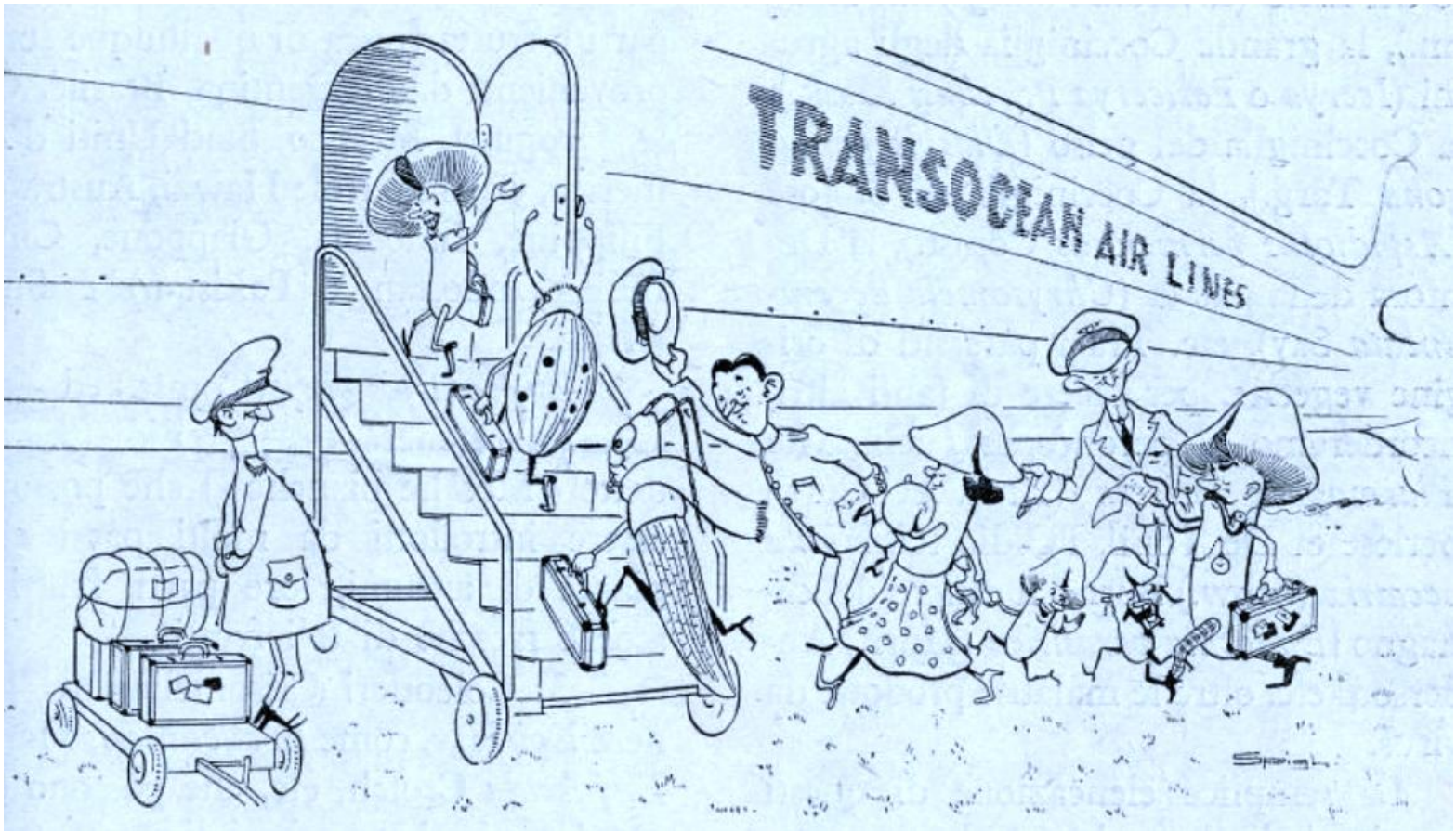
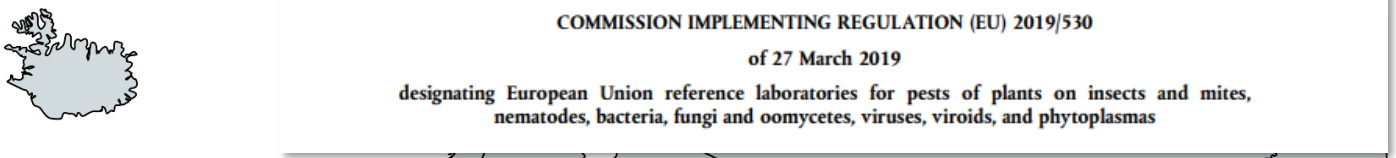


Andrea Taddei – EURL for Insects and Mites

Giornata di formazione sulla sorveglianza fitosanitaria in Lombardia
ERSAF - Servizio Fitosanitario Regione Lombardia, Milano, 10 Dicembre 2020

The EU Reference Laboratories (EURL)

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



29.3.2019 EN Official Journal of the European Union L 88/19

COMMISSION IMPLEMENTING REGULATION (EU) 2019/530

of 27 March 2019

designating European Union reference laboratories for pests of plants on insects and mites, nematodes, bacteria, fungi and oomycetes, viruses, viroids, and phytoplasmas

EURL for fungi and oomycetes:
ANSES (Nancy, France)

EURL for nematodes (consortium):
ANSES (Rennes, France)
ILVO (Merelbeke, Belgium)

Insects and mites (consortium):
ANSES (Montpellier, France)
AGES (Vienna, Austria)

EURL for bacteria (consortium):
NVWA-NRC (Wageningen, Netherlands)
ILVO (Merelbeke, Belgium)
CREA-DC (Rome, Italy)
NIB (Ljubljana, Slovenia)

EURL for viruses, viroids and phytoplasmas (consortium):
NVWA-NRC (Wageningen, Netherlands)
CREA-DC (Rome, Italy)
NIB (Ljubljana, Slovenia)

Created with mapchart.net

EURL for insects and mites

A consortium between:

