COST STSM Reference number: WG4
Period: December 2010, 4 weeks
COST action: FA0807
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STSM Topic: Study the fitness of the chrisantemum yellows phytoplasma vector, *Macrosteles quadripunctulatus*, on SAP11 expressing *Arabidopsis thaliana* and verify the possibility of producing a cDNA library from leafhopper gut.

Background

Various investigations have shown that membrane and secreted proteins of phytoplasmas directly interact with host cell factors and hence probably mediate specific interactions with plant and insect hosts. One class of membrane-associated proteins are immunodominant membrane proteins, which were shown to immunoprecipitate with insect gut proteins. In addition, the lab of dr S. Hogenhout (JIC) identified the secreted AY-WB protein SAP11, which targets plant cell nuclei (Bai et al., 2009) and interacts with plant transcription factors that are involved in plant development and regulation of Lox genes of the jasmonate (JA) synthesis pathway (Sugio et al. 2010). *Macrosteles quadrilineatus* fecundity is improved on SAP11-expressing lines of *A. thaliana* that also produce less JA and lox2-silenced lines (Sugio et al., 2010). Thus, SAP11 plays a role in the improved fecundity of this leafhopper on AY-WB-infected plants (Sugio et al. 2010).

Purpose of the visit

We plan to further investigate the roles of the membrane and secreted phytoplasma proteins in determining host specificity through the study of *M. quadripunctulatus* fecundity on AY-WB-infected and SAP11-expressing *A. thaliana* plants in order to determine whether *M. quadripunctulatus* can vector AY-WB and whether SAP11 activity is specific to *M. quadrilineatus* (the vector of AY-WB) or also other *Macrosteles* vector species.

Work carried out during the visit

Establishment of a *M. quadripunctulatus colony.*
To start a new colony of the phytoplasma vector *M. quadripunctulatus*, female adults were caged on oat plants for an oviposition period of one week in Torino laboratory. After insect removal, the plants were sent to the JIC in Norwich. Upon arrival, they were transferred to a growth chamber at 24 °C, 16:8 L:D. The colony was maintained by caging adults on potted oat plants for one week oviposition periods and maintaining rearing plants in the growth chamber until adults of the new generation emerged.
Transmission of aster yellows witches'broom phytoplasma by *M. quadripunctulatus*.
To determine whether *M. quadripunctulatus* can vector aster yellows witches' broom strain (AY-WB) of the '*Candidatus* Phytoplasma asteris', healthy nymphs from the previously established colony were allowed to feed on AY-WB infected *Arabidopsis thaliana* plants for one week. They were then transferred on healthy oat plats for two weeks to complete the latency period, after which males only were transferred on healthy *A. thaliana* plants for

an inoculation access period of three days. After this period, the insects were removed and stored at -20°C, while the plants were drench treated with thiamethoxam (5 mg a.i./plant) and kept in the greenhouse (day/light) to monitor symptom appearance.

Fitness of *M. quadripunctulatus* on SAP11 transgenic *Arabidopsis thaliana* plants.
The secreted AY-WB protein SAP11, which targets plant cell nuclei (Bai et al., 2009) and interacts with plant transcription factors (Sugio et al. 2010), has been already identified by the lab of Dr S. Hogenhout (JIC). In order to determine whether SAP11 activity is specific to *M. quadrilineatus* (the natural vector of AY-WB) or also other *Macrosteles* vectors, we evaluated the fecundity of *M. quadripunctulatus* on SAP11 expressing and AY-WB infected *A. thaliana* plants. Upon symptom development (two weeks after leafhopper inoculation) AY-WB infected SAP11 *A. thaliana* plants, were exposed to healthy ovipositing females together with males of *M. quadripunctulatus* and then determine the number of nymphs 2-3 weeks later. The same procedure was followed for *M. quadripunctulatus* on isogenic *A. thaliana*. SAP11 and isogenic *A. thaliana* plants were maintained in the climatic chamber until egg hatching and nymph counting. The mean number of nymphs per female per day was calculated by dividing the total number of progeny nymphs (obtained in each oviposition period) by the total number of female/days of life (D'Amelio et al., 2008).

During the stage, the possibility of sequencing the transcriptome of *M. quadripunctulatus* midguts in order to identify proteins of this leafhopper that directly interact with CY Amp has been discussed. Mass spectrometry data of peptides of proteins that immunoprecipitated with CY Amp have already been obtained. However, the identities of majority of these peptides are unknown because of lack of sequence data. Transcriptome sequences will allow identification of these peptides and subsequent identification of candidates that act as receptors for CY Amp. Following dissection of *M. quadripunctulatus* adults and RNA extraction from leafhopper midguts, RNA yield and quality has been evaluated in order to set up the proper protocols for obtaining a cDNA library, that will likely represents a further work step towards the understanding of phytoplasma-vector interactions.

Experiments are currently in progress and results are expected in the next future.

REFERENCES

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