



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



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**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**

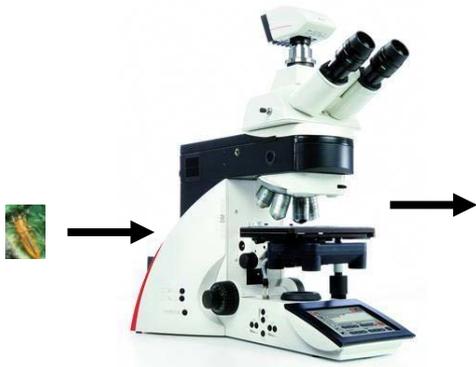
The challenge of identifying insects

Too many bugs so little time!

Between 2 millions and 30 millions of arthropods species

(Erwin, T. L. 1983. Tropical forest canopies: the last biotic frontier. Bulletin of the Entomological Society of America, Volume 29: 14-19)

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

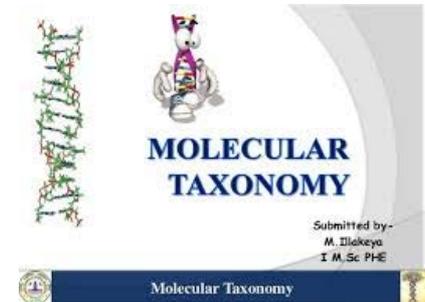
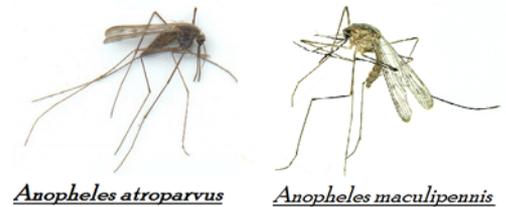