- Entomology and Invasive Plants Unit of ANSES Plant Health Laboratory (Montpellier, France)
- Institute for Sustainable Plant Production of AGES (Vienna, Austria)

ANSES (French Agency for Food, Environmental and Occupational Health & Safety), created in 1996.
The Unit is located in CBGP (Center for Biology and Management of Populations) since january 2010
Activities as NRL: entomology (since 1996), invasive plants (since 2008)

AGES (Austrian Agency for Health and Food Safety) since 2002 (Health and Food Safety Act (GESG))
Institute for Sustainable Plant Production is NRL for Insects and Mites, Nematodes, Viruses, Viroids and Phytoplasmas, Bacteria, Fungi and Oomycetes
18 Federal Agencies & Offices merged
1,400 Staff, 900,000 samples tested with 7.2 million individual analyses/year

EURL for insects and mites: 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

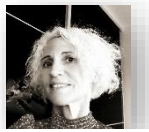
David Ouvrard
- Homoptera
- Coccoidea
- General entomology

EURL Staff- ANSES

Jean-Marie Ramel
- Lepidoptera
- Hymenoptera

Andrea Taddei
- Lepidoptera
- Diptera
- DNA barcoding

Arturo Goldarazena
- Coleoptera
- Thysanoptera
- Acari



Sylvie Dubois
- Administrative support

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

Richard Gottsberger
- Molecular identification by PCR
- DNA barcoding
- General bacteriology

EURL Staff- Molecular Unit - AGES

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

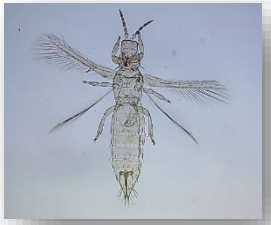
EURL Staff- Morphological Unit - AGES

Claudia Heiss
- Technician

Christina Lippitz
- Technician

Doris Busch
- Administration

Equipment for morphological identification

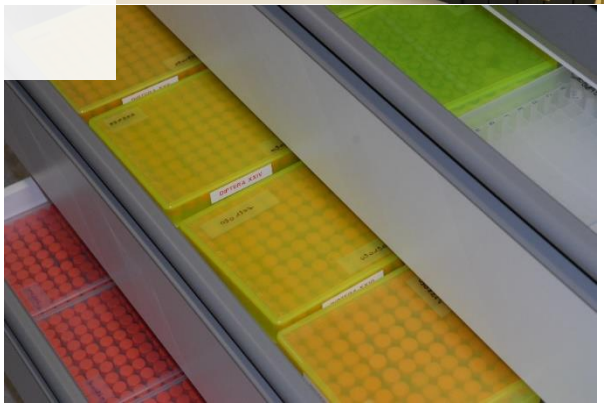


Specimens and slides collections

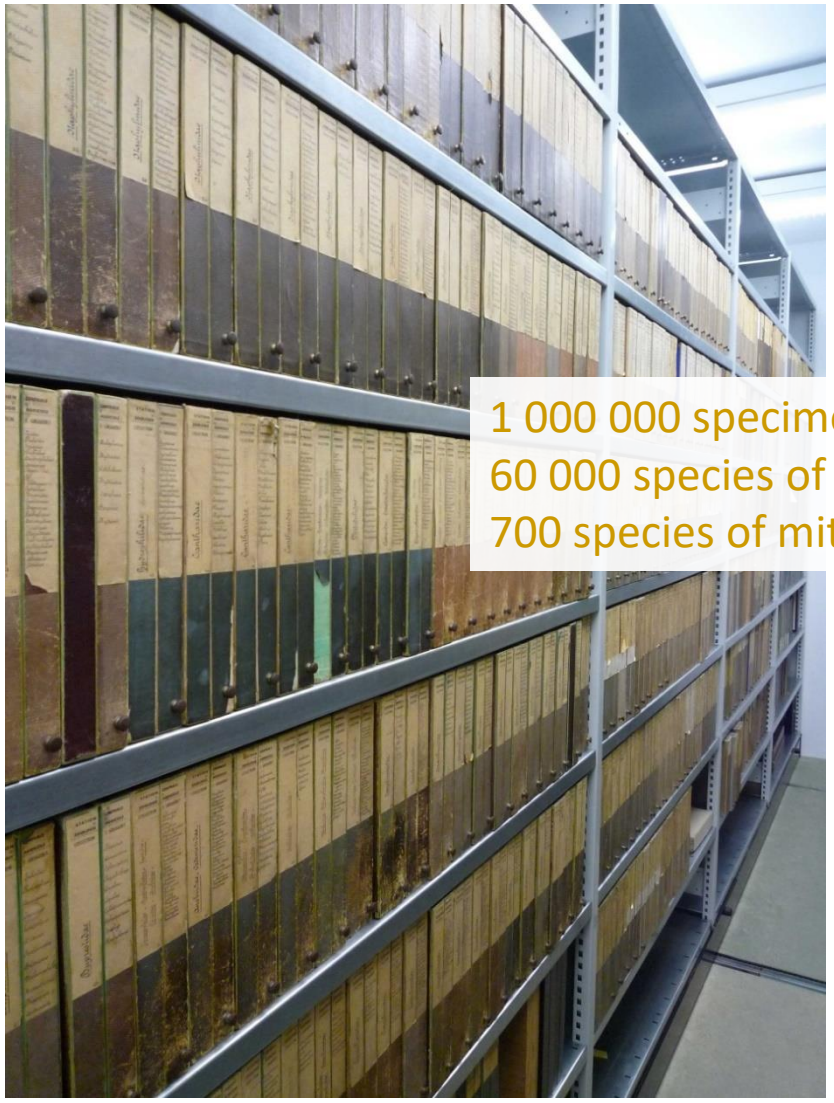
Main taxonomic groups covered:

