Imperial College, and a 63X objective, we could localize the C3 effector in cytoplasm and nucleocytoplasm, the C9, C11 and the C30 effectors clearly to the plasma membrane, and for some of them to the nucleus. The C6 effector does not seem to co-localize with the RFP Golgi marker that labeled little vesicles in red, and localizes to cytoplasm and unknown vesicles, and the C26 effector that we thought expressed in the plasma membrane appeared to be nucleocytoplasmic, because it doesn't co-localize with the RFP-StRem1.3.

Three, five and seven days post infection, we did several attempts to observe in the agroinfiltrated inoculated leaves *P.tabacina* infection and presence of haustoria. Unfortunately, no infection was visible, probably because infection success is decreased in agroinfiltrated leaves due to general plant defense reactions. Anyway, Tolga showed me several confocal pictures and videos of leaves with haustoria they did earlier, to be able to recognize them in future experiments.

Figure 1: Confocal images showing the localizations in *N. benthamiana* cells of *P. halstedii* effectors C3 (A), C9 (B), C11 (C), C30 (D), C6 (E) and C26 (F) labeled with YFP (yellow) together with RFP-StRem1.3 (red) (A-D and F) or with the RFP-Golgi marker (E). Autofluorescence of chlorophyll in chloroplasts appears in blue.

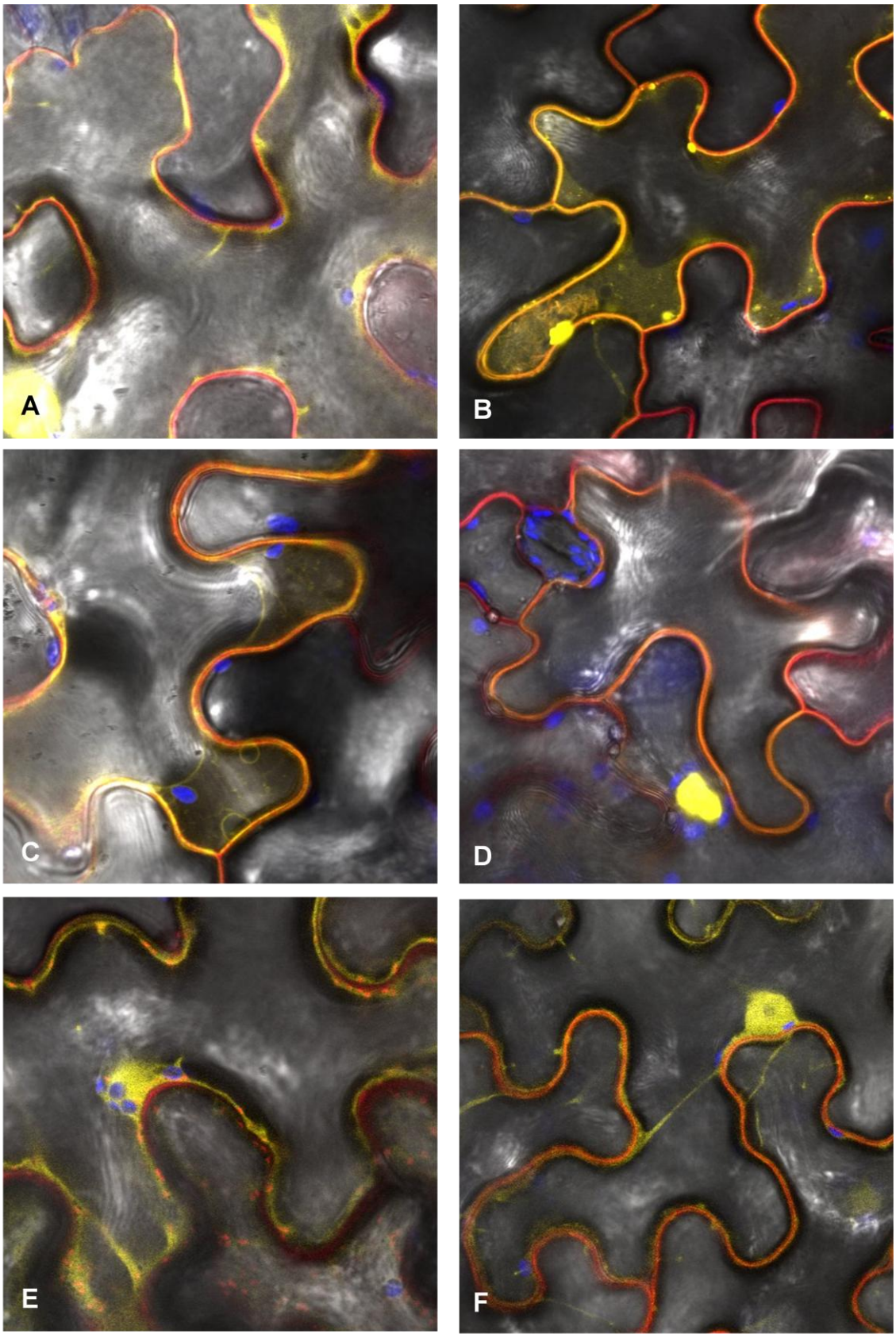


Figure 1

2. We also get through all the confocal pictures we did in Toulouse to discuss with Tolga possible localizations of the 24 other effectors of *P. halstedii*.