Intra-specific variation



Harmonia axyridis

Sibling (cryptic) species



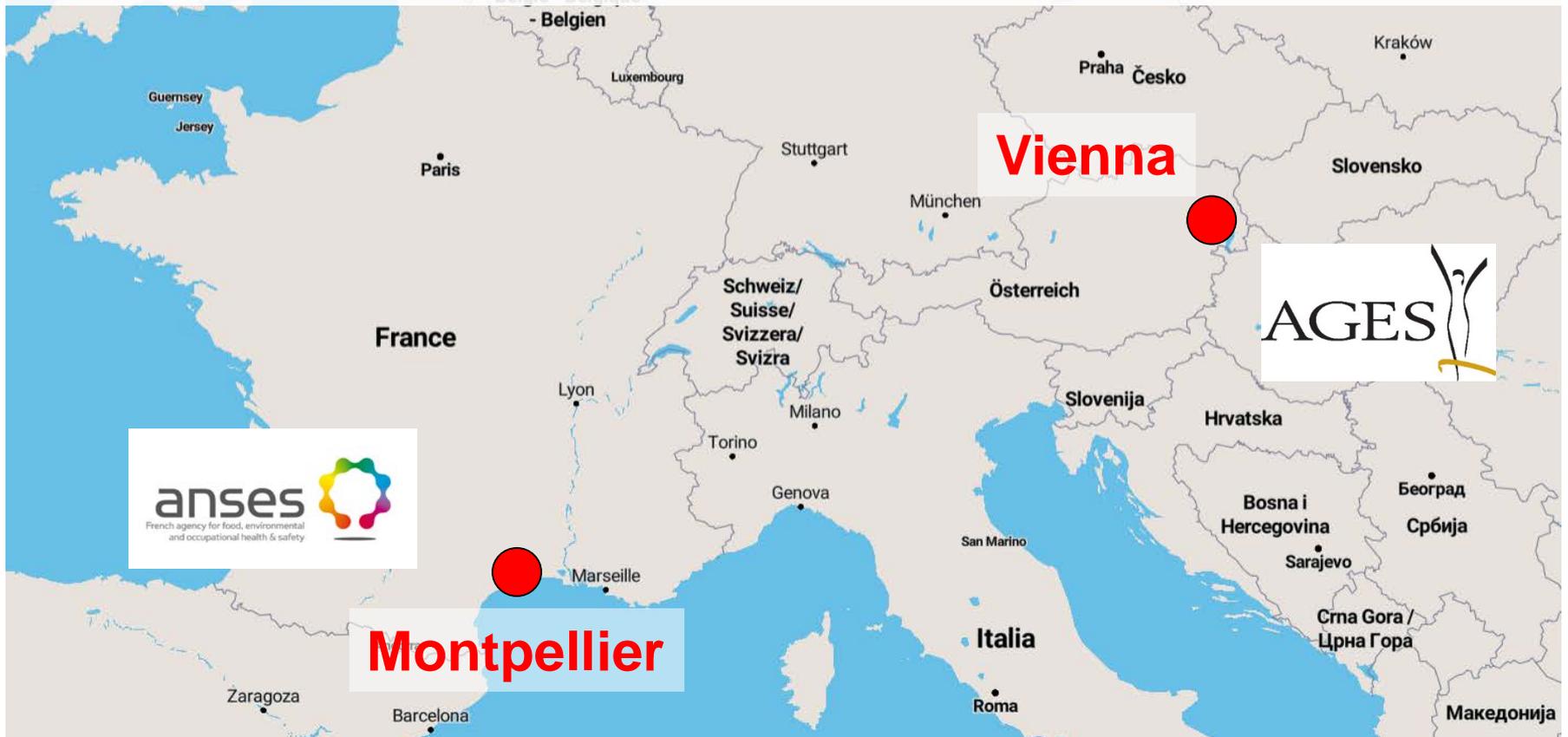


European Reference Laboratory for Insects and Mites

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

A consortium between:

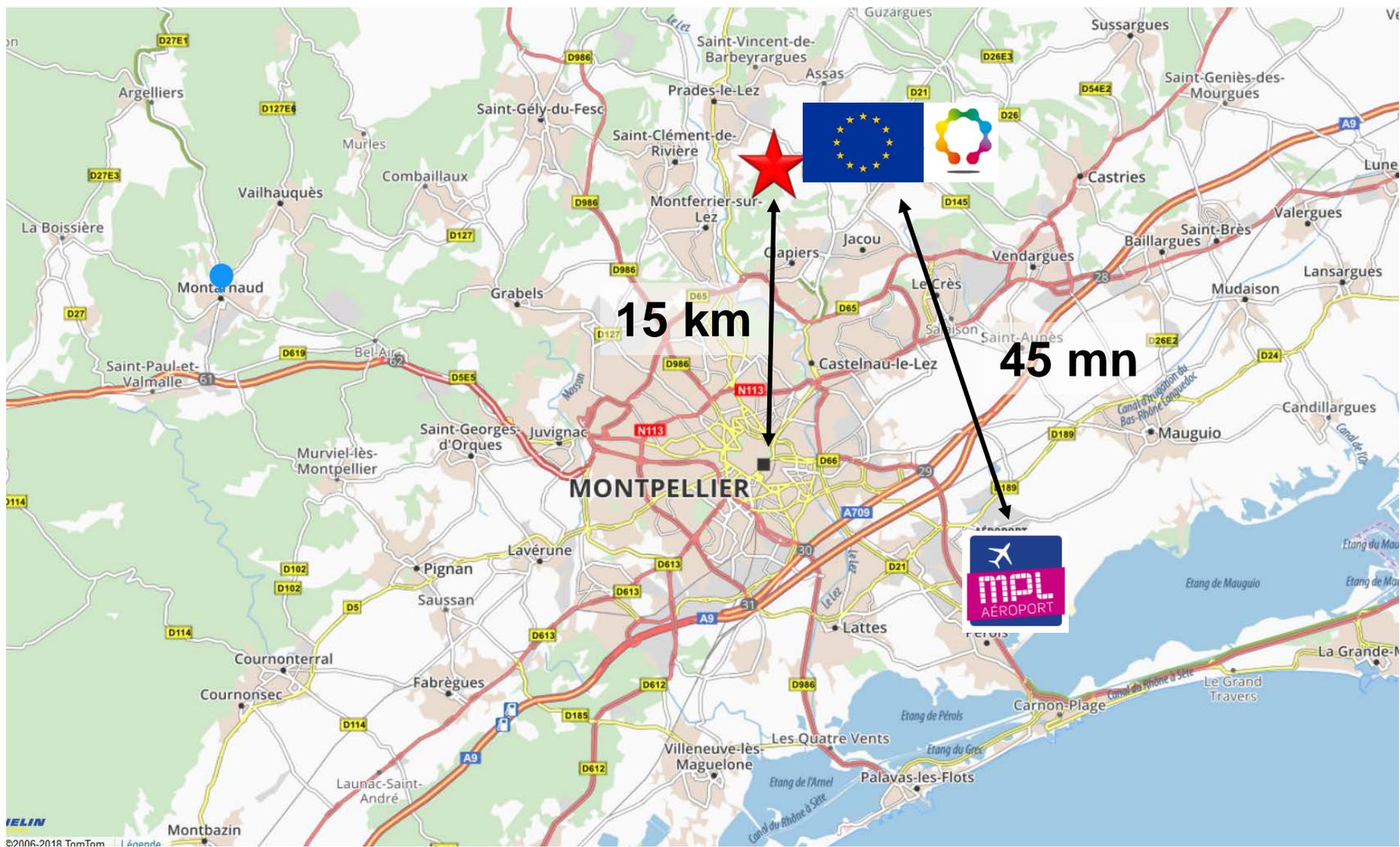
- Entomology and Invasive Plants Unit of ANSES Plant Health Laboratory (Montpellier, France)
- Department for Molecular Diagnostics of Plant Diseases of the AGES (Vienna, Austria)





