Project Proposal

- **Background**

Pathogenicity of most *Xanthomonas* relies on the efficient translocation of so-called effector proteins (T3E) by the type 3 secretion system directly into the plant cell. T3E are known to interfere with plant immunity and physiology to promote bacterial pathogenicity. Yet, the T3E are usually non-essential for pathogenicity taken individually. The effectome i.e., the complete repertoire of T3E, is classically composed of 15-40 T3E in *Xanthomonas* which is collectively essential for pathogenicity. Understanding the behaviour of the effectome is thus complex and most studies consist in the analysis of individual T3E. We currently know the biological targets for only few *Xanthomonas* T3E.

The archetypal T3E target is the basal plant immunity also known as PTI (PAMP-triggered immunity). PTI is a generic immune response to conserved microbial molecular patterns (PAMPs) such as the flagellin peptide flg22. While some T3E interfere with signal transduction downstream of PAMP receptors, others modulate plant physiology, transcriptome, vesicular trafficking, autophagy or proteasome activity. My group, with others has recently shown the importance of autophagy and proteasome activity for plant immunity against *Pseudomonas syringae* and *X. euvesicatoria* in Arabidopsis and pepper (Üstün et al., 2016; Üstün et al., 2018; Üstün et al., 2013).

X. campestris pv. *campestris* (*Xcc*) is a vascular pathogen and the causal agent of black rot disease of Brassicaceae of both economic and academic importance such as *B. oleracea* and Arabidopsis. Pathogenicity of *Xcc* relies in part on its T3SS and 28 T3E candidates as known for the reference strain 8004. In collaboration with the Noël group (LIPM, Toulouse, France, www.xantho.fr), we have obtained *Agrobacterium* strains to transiently express the 28 individual T3E *in planta* and transgenic Arabidopsis plants expressing 20 of the 28 T3E genes under an inducible promoter.

- **Scientific question**

In the frame of the STSM project, we wish to identify which of the T3E from *Xcc* interfere with autophagy/proteasome pathways or PTI in order to functionally characterize the *Xcc* effectome in a systematic manner.

- **Planned activities**

The objective is to screen for the impact of Xcc T3E expression in both transient expression in a nonhost context (*N. benthamiana*) and stable expression in transgenic host plants (Arabidopsis).

<u>Screening for T3E interference on autophagy and proteasome activity in *N. benthamiana*</u>

1) We will start analyzing T3E-dependent modulation of autophagy using our quantitative autophagy assay. This assay is based on *Agrobacterium*-mediated transient expression of *Renilla* luciferase

(RLUC) fused to ATG8a together with free Firefly luciferase (FLUC) in *N. benthamiana* leaves. RLUC-ATG8a is incorporated to autophagosomes and targeted for degradation, while cytoplasmic FLUC serves as internal reference for the expression level (Üstün et al., 2018). Further validation of T3Es and their involvement in autophagy will be carried out by using biochemical methods looking into the degradation rate of known autophagy marker proteins (e.g. ATG8 and NBR1 see WP1) after transient expression of the effectors in *N. benthamiana* or in transgenic Arabidopsis lines that express individual T3Es under an inducible promoter. 2) In a similar approach we will assess whether T3Es alter proteasome activity in plants. Relative proteasome activity in total protein extracts will be determined by monitoring the breakdown of the fluorogenic peptide suc-LLVY-AMC after transient expression of T3Es in *N. benthamiana* or in transgenic Arabidopsis plants (Üstün & Börnke, 2017).

Screening for T3E-mediated PTI suppression in *N. benthamiana* and Arabidopsis measured by ROS production and MAPK activation in response to flg22

We have previously established that T3Es from *X. euvesicatoria* are able to suppress PTI (Üstün et al., 2013, our unpublished data). Thus, it is likely that *Xcc* T3Es use similar mechanisms to dampen plant immunity. Our lab has established the analysis of different PTI responses and performs these assays routinely. Given that, we plan to monitor PTI responses (ROS burst, MAPK activation) after transient expression of T3Es in *N. benthamiana* and/or using the transgenic Arabidopsis plants.

The proposed work program should occupy two full months in order to conduct both PTI and Autophagy/proteasome assays and to be able to reproduce them at least three times for all effectors in *N.benthamiana* and *Arabidopsis* transgenic lines.

The trainee will also attend ZMBP meetings and is expected to present his work in the group meeting.

- **Expected results**

List of *Xcc* effectors that interfere *in planta* with autophagy, proteasomal function and PTI in *N. benthamiana* and/or Arabidopsis.

- **Planned period of time**

2 full months: october-november 2020

- **Cost estimates for subsistence of the STSM grantee**

Where: Tübingen, Germany

Accomodation (october-november 2020): 1000€/month = 2000€

Meals 20€/day: 1200 euros

Travel expenses: 600€

Total requested budget: 3800€