EURL mandate on insects and mites: a mandate for 240 regulated organisms!

PHILIPPE REYNAUD – Entomology and Invasive Plants unit
ANSES/ Plant health laboratory
755 avenue du Campus Agropolis CS 30016 34988 Montferrier sur Lez – FRANCE

RICHARD GOTTSBERGER – Institute for Sustainable Plant Production
AGES
Spargelfeldstr. 191, 1226 Wien - AUSTRIA

Workshop for Heads of Plant Pest Diagnostic Laboratories
Maisons-Alfort (FR) - 2019-09-09/11
1/ Insects and mites: general considerations
2/ EURL presentation Anses Montpellier
3/ EURL presentation AGES Vienna
4/ Work programme EURL
5/ Communication
6/ Accreditation issue
The challenge of identifying insects

Too many bugs so little time!

Between 2 millions and 30 millions of arthropods species


Tiny size (microscopic insects and mites that require complex slide mountings and powerful microscopes)

Intra-specific variation

Sibling (cryptic) species

Behavior studies

Harmonia axyridis
European Reference Laboratory for Insects and Mites

Designated under the Commission Implementing Regulation (EU) 2019/530 of 27 March 2019

A consortium between:

- Entomology and Invasive Plants Unit of ANSES Plant Health Laboratory (Montpellier, France)
- Department for Molecular Diagnostics of Plant Diseases of the AGES (Vienna, Austria)
15 km

45 mn
EURL Staff

Philippe Reynaud
- Head of EURL Laboratory
- Thysanoptera
- Acari
- General entomology

Raphaëlle Mouttet
- Deputy Head of EURL Laboratory
- Quality Assurance Manager
- Coleoptera
- DNA barcoding

Valerie Balmes
- Diptera
- Aphididae
- General entomology

Jean-Marie Ramel
- Lepidoptera
- Hymenoptera

Andrea Taddei
- Lepidoptera
- Diptera
- DNA barcoding

Arturo Goldarazena
- Coleoptera
- Thysanoptera
- Acari

X [to be recruited]
- Homoptera
- Coccoidea
- General entomology

And also Aude Chabirand and Géraldine Anthoine
Created in 1996

Located In CBGP (Center for Biology and Management of Populations) since January 2010

[the CBGP : a joint research unit INRA/IRD/CIRAD/AGRO.M) carries out research in the fields of systematics, genetics and ecology relevant to the management of populations and communities of organisms for the purposes of agriculture, public health and biodiversity]

Activities:

• entomology (since 1996)
• invasive plants (since 2008)
Equipment for morphological identification ANSES
Main taxonomic groups covered:

- Thysanoptera
- Hemiptera Sternorrhyncha
- Diptera larvae
- Lepidoptera genitalia
- Coleoptera genitalia
- Acari

- 30 000 tubes (300 000 specimens)
- 15 000 slides
- More than 400 families
- More than 4000 species
Vouchers collection CBGP

1 000 000 specimens
60 000 species of insects
700 species of mites
Specialized bibliography
AGES – Austrian Agency for Health and Food Safety

Data, Facts, Figures

• Founded on June 1, 2002
• Fusion of 18 Federal Agencies and Offices

• Health and Food Safety Act (GESG)
• Republic of Austria
  Owner and Main Contracting Authority

• 1,400 Staff
  • 900,000 samples tested with
  • 7.2 million individual analyses/year
Equipment and laboratory facilities for molecular identification

State of the art equipment and `forward flow` principle

Dedicated PCR working areas

MIC cycler

Capillary electrophoresis

DNA /RNA extraction and purification

Mastermix preparation

Post – PCR (Analysis of amplification products)
- Capillary electrophoresis/gel electrophoresis
- Preparation of PCR amplification products
- Sequence analysis (Geneious)
Quality assurance system of the Molecular Unit - AGES

ISO 17025:2005 accreditation for detection and identification of plant pathogenic pests since 2005

Accreditation of horizontal SOPs on a technical level
✓ Qualitative detection of plant pathogens by endpoint PCR
✓ Qualitative detection of plant pathogens by realtime PCR
  • E.g. identification of *Thrips palmi* by realtime PCR

✓ DNA barcoding for insects submitted for accreditation June 2019

Implementation of new ISO 17025:2017 (re-accreditation October 2019)
EURL – Morphological Unit - AGES

Sylvia Blümel
- Quality assurance
- General entomology

Christa Lethmayer
- Coleoptera
- Diptera
- Homoptera
- General entomology
- Reference collection

Katharina Wechselberger
- Coleoptera
- Lepidoptera
- General entomology

Anna Moyses
- Thysanoptera
- Coleoptera
- Lepidoptera
- General entomology

Gudrun Strauss
- Auchenorrhyncha
- Coleoptera
- General entomology

Alois Egartner
- Diptera (Tephritidae)
- Coleoptera
Equipment & bibliography for morphological identification - AGES

- Stereomicroscopes
- Controlled climate chambers for insect rearing
- Microscopes
- Greenhouse facility and secure chambers

Bibliography:
- Hard copies
- Web based identification keys/tools
- Electronic database
Vouchers and slide collection - AGES

Separate rooms for dry and alcohol collection
Vouchers working collection since 1904
Vouchers for 160 different species (Annexes EU-2000/29)

Main taxonomic groups covered
- Cicadina
- Psyllina,
- Aleyrodina,
- Coccina,
- Aphididae,
- Heteroptera,
- Coleoptera,
- Lepidoptera,
- Thysanoptera
- Diptera
Work Programme
## Priority pests