anses
French agency for food, environmental
and occupational health & safety





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CBGP





EURL Staff



Philippe Reynaud



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



Jean-Marie Ramel



- Lepidoptera
- Hymenoptera



Raphaëlle Mouttet



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



Andrea Taddei



- Lepidoptera
- Diptera
- DNA barcoding



Valerie Balmes



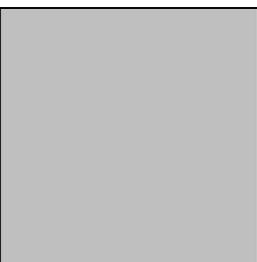
- Diptera
- Aphididae
- General entomology



Arturo Goldarazena



- Coleoptera
- Thysanoptera
- Acari



X [to be recruited]



- Homoptera
- Coccoidea
- General entomology

And also Aude Chabirand and Géraldine Anthoine



EURL Facilities **ANSES**

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]

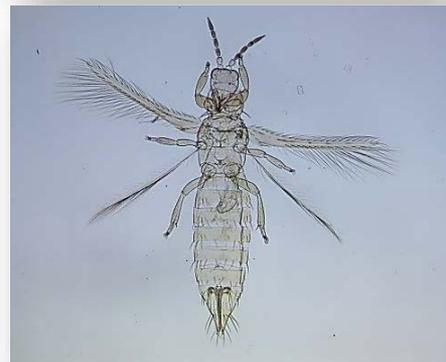
Activities :

- entomology (since 1996)
- invasive plants (since 2008)





Equipment for morphological identification **ANSES**





Slides collections - 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

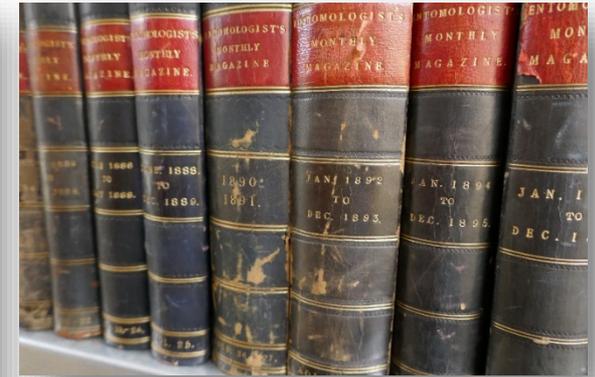


1 000 000 specimens
60 000 species of insects
700 species of mites





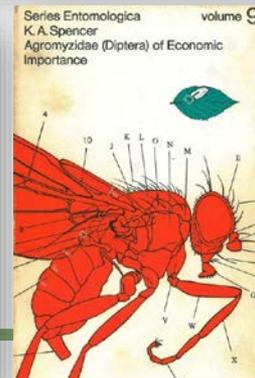
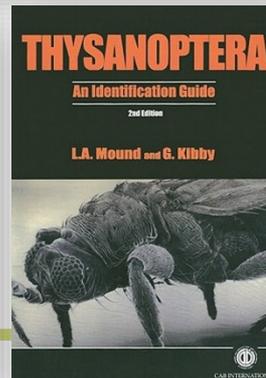
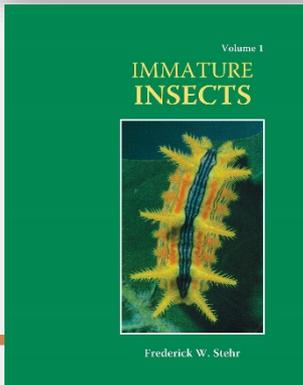
Specialized bibliography