- ✓ 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

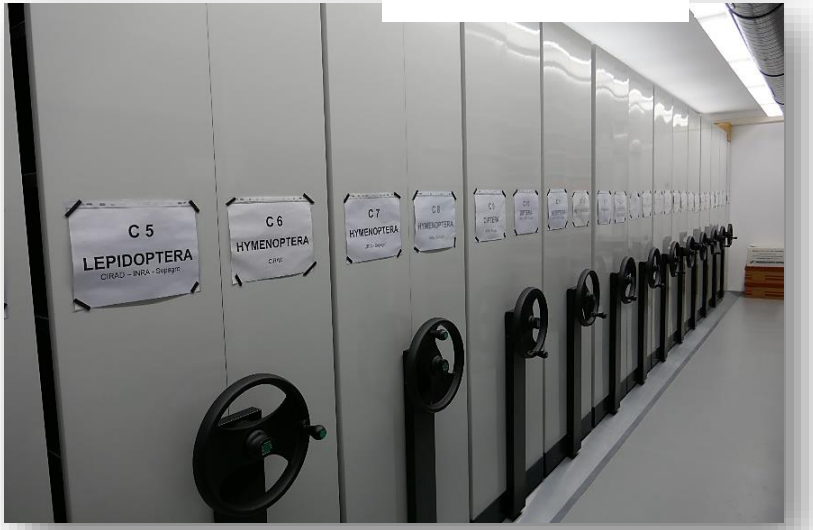


Vouchers collection CBGP

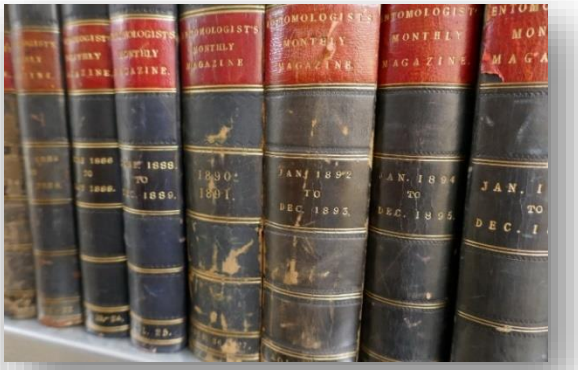


1 000 000 specimens
60 000 species of insects
700 species of mites

CBGP
Centre de Biologie pour la Gestion des Populations



Specialized bibliography



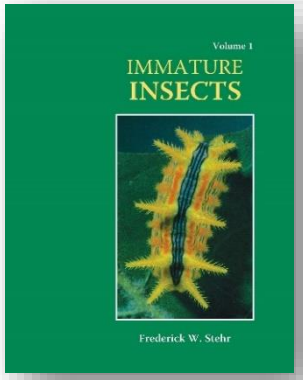
Biobliography:

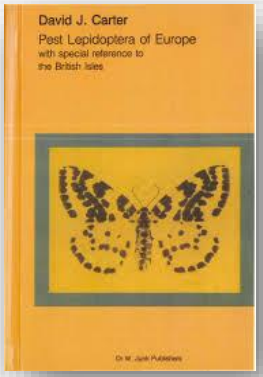
- hard copies
- web based identification keys/tools
- electronic database

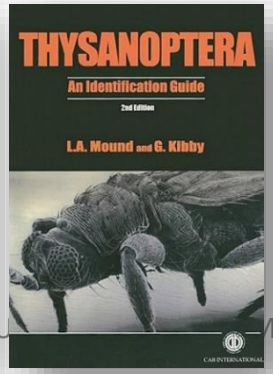
Zoological Record®

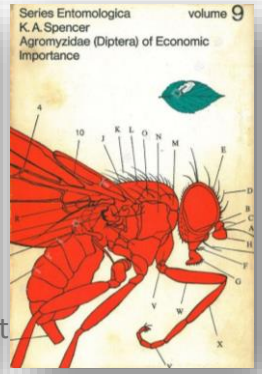
CAB ABSTRACTS

ELSEVIER Scopus



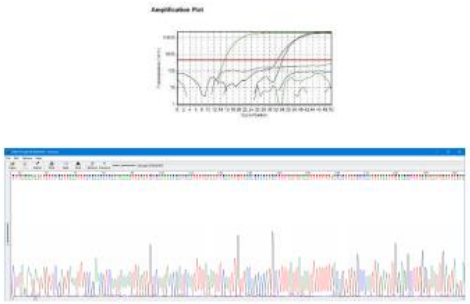






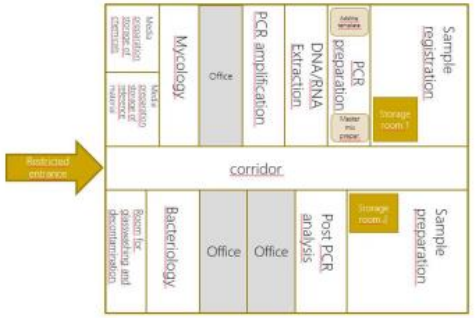
Equipment and laboratory facilities for molecular identification

State of the art equipment



MIC cycler

Capillary electrophoresis



Dedicated PCR working areas

DNA /RNA extraction and purification

Mastermix preparation

PCR amplification

Post – PCR (Analysis of amplification products)

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

We are designated as quarantine station

Reisenzein, EURL, 13/15 Oct. 2020

This EURL mission is carried out through different activities planned in two-year work programmes and based on the **Regulation (EU) 2017/625**, specific instructions of the European Commission and the needs of the European Union National Reference Laboratories (EU NRLs) network.

The work programme is structured around 4 main axes, which in turn include several subactivities:

1. to ensure availability and use of high quality methods and to ensure high quality performance by EU NRLs:
   - validation and the selection of reference protocols,
   - cooperate with NRLs and Commission to develop high standard methods of analysis,
   - provision of reference material (both as specimens and DNA),
   - organization of inter-laboratories proficiency tests;

2. to provide scientific and technical assistance to EU NRLs:
   - organization of training courses,
   - provision of relevant up-to-date scientific and technical information to the EU NRLs network through a dedicated website, a newsletter and workshops;

3. to provide scientific and technical assistance to the European Commission and other organizations, such as the European Food and Safety Authority (EFSA) and laboratories in third countries:
   - performance of confirmatory analysis at request of the European Commission or EU NRLs;

4. to establish a list of recommended reagents for molecular biology and reference collections of specimens and DNA.