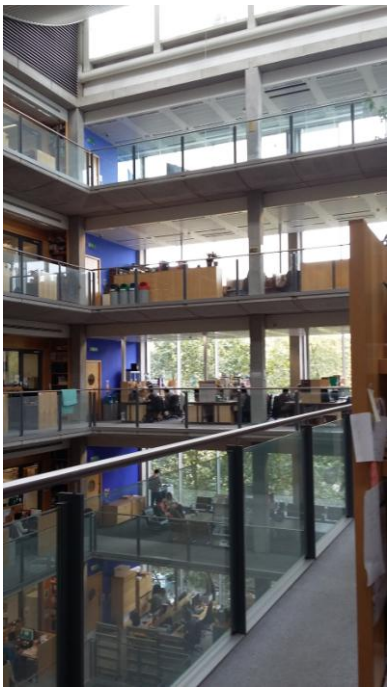
3. In the time left between agroinfiltrations and confocal analyses, I looked for homologies of our 30 conserved effectors to other oomycetes, in particular to *P. tabacina*. I also did blastp searches in nr non redundant sequences of NCBI in order to find the best homologs either in the sequenced *P.halstedii* strain (Sharma et al., 2015) or in all other organisms. Only 4 *P.halstedii* conserved effectors gave a blastp hit (e-value < 0.001) with *P.tabacina* RXLRs (Derevnina et al., 2015), (the maximal hit score was 3E-49 with 50% identity between both proteins) suggesting that the majority of RXLRs are specific to each of these both biotrophic oomycetes. Blastp analyses on nr database gave of course major hits on the proteins predicted from *P. halstedii* sequenced genes, however we still have 4 proteins that were not present in the published sequenced genome and 8 hypothetical proteins that were not predicted as RXLR. The other blastp best hits were found with other oomycete predicted proteins, 8 *P. halstedii* RXLR having homology (e-value <1. 10-5) with hypothetical proteins from *Phytophthora parasitica* and 2 others with putative secreted RxLR effector proteins from *Phytophthora infestans*. The 20 others RXLR are specific to *P. halstedii*.

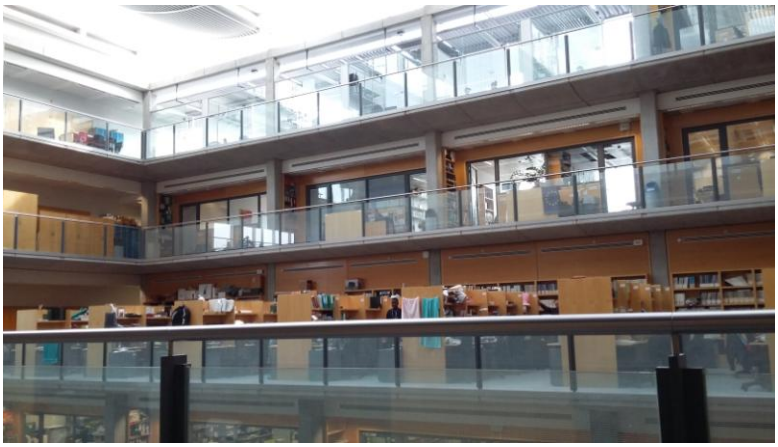
Future collaboration with the host institution (if applicable):

We discussed with Dr Bozkurt of possible collaborations on some *P. halstedii* effectors, whose localization could be of interest for them. I also took the opportunity to be in England to meet research principal investigators, to discuss my research projects (Dr Murray Grant, Pr John Mansfield and Pr. Pietro Spanu).

During my stay, I gave a seminar to the lab of Tolga Bozkurt and Pietro Spanu.

Here are two pictures taken in the Sir Alexander Fleming Building for Life Sciences, at Imperial College.





- Projected publications/articles related to or resulting from the STSM:
  To be discussed later on.

- Confirmation by the host institution of the successful execution of the STSM (this must consist of a signed letter from the host institution):
  See attached latter

Other comments (if any):

This was for me a very fruitful visit, both on the research side and on the linguistic side, *i.e.* a complete immersion in the English language for 2 weeks. This should also be taken into consideration for the attribution of STSM grants to non –english natives willing to improve their English level, it is such an important part of our scientific work.