

Minutes training school for molecular identification of psyllid vectors Montpellier 2012

Monday, October 1st 2012

Welcome of the participants by Nicolas Sauvion, and overview on the activities of the research unit Biology and Genetics of Plant-pathogen Interactions (BGPI) (<http://umr-bgpi.cirad.fr/index-uk.htm>). Visit of the Center for Biology and Management Populations (CBGP) (<http://www1.montpellier.inra.fr/cbcp/?q=en>), in particular the insect collection (60,000 species ; >1 million specimens) and the library compassing all fields of entomology. Finally, presentation of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) by Philippe Reynaud and visit of its national reference laboratory of Entomology at Montpellier.

Tuesday, October 2nd 2012

Astrid Cruaud and Jean-Yves Rasplus (INRA Researchers, CBGP) gave a comprehensive presentation on the barcoding flowchart, from specimen collection to sequence library validation. During a 3 hours session conceptual aspects and practical problems, in particular, the interest of barcoding as a tool for molecular identification of insects was discussed. The accent was put on the interest (even more the necessity) of a closer collaboration between traditional taxonomists and molecular biologists.

In a first practical session in the afternoon, the attendees were separated in two parallel groups. The first group started with the morphological identification of psyllids using diverse identification keys taught by the trainers David Ouvrard (NHM London) and Barbara Jarausch (AIPlanta-IPR). The second group, led by Nicolas Sauvion (INRA Researcher, BGPI) and supported by the trainer Wolfgang Jarausch (AIPlanta-IPR) demonstrated the flowchart from field sampling to DNA extraction and the good laboratory practice used in the Montpellier unit (ISO 9001 label) to avoid any risk of contamination between sampling DNA extraction and PCR analysis. Conservation methods of psyllids were discussed: 95 % alcohol at -20°C is the good condition for a long term conservation of samples used for molecular analysis; 70 % alcohol is better for specimens dedicated for morphological identification. Attendees were enabled to identify their own psyllid material (numbering of samples, filling of a datasheet for each sample for the traceability of these samples,...) before starting the process of DNA extraction in plate. Two DNA extraction protocols were detailed: the “classical” CTAB protocol, and a non destructive protocol using a TNES buffer. The second one is used in routine since two years in Nicolas Sauvion’s lab for molecular analysis on adult or nymph psyllids.

Wednesday, October 3rd 2012

Gaël Kergoat (INRA Researcher, CBGP) opened the meeting with an introductory presentation on “Integrative taxonomy in the age of the molecule”. He demonstrated how a multiple-disciplinary approach (biology, traditional taxonomy, molecular phylogeny,...) is useful and even necessary for species delimitation, and at a more general point of view to exploring biodiversity. Jean Peccoud (Post-DOC, INRA Rennes) completed the concept of species, biotypes, races, and ecological speciation in his talk “Host-specialized races in phytophagous insects: cryptic diversity below the species boundary”. In particular, he

reviewed the literature covering speciation through host-plant specialisation in a well-studied model, the pea aphid complex, *Acyrtosiphon pisum*, which encompasses numerous biotypes that parasitize different legume host species worldwide.

After the explanatory morning presentations, the previous concept of two parallel practical sessions were continued during the afternoon, with one part of morphological identification and a second for molecular analysis. Additionally, within a third practical session, Jean Peccoud demonstrated how to analyse sequences from raw data to phylogeny analysis using the Geneious software (<http://www.geneious.com/>), including primer design and blasting of similarity between sequences.

Thursday, October 4th 2012

David Ouvrard gave a presentation on the macroevolutionary history of Psylloidea. He described a revised classification for this insect group based on a very recent publication published together with D. Burckhardt in *Zootaxa* (2012 ; 3509: 1-34), incorporating relevant literature as well as unpublished ongoing morphological and molecular studies of the world fauna. As a resumé: there is no doubt that the Psylloidea constitute a monophyletic group, but at the moment it is difficult to get a clear picture of several taxa within this group. For example, the Triozidae is a species-rich, probably monophyletic family. In contrast, most of the genera are ill-defined and artificial, and the phylogenetic relationships between genera remain largely unknown. The Psyllinae seem less problematic; subdivisions into subfamilies are supported by convincing characters, but again the phylogenetic relationships between genera remain often unknown.

In a conclusive presentation, Nicolas Sauvion described in detail the literature concerning the psyllid vectors of Apple Proliferation (AP), Pear Decline (PD) and European Stone Fruit Yellows (ESFY). He noticed that almost all the epidemiological studies are focused on a species and a country, and rarely take into account the question at the pertinent scale (i.e. distribution area of the disease and/or vector). The problem of the ambiguous term “putative vector” was also debated. Within the forum (attendees, trainers) the questions were raised: at an epidemiological point of view, is it necessary to take into account only true vectors (i.e. for which the capacity to transmit was proved), or is it also necessary to take into account potential vectors (e.g. *C. pyricola* for PD in Austria)? All the discussions of the week showed the necessity of studies at a large scale, and so the interest of a project as the COST for exchanges of materials between laboratories.

In the last practical afternoon session, the attendees participated alternately to one of the three practical sessions: morphological identification, molecular analysis, and sequence analysis.

Friday, October 5th 2012

The workshop was closed with a demonstration of the several important steps of the TNES protocol for DNA extraction, and with a final discussion on the means to answer questions on the epidemiology of diseases transmitted by aerial vectors. For example, Nicolas Sauvion showed possibilities how to determine possible distances and trajectories of dissemination of ‘*Ca. P. prunorum*’ by its psyllid vector *C. pruni* using an approach combining population genetics and molecular epidemiology.

Remark : numerous documents (talks, references, protocols,...) can be downloaded on a website. For more information contact: sauvion@supagro.inra.fr