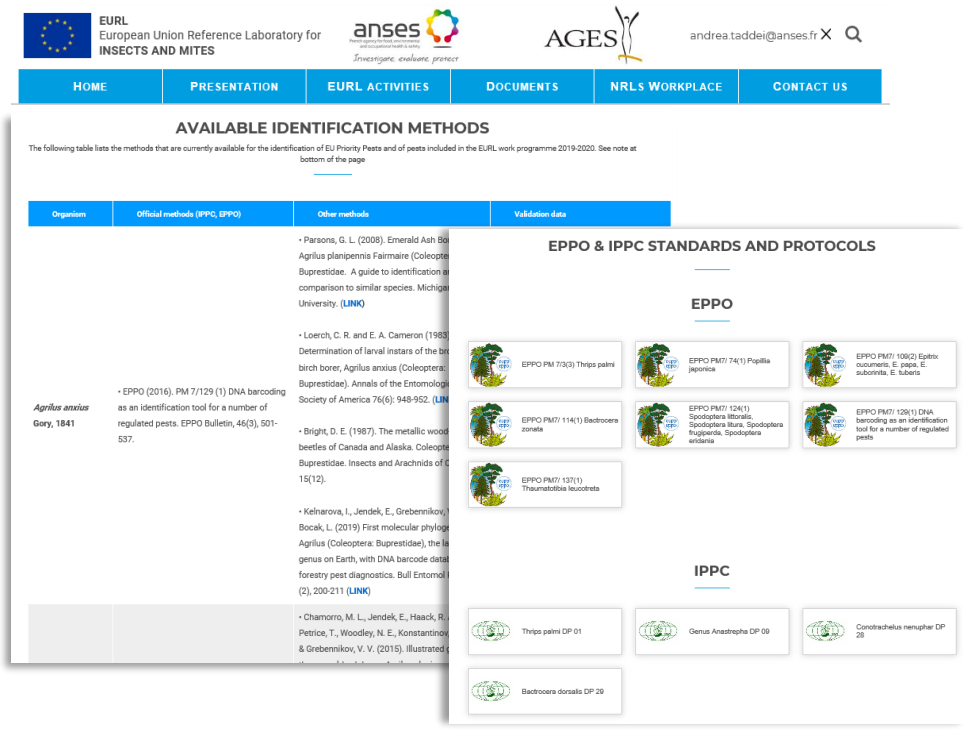
SPECIAL FOCUS ON EU PRIORITY PESTS!

1. Reference methods for identification
Selection of methods

- Extensive literature search (ELS), selection, pre-testing of methods and validation
- List of available identification methods for Priority pests
- List of useful bibliography for the identification of the main taxonomic groups
- Contribution to the production of official methods (EPPO, IPPC)

EU priority arthropod pests (Commission Delegated Regulation (EU) 2019/1702)

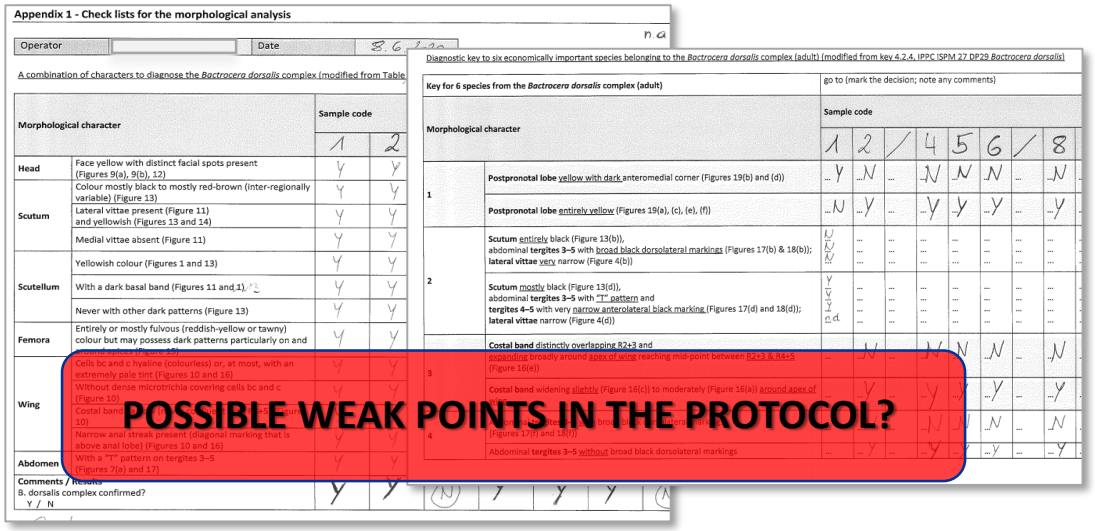
Order	Family	Name	EU	No	Yes
Coleoptera	Buprestidae	*Agrilus anxius*	Annex II A	●	
Coleoptera	Buprestidae	*Agrilus planipennis*	Annex II A	●	
Coleoptera	Cerambycidae	*Anoplophora chinensis*	Annex II B	●	
Coleoptera	Cerambycidae	*Anoplophora glabripennis*	Annex II A	●	
Coleoptera	Cerambycidae	*Aromia bungii*	Annex II B	●	
Coleoptera	Curculionidae	*Anthonomus eugenii*	Annex II A	●	
Coleoptera	Curculionidae	*Conotrachelus nenuphar*	Annex II A		●
Coleoptera	Rutelidae	*Popillia japonica*	Annex II B		●
Diptera	Tephritidae	*Anastrepha ludens*	Annex II A		●
Diptera	Tephritidae	*Bactrocera dorsalis*	Annex II A		●
Diptera	Tephritidae	*Bactrocera zonata*	Annex II A		●
Diptera	Tephritidae	*Rhagoletis pomonella*	Annex II A	●	
Hemiptera	Triozidae	*Bactericera cockerelli*	Annex II A	●	
Lepidoptera	Lasiocampidae	*Dendrolimus sibiricus*	Annex II A	●	
Lepidoptera	Noctuidae	*Spodoptera frugiperda*	Annex II A		●
Lepidoptera	Tortricidae	*Thaumatotibia leucotreta*	Annex II A		●
Total				9	7