Zoological Record®



ELSEVIER
Scopus





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

 Federal Ministry
Labour, Social Affairs, Health
and Consumer Protection

 Federal Ministry
Republic of Austria
Sustainability and Tourism

- **1,400 Staff**
- 900,000 samples tested with
- 7.2 million individual analyses/year



EURL team AGES





Helga Reisenzein AGES  

- Deputy Head of EURL Laboratory
- Molecular identification by PCR
- DNA barcoding
- General phytoplasmology
- Quality assurance



Richard Gottsberger AGES  

- Molecular identification by PCR
- DNA barcoding
- General bacteriology



Thomas Leichtfried AGES  

- Molecular identification by PCR
- DNA barcoding
- Molecular virology/mycology



Claudia Heiss AGES  

- Technician



Christina Lippitz AGES  

- Technician



Doris Busch AGES  

- Administration

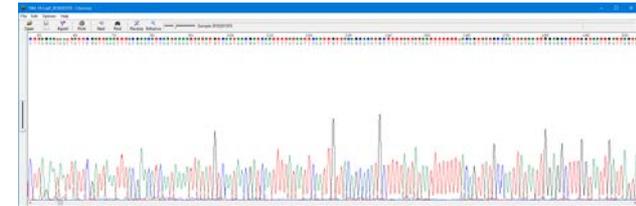
State of the art equipment and `forward flow` principle



MIC cycler



Capillary electrophoresis



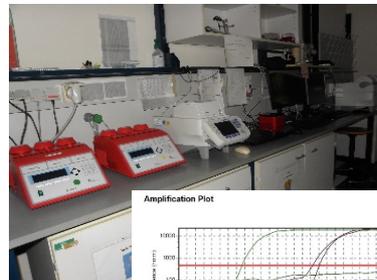
Dedicated PCR working areas



DNA /RNA extraction and purification



Mastermix preparation



PCR



Post – PCR (Analysis of amplification products)

- Capillary electrophoresis/gel electrophoresis
- Preparation of PCR amplification products
- Sequence analysis (Geneious)



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)**



Sylvia Blümel



- Quality assurance
- General entomology



Katharina Wechselberger



- Coleoptera
- Lepidoptera
- General entomology



Christa Lethmayer



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



Anna Moyses



- Thysanoptera
- Coleoptera
- Lepidoptera
- General entomology



Gudrun Strauss



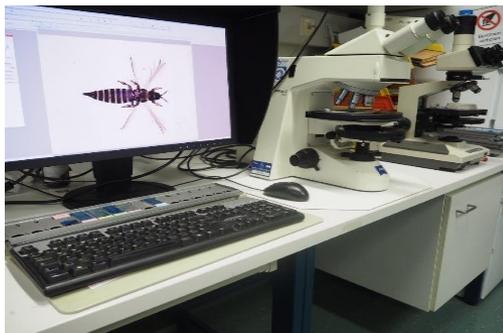
- Auchenorrhyncha
- Coleoptera
- General entomology



Alois Egartner



- Diptera (Tephritidae)
- Coleoptera



stereomicroscopes



controlled climate chambers for insect rearing



microscopes



Greenhouse facility and secure chambers



Biobibliography:

- **hard copies**
- **web based identification keys/tools**
- **electronic database**



collection platform



Insect drawer



Insect drawer

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



safety cupboard alcohol collection



Main taxonomic groups covered

- ✓ Cicadina
- ✓ Psyllina,
- ✓ Aleyrodina,
- ✓ Coccina,
- ✓ Aphididae,
- ✓ Heteroptera,
- ✓ Coleoptera,
- ✓ Lepidoptera,
- ✓ Thysanoptera
- ✓ Diptera



slide collection

Work Programme



Priority pests

Species	Method ?	PT morph	PT mol	Work on Method	Build up expertise	Year
<i>Spodoptera frugiperda</i>	EPPO	2019	2019	X		1
<i>Epitrix spp.</i>	No			X	X	1
<i>Thrips palmi</i>	EPPO	2020	2020	X		2
<i>Agrilus planipennis</i>	No			X	X	1-2
<i>Bactrocera zonata</i>	EPPO			X	X	1-2
<i>Bactrocera dorsalis</i> complex	IPPC			X	X	1-2
<i>Anoplophora chinensis</i>	No			X	X	2
<i>Anoplophora glabripennis</i>	No			X	X	2
<i>Aromia bungii</i>	No			X	X	2
<i>Popillia japonica</i>	EPPO	2020		X		2
<i>Thaumatotibia leucotreta</i>	Soon			X	X	2
<i>Agrilus anxius</i>	No			X	X	1-2



Work programme

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.

