COST Action FA0807 Integrated Management of Phytoplasma Epidemics in Different Crop Systems

Short-term Scientific Mission (STSM) Report

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STSM Topic: Multi gene characterization of phytoplasmas from fruit trees in Canada and in other fruit growing Countries

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Purpose of the visit

Since phytoplasmas have been reported in fruit crops in Canada, the aim of this STSM was the comparison of phytoplasma strains infecting fruit trees in Canada with strains from the same species detected in Serbia, Italy and other countries on other genes than 16Sr RNA.

Description of the work

During the visit, several pear and blueberry orchards, vineyards and vegetable crops open fields as well as greenhouses and surrounding areas were surveyed. No phytoplasma related symptoms were observed on vegetable crops in fields while typical phytoplasma related symptoms have been observed in pear orchards and on wild population of mulberry trees. However, some nonspecific symptoms have been also observed on blueberry plants. The surveys were carried out during the first week of the work in order to collect the material before the season was too advanced to recognize symptoms and to collect samples in the best conditions for the processing.

Collected samples were labeled with specific codes and midribs and phloem scrapes were prepared and used for nucleic acid extraction and parts of them were maintained at -80°C.
More samples (22) collected during June/July of 2011 and 2012 from symptomatic peach, nectarine, pear and other fruit trees that were available in the lab as freeze dried material were also employed for the testing to verify phytoplasma presence in order to verify the performance of infected samples collected in different seasons, and maintained under different conditions.

List of the samples collected

Plant species	No of samples	Symptoms	Locality
Pear	12	Reddening of leaves and upward curling	Ruthven
Mulberry	3	Little leaf and leaf malformation	Harrow
Grapevine	4	A specific reddening or yellowing, down word curling of the leaves, paper like texture of leaves	Kingsville
Cirsium arvense	7	Yellowing and stunting	Leamington
Asparagus spp.	4	Witches' broom	Harrow
Blueberry	15	A specific	Colchester
Hydrangea	5	Stunting and reddening of the leaves	Kingsville
Total samples	45		

The newly collected and the freeze dried samples were subjected to DNA extraction using a CTAB protocol (Angelini *et al.*, 2001).
Aliquots of 1 to 2 g were maintained at -80°C or as freeze dried samples in order to assess different extraction methods if needed when multiple gene analyses will be carried out on positive samples.
The extracted DNAs were divided in batches for the parallel analyses in the two laboratories.
A total of 72 samples was prepared, 30 of which were further processed by preliminary PCR and nested PCR assays targeting the 16S rRNA gene to verify phytoplasma presence in samples subjected to DNA extraction. The majority of the samples were positive and RFLP and/or sequencing to verify phytoplasma identity are in progress. Testing of the other processed samples will be carried out in parallel in the two laboratories so results will allow verifying phytoplasma populations present in Canadian fruit trees orchards and comparison between them and those present in the European countries.

Collection of *Cirsium arvense* samples in the border of a vineyard.

Symptoms of possible phytoplasma presence in hydrangea in a nursery located in the fruit trees growing areas surveyed.

Wild mulberry plant with small leaves and witches broom formations.

Pear orchard with clear symptoms of pear decline.

During the STSM I gave a seminar titled “Phytoplasma impact on crops and their influence on food quality” to explain the impact of these diseases in the chain of fruit production. The seminar was presented at the Guelph Food Research Centre, Agriculture and Agri-Food Canada, and it was attended by a number of specialists of the center. Interesting discussion followed the seminar about phytoplasma detection methods and also on possible impact of phytoplasma presence in food, especially fruits.

Conclusion and future collaboration

An informal collaboration was agreed on the full identification and molecular characterization of phytoplasmas detected in all collected samples. Selected phytoplasma strains will be then subjected to a multi gene characterization that will be carried out in Canada and in Serbia in parallel experiments in order to double verify results and to carry out comparison of strains with those present in other European countries on fruit trees. The data will be very useful to implement management of phytoplasma – associated diseases in COST countries as well as in Canada fruit tree orchards and vineyards. Moreover, results will allow strengthening of collaboration between Canada and European countries on phytoplasma research.

References

ANGELINI E., CLAIR D., BORGO M., BERTACCINI A., BOUDONPADIEU E., 2001. Flavescence dorée in France and Italy. Occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder phytoplasma. *Vitis,* 40: 79-86.