- 2019-2020: Validation of diagnostic protocols for the morphological and molecular identification of ***Bactrocera zonata*** (EPPO PM7/114 (1)), ***Bactrocera dorsalis*** (IPPC ISPM 27 – DP 29) and ***Epitrix*** spp (EPPO PM7/ 109 (2))
- 2019-2020: Validation of diagnostic protocols for the molecular identification of ***Thrips palmi*** and ***Spodoptera frugiperda***

Objective:
Provide evidence that the selected protocols are suitable to perform routine identification of the pest by evaluating the performance characteristics of the protocol

Performance criteria:
Specificity
Sensitivity
Accuracy
Repeatability
Reproducibility



Appendix 1 - Check lists for the morphological analysis

Operator | Date

A combination of characters to diagnose the *Bactrocera dorsalis* complex (modified from Table

Morphological character		Sample code 1	2
Head	Face yellow with distinct facial spots present (Figures 9(a), 9(b), 12)	Y	Y
Scutum	Colour mostly black to mostly red-brown (inter-regionally variable) (Figure 13)	Y	Y
	Lateral vittae present (Figure 11) and yellowish (Figures 13 and 14)	Y	Y
	Medial vittae absent (Figure 11)	Y	Y
Scutellum	Yellowish colour (Figures 1 and 13)	Y	Y
	With a dark basal band (Figures 11 and 11)	Y	Y
	Never with other dark patterns (Figure 13)	Y	Y
Femora	Entirely or mostly fulvous (reddish-yellow or tawny) colour but may possess dark patterns particularly on and	Y	Y
Wing	Cells bc and c hyaline (colourless) or, at most, with an extremely pale tint (Figures 10 and 16)	Y	Y
	Without dense microtrichia covering cells bc and c (Figure 10)	Y	Y
	Costal ban	Y	Y
	Narrow anal streak present (diagonal marking that is above anal lobe) (Figures 10 and 16)	Y	Y
Abdomen	With a "T" pattern on tergites 3–5 (Figures 7(a) and 17)	Y	Y
Comments / Results B. dorsalis complex confirmed? Y / N		Y	Y

Diagnostic key to six economically important species belonging to the *Bactrocera dorsalis* complex (adult) (modified from key 4.2.4, IPPC ISPM 27 DP29 *Bactrocera dorsalis*)

Key for 6 species from the *Bactrocera dorsalis* complex (adult)		go to (mark the decision; note any comments) Sample code 1	2	/	4	5	6	/	8
1	Postpronotal lobe yellow with dark anteromedial corner (Figures 19(b) and (d))	Y	N		N	N	N		N
	Postpronotal lobe entirely yellow (Figures 19(a), (c), (e), (f))	N	Y		Y	Y	Y		Y
2	Scutum entirely black (Figure 13(b)), abdominal tergites 3–5 with broad black dorsolateral markings (Figures 17(b) & 18(b)); lateral vittae very narrow (Figure 4(b))	N							
	Scutum mostly black (Figure 13(d)), abdominal tergites 3–5 with "T" pattern and tergites 4–5 with very narrow anterolateral black marking (Figures 17(d) and 18(d)); lateral vittae narrow (Figure 4(d))	Y							
3	Costal band distinctly overlapping R2+3 and expanding broadly around apex of wing reaching mid-point between R2+3 & R4+5 (Figure 16(e))		N		N	N	N		N
	Costal band widening slightly (Figure 16(c)) to moderately (Figure 16(a)) around apex of				Y		Y		Y
4	(Figures 17(f) and 18(f))				N		N		N
	Abdominal tergites 3–5 without broad black dorsolateral markings		Y		Y		Y		Y

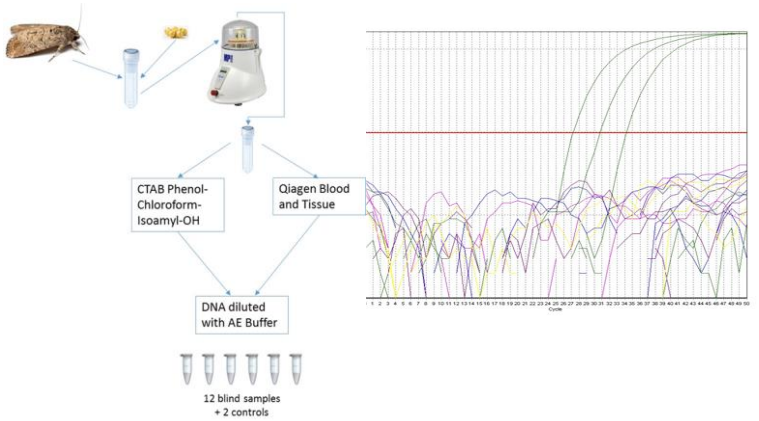
POSSIBLE WEAK POINTS IN THE PROTOCOL?

- 2019: two Proficiency Tests on ***Spodoptera frugiperda*** (one with a morphological method and one with a molecular method) with detailed reports and follow-up actions.
- 2020: two Proficiency Tests on ***Thrips palmi*** (morphological and molecular methods) under progress

Upcoming:

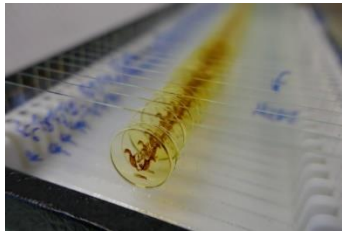
- 2021: Organization of one Proficiency Tests (morphological method) on ***Popillia japonica*** planned
- 2021: Organization of one Proficiency Tests (molecular method) on ***Bactrocera zonata*** planned