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



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

- 2019 → **initial evaluation** of the needs for reference specimens. **First supplies**
- 2020 → Continuation, as a **permanent task**.



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

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



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

- 2019 → **studies in progress** for all the target species
- 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 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*

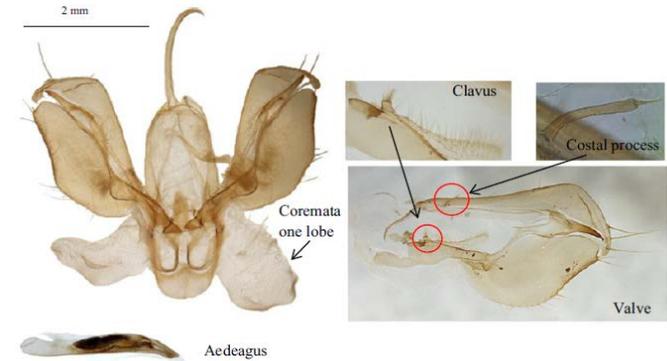
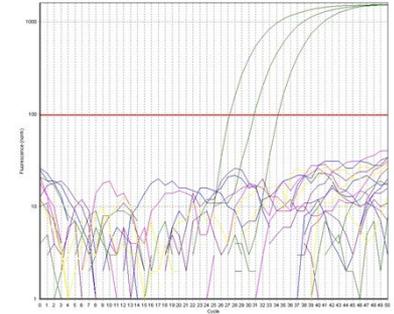


Fig. 63 Male genitalia *Spodoptera frugiperda*. Photos © J. Brambila, USDA-APHIS-PPQ.

© 2015 OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 45, 410–444

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

Identification of *S. frugiperda* by molecular methods



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

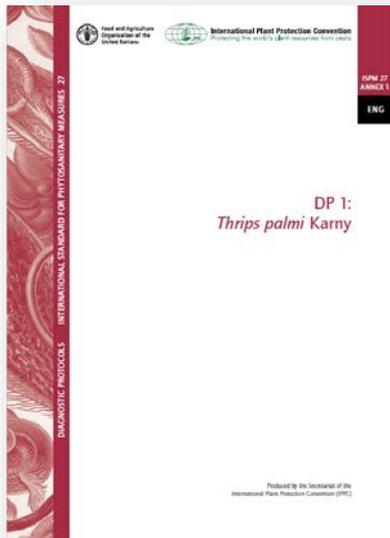
PM 7/124 (1)

Diagnostics
Diagnostic

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



Bulletin OEPP/EPP Bulletin (2018) 48 (3), 446-460

ISSN 0250-8052. DOI: 10.1111/epp.12545

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

PM 7/3 (3)

Diagnostics
Diagnostic

PM 7/3 (3) *Thrips palmi*



Proficiency Test on the identification of *Popillia japonica*



Identification of *Popillia japonica* based on morphological analysis of the adult

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

PM 7/74 (1)

