

## SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

**Action number: CA16107**

**STSM title: Exploring the Naturalis Biodiversity Center herbarium for *Xanthomonas* symptoms on Rutaceae and Euphorbiaceae in order to reconstruct the evolution and emergence of diseases on Citrus and Cassava**

**STSM start and end date: 12/11/2017 to 24/11/2017**

**Grantee name: Dr Lionel Gagnevin**

### PURPOSE OF THE STSM

The objectives of this short term scientific mission were to assess the existence of traces of ancient diseases on plant specimens collected for botanical purposes and conserved in herbaria and, if possible, sample them for genetic analyses. The different herbaria in the Netherlands were regrouped in the recent years to constitute the “Dutch Herbaria” collections at the Naturalis Biodiversity Center in Leiden. It contains the former herbaria of Leiden, Wageningen, Utrecht and Amsterdam as well as historical collections. It is now the fifth largest herbarium in the world with almost 7,000,000 specimens. In addition to being exceptionally rich this herbarium hosts collections that complement those of other collections we have prospected before on a botanical point of view as well as on a geographical point of view. For example, it is believed that the Malay-Indonesian area is the area where Citrus canker may have emerged and/or diversified but this area is little represented in other collections for historical colonial reasons. When symptoms of the corresponding diseases are present, a small piece of the specimen is excised with the agreement of the curator for future genetic analyses.

The mission focused on all Citrus species which may harbour symptoms of bacterial canker (*Xanthomonas citri* pv. *citri*) and on cassava (*Manihot esculenta*) which may harbour symptoms of vascular bacteriosis (*Xanthomonas phaseoli* pv. *manihotis*), which are both models for the studies concerning interactions with the plant, evolution of bacterial pathogens, the mechanism of disease emergence, etc., and for which we already have collected ancient samples from 9 other herbaria in Paris, London, Bogota, Washington, New-York, Mauritius and Cape-Town.

The results of the genetic analyses from these samples will help enrich evolutionary and epidemiological models to predict disease emergence, as well as provide reliable past time-points for an absolute datation of evolutionary events.

### DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

This STSM consisted of 8 days of work at the different locations where the Dutch Herbaria are temporarily stored until a dedicated building is ready for them. The Wageningen collection is gathered in one warehouse, the Leiden, Utrecht and Amsterdam collections are in another. First, an inventory of the available resources was performed using the web-hosted database. Secondly, the targeted specimens were visually inspected one by one for canker or vascular bacteriosis. At least 1155 specimens of Citrus (all species) and 736 specimens of *Manihot* (mostly the species *esculenta*) were inspected. Several “neighboring” genera to Citrus were inspected as well as they might be alternate hosts of the bacterium and they are sometimes mis-identified Citrus species. Specimen with potential

symptoms were put aside and re-evaluated during a second round. Then, with the agreement of the collections' curator, a small sample (usually less than 1cm<sup>2</sup>) is excised and placed in a bag. The appropriate metadata (origin of specimen, date, genus, species and/or cultivar) are recorded for each sample. A label indicating that plant material was removed for pathogen DNA analyses is placed near where the excision was done. When possible the sample is not taken from the specimen itself but from detached leaves conserved in pockets on the plate.

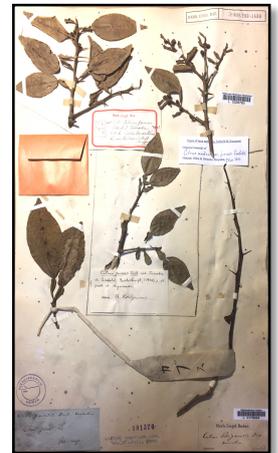
A seminar was given to the scientists of the Naturalis Biodiversity Center exposing the objectives, approaches and first results of the global project.



#### **DESCRIPTION OF THE MAIN RESULTS OBTAINED**

A total of 73 samples from Citrus and other rutaceae, the oldest dating from 1824 were excised. Dr Jan Wieringa, curator, allowed me to sample 4 specimens collected by Philipp von Siebold in Japan in the 1820's (*pictured*), which would be the oldest report of the disease if confirmed, predating the first observation of the disease worldwide in 1899 (in Japan). For Citrus, this number exceeds the usual yield of diseased samples during herbarium explorations, probably because a lot of specimens in the Dutch collection were from the Malayan peninsula and Indonesia where the disease is hypothesised to have emerged.

A total of 17 samples from *Manihot esculenta* and a few other *Manihot* species were excised, the oldest from 1837. In contrast to our previous herbarium exploration this number is low, probably because the Dutch herbaria are not especially oriented towards collections from South-America (except for Suriname) where the disease might have emerged. Nevertheless, the Dutch collections complemented well other collections for cassava specimens from Africa (an alternative hypothesis describes the emergence of vascular blight from Africa).



#### **FUTURE COLLABORATIONS (if applicable)**

One objective of our global project on herbarium resources is to create a link between herbaria curators and scientists working on the preservation of these collections and scientists working on pathogen evolution who might benefit from these resources. An important aspect of this is the availability of data produced after a sampling campaign to other scientists who might need it in the future. Therefore, like with other herbaria, the scientific teams of the Biodiversity Naturalis Center in Leiden will be informed and associated with this process of sharing information and data, as well as the academic output of the project.