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



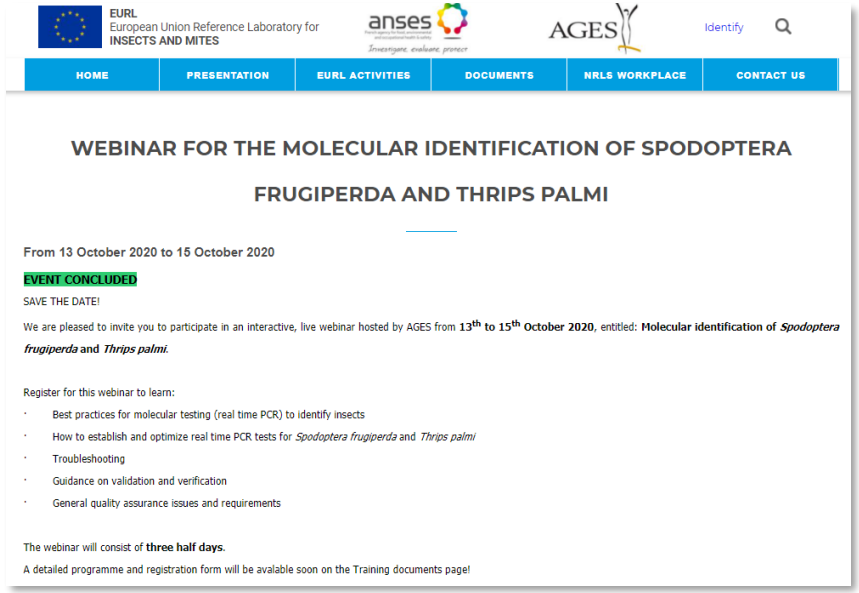
Objective:
evaluate the ability of participating laboratories to identify a pest through morphological and/or molecular analysis by evaluating the accuracy of their results

ISO 17043


2. Scientific and technical assistance to EU NRLs
Training courses and webinars

- Training sessions based on the results of PTs, actual needs and suggestions from NRLs (collected through a survey conducted in 2020 and an online form available on the website)



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WEBINAR FOR THE MOLECULAR IDENTIFICATION OF SPODOPTERA FRUGIPERDA AND THRIPS PALMI

From 13 October 2020 to 15 October 2020

EVENT CONCLUDED

SAVE THE DATE!

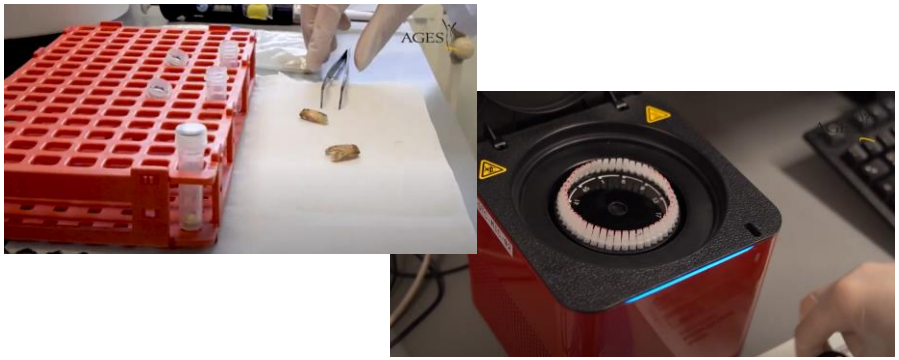
We are pleased to invite you to participate in an interactive, live webinar hosted by AGES from 13th to 15th October 2020, entitled: **Molecular identification of *Spodoptera frugiperda* and *Thrips palmi*.**

Register for this webinar to learn:

- Best practices for molecular testing (real time PCR) to identify insects
- How to establish and optimize real time PCR tests for *Spodoptera frugiperda* and *Thrips palmi*
- Troubleshooting
- Guidance on validation and verification
- General quality assurance issues and requirements

The webinar will consist of **three half days**.

A detailed programme and registration form will be avalable soon on the Training documents page!



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TRAINING SESSION ON THE MORPHOLOGICAL IDENTIFICATION OF SPODOPTERA FRUGIPERDA

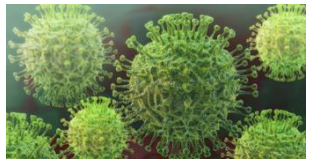
From 08 July 2020 to 09 July 2020

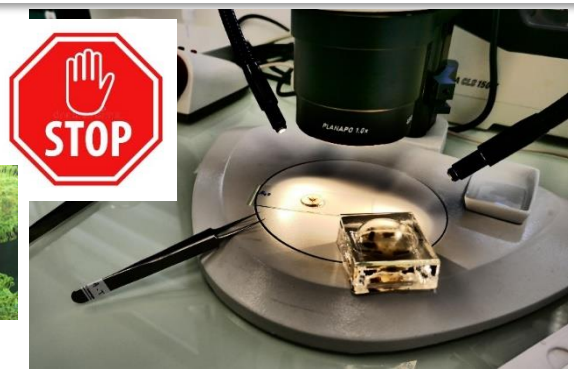
POSTPONED - **Training session on the morphological identification of *Spodoptera frugiperda*, including practical part on dissection technique and preparation of slide-mounted genitalia.**

The training will take place in the **EURL facilities in Montferrier-sur-Lez** (Montpellier) and will last **1,5 days.** A maximum number of 5 participants is admitted.

The costs will be reimbursed for **only one NRL representative per EU Member State** (travel, hotel expenses and daily allowance).