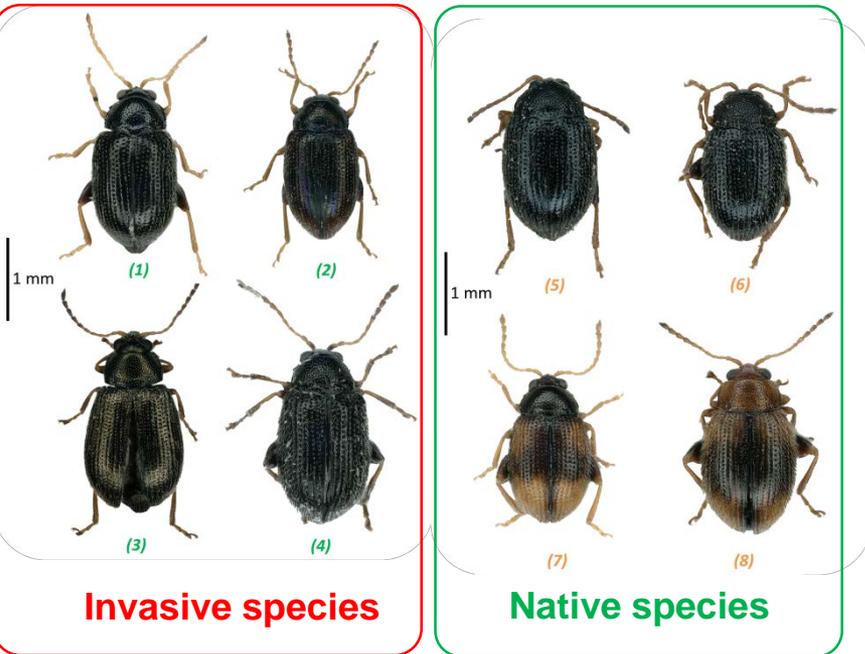
Diagnostics¹
Diagnostic

Popillia japonica





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

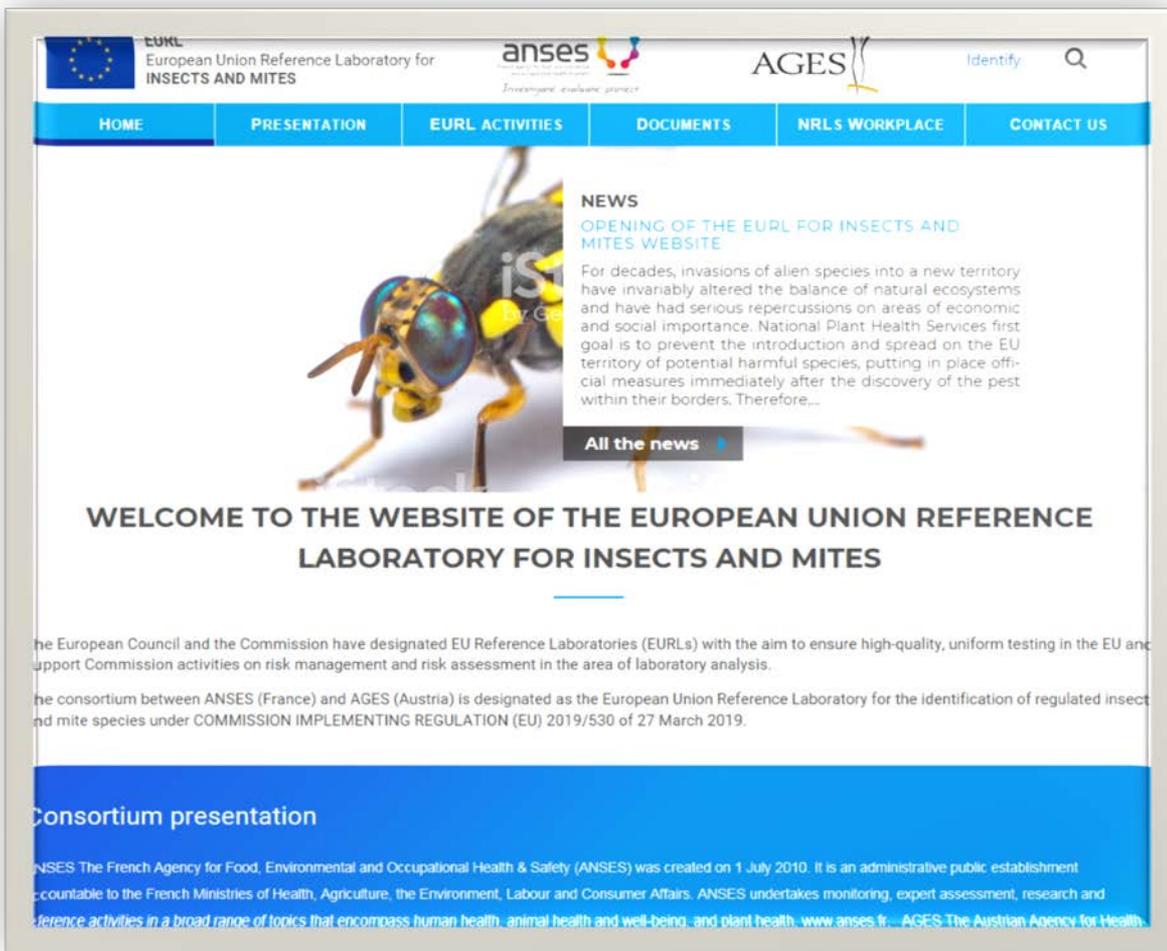
➤ Updated official protocols and bibliography

➤ Reference material and approved reagents

➤ NRLs network and contacts

➤ And much more ...

➤ <https://eurl-insects-mites.anses.fr>



EU National Reference Laboratories (NRLs)

list not available yet !