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>PT morph</th>
<th>PT mol</th>
<th>Work on Method</th>
<th>Build up expertise</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Spodoptera frugiperda</em></td>
<td>EPPO</td>
<td>2019</td>
<td>2019</td>
<td>X</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Epitrix spp.</em></td>
<td>No</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>1</td>
</tr>
<tr>
<td><em>Thrips palmi</em></td>
<td>EPPO</td>
<td>2020</td>
<td>2020</td>
<td>X</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Agrilus planipennis</em></td>
<td>No</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>1-2</td>
</tr>
<tr>
<td><em>Bactrocera zonata</em></td>
<td>EPPO</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>1-2</td>
</tr>
<tr>
<td><em>Bactrocera dorsalis</em> complex</td>
<td>IPPC</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>1-2</td>
</tr>
<tr>
<td><em>Anoplophora chinensis</em></td>
<td>No</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>2</td>
</tr>
<tr>
<td><em>Anoplophora glabripennis</em></td>
<td>No</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>2</td>
</tr>
<tr>
<td><em>Aromia bungii</em></td>
<td>No</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>2</td>
</tr>
<tr>
<td><em>Popillia japonica</em></td>
<td>EPPO</td>
<td>2020</td>
<td></td>
<td>X</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Thaumatotibia leucotreta</em></td>
<td>Soon</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>2</td>
</tr>
<tr>
<td><em>Agrilus anxius</em></td>
<td>No</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>1-2</td>
</tr>
</tbody>
</table>
The functions and duties of the EURL for Insects and Mites are laid in the art. 94 of the Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 as follows:

- **TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.**
  
  (provide reference methods; provide reference materials; organise inter-laboratory comparative testing or proficiency tests; cooperate with NRLs and Commission to develop high standard methods of analysis)

- **TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs**
  
  (Coordinate practical arrangements necessary to apply new methods of laboratory analysis; conduct training courses for staff NRLs; provide information on relevant national, Union and international research activities)

- **TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS**

- **REAGENTS AND REFERENCE COLLECTIONS**

A questionnaire will soon be presented to all EU NRLs in order to collect information on their expertise, facilities and available staff and their expectations for future EURL activities, such as their technical and scientific support needs.
Sub-activity **1.1** Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.

- **Survey**
  - 2019: Initial list of **recommended methods**
  - 2020: **assessment** of candidate methods

Sub-activity **1.2** Providing reference materials to national reference laboratories

- **initial evaluation** of the needs for reference specimens. **First supplies**
  - 2019: Continuation, as a **permanent task**.

Sub-activity **1.3** Coordinating the application of methods

- **2 proficiency tests**
  - 2019
  - 2020: **3 proficiency tests** and subsequent **follow-up actions**

Sub-activity **1.4** develop methods of analysis, testing or diagnosis of high standards

- **studies in progress** for all the target species
  - 2019
  - 2020: **reports available** for *Bactrocera zonata* and *Bactrocera dorsalis complex*. 
Sub-activity 2.1 apply new methods and informing national reference laboratories of advances in this field.

- EURL provides high-quality support and technical support

Sub-activity 2.2 Conducting training courses for staff from national reference laboratories

- Several training courses are organized (total number of participants to be defined according to needs identified in sub-activity 1.1)

Sub-activity 2.3 Providing information on research activities to national reference laboratories.

- establishment of a scientific survey
- Extensive literature study
- participation in international events
- Communication (Website, Workshop...
Sub-activity 3.1 Providing scientific and technical assistance to the Commission within the scope of their mission

- Communication of relevant information to the Commission.
- Provide scientifically based advice to the Commission

Sub-activity 3.2 Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA)

- Permanent task

Sub-activity 3.3 Assisting actively in the diagnosis of outbreaks

- Confirmatory diagnosis results
- Support documents and trainings are provided

Sub-activity 4.1 Verification of the quality of reagents and lots of reagents used for the diagnosis of pests of plants.

- Tests
- List of recommended DNA extraction kits

Sub-activity 4.2 Reference collections of pests of plants and/or reference strains of pathogenic agents

- Start of the EURL Reference collection and Working collection
- Improvement of the collections (permanent task)
Proficiency Test on the identification of *Spodoptera frugiperda*

Identification of *S. frugiperda* based on morphological analysis of male genitalia structure

Identification of *S. frugiperda* by molecular methods

Fig. 63 Male genitalia *Spodoptera frugiperda*. Photos © J. Brambila, USDA-APHIS-PPQ.

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 7/124 (1) *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania*
Proficiency Test on the identification of *Thrips palmi*

Identification of *Thrips palmi* based on morphological analysis of the adult female

Identification of *Thrips palmi* using molecular methods
Identification of *Popilia japonica* based on morphological analysis of the adult
Taxonomical study of the South american species of *Epitrix*

- Very **small** (1,5 mm)
- Many native species in Europe
- But also **invasive species** from South America
- Some are a **potential threat** for European **potato** crops
- Also still a **lot of South american species**
- The diversity of associated to wild and cultivated solanaceae plants is not well known
- The total number of species remain unkown
- Taxonomy is difficult, based in the dissection of genitalias (spermatecs and aedeagus)

1. **Memorandum of Understanding** with Universidad La Molina Peru, Entomological Museum of UNALM and International Potato Center CIP and ANSES

2. **Sampling in Peru**

3. **Morphological studies DNA Barcoding**

**Preparation of the Training Course for EU National Reference Labs**
Communication
Website

- Promotion of EURL activities
  - PTs
  - Training Sessions
  - Workshops
- Updated official protocols and bibliography
- Reference material and approved reagents
- NRLs network and contacts
- And much more …

- [https://eurl-insects-mites.anses.fr](https://eurl-insects-mites.anses.fr)

**EU National Reference Laboratories (NRLs)**

list not available yet !