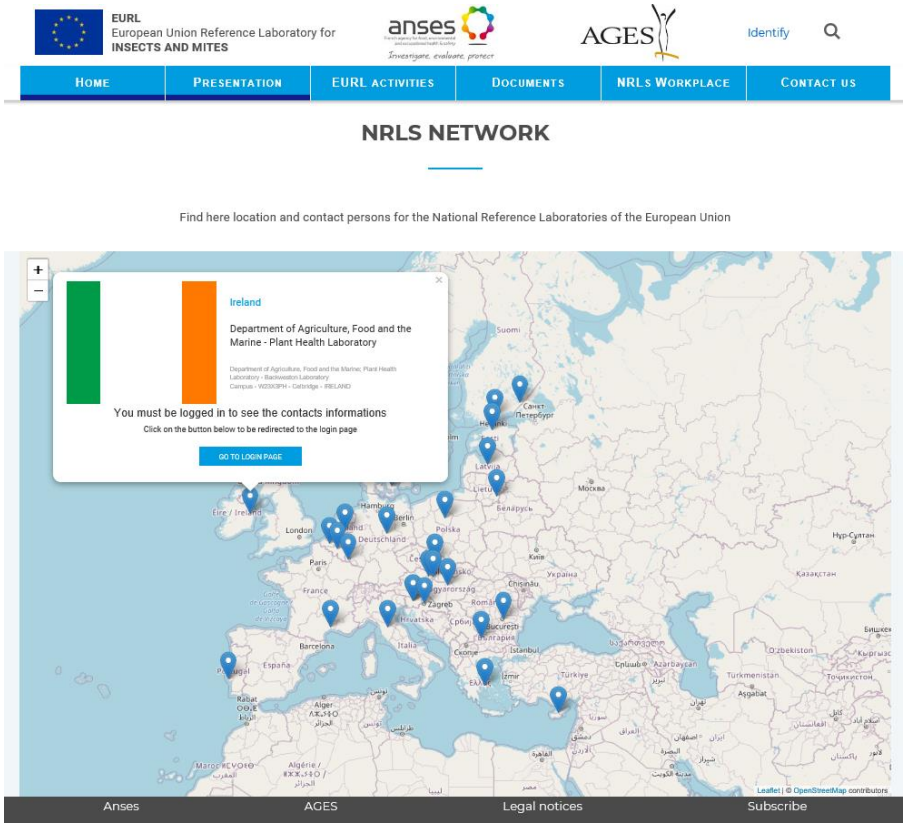
Full Programme and Registration Form are available on the EURL website Training Documents page. Please, fill the Registration form and return it before **May 29th** to **eurl-insects-mites@anses.fr**



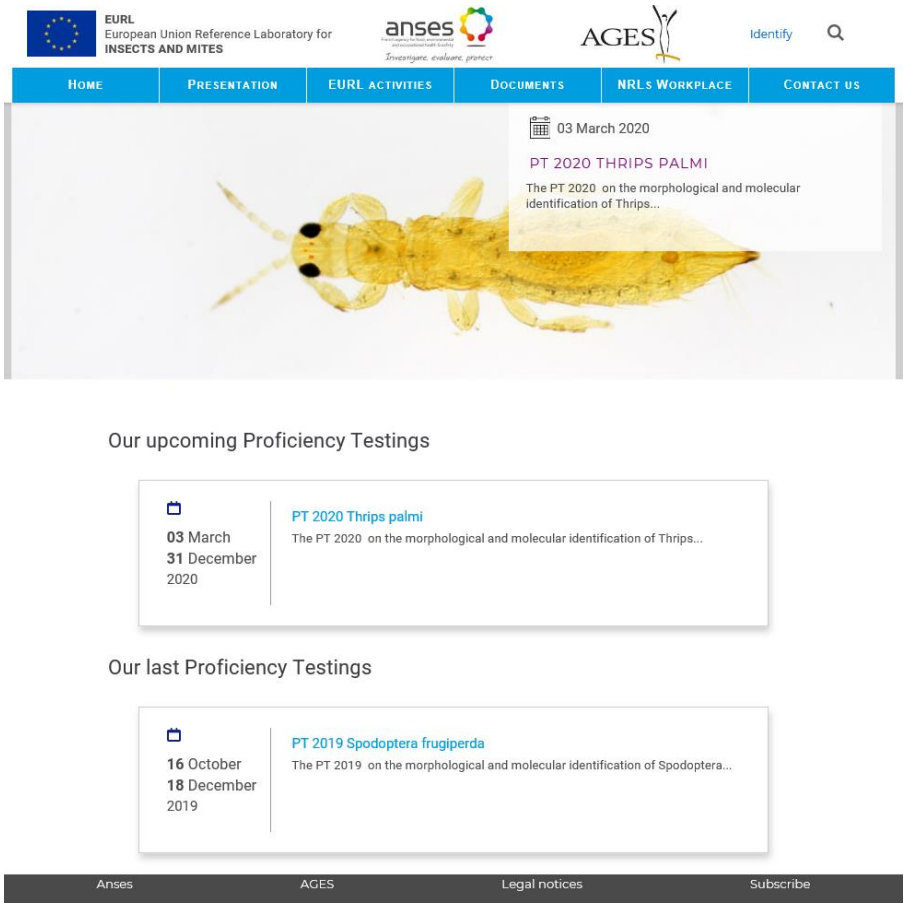


- A website has been implemented. It includes useful information for the NRLs network:
  - The work programme
  - Main European regulation in Plant Health

- A website has been implemented. It includes useful information for the NRLs network:
  - The work programme
  - Main European regulation in Plant Health
  - A network map of NRLs with contacts details



- A website has been implemented. It includes useful information for the NRLs network:
  - The work programme
  - Main European regulation in Plant Health
  - A network map of NRLs with contacts details
  - Information about the planned, ongoing and completed Proficiency Tests, Training sessions and Workshops



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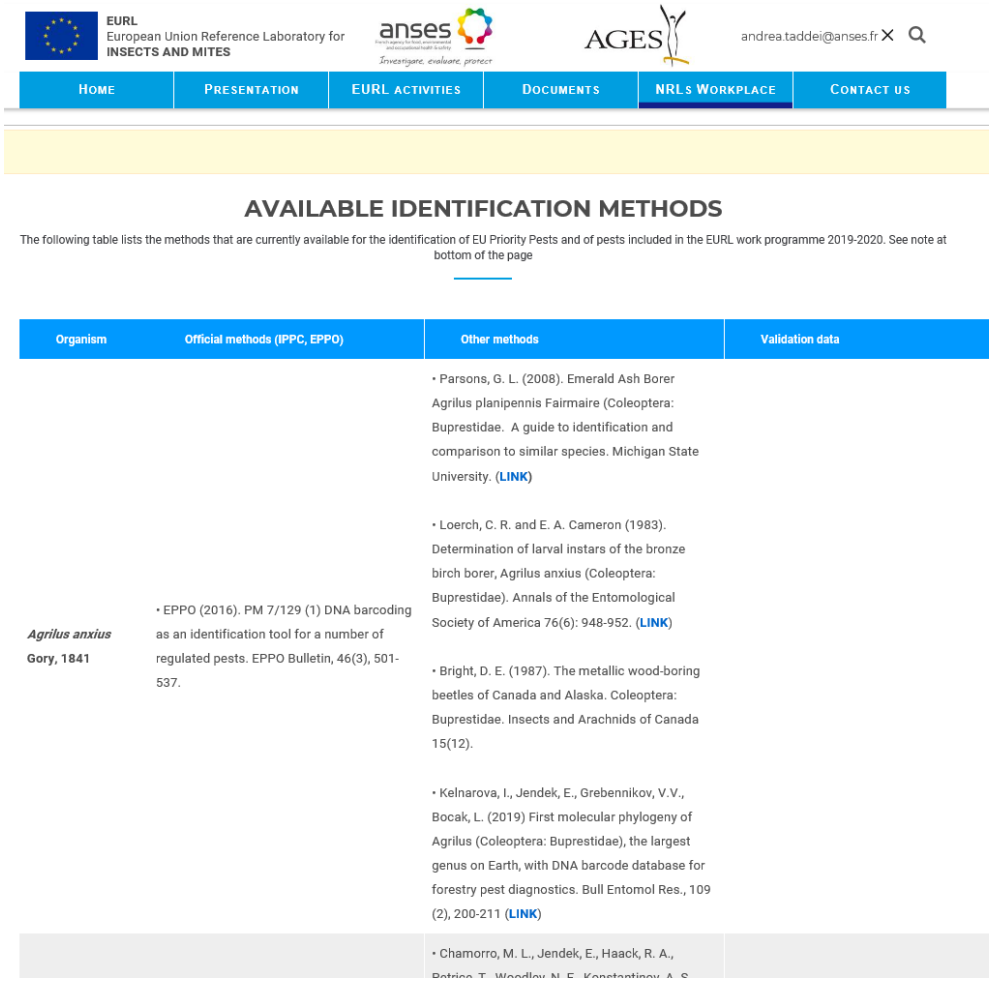
  - Main European regulation in Plant Health
  - A network map of NRLs with contacts details
  - Information about the planned, ongoing and completed Proficiency Tests, Training sessions and Workshops
  - A review of the available methods is published on the webpage of the EURL



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andrea.taddei@anses.fr

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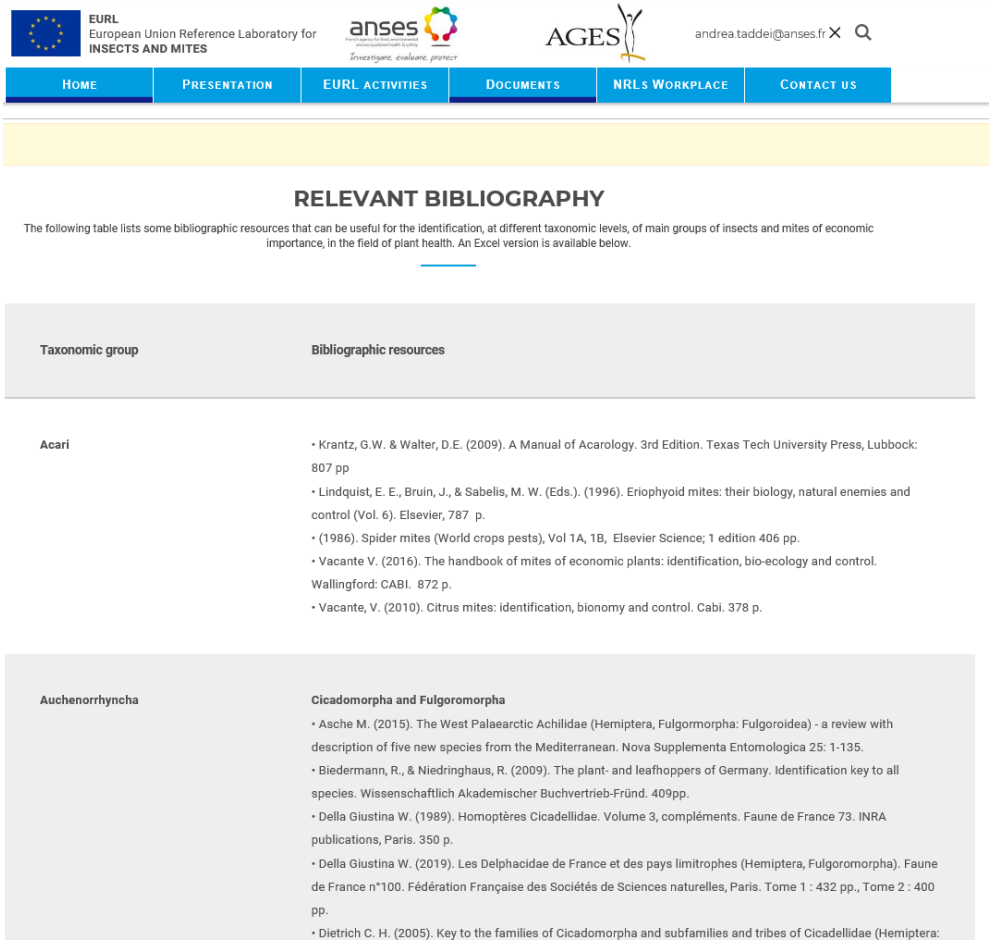
AVAILABLE IDENTIFICATION METHODS

The following table lists the methods that are currently available for the identification of EU Priority Pests and of pests included in the EURL work programme 2019-2020. See note at bottom of the page

Organism	Official methods (IPPC, EPPO)	Other methods	Validation data
Agrilus anxius **Gory, 1841**	• EPPO (2016). PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. EPPO Bulletin, 46(3), 501-537.	• Parsons, G. L. (2008). Emerald Ash Borer Agrilus planipennis Fairmaire (Coleoptera: Buprestidae. A guide to identification and comparison to similar species. Michigan State University. (LINK) • Loerch, C. R. and E. A. Cameron (1983). Determination of larval instars of the bronze birch borer, Agrilus anxius (Coleoptera: Buprestidae). Annals of the Entomological Society of America 76(6): 948-952. (LINK) • Bright, D. E. (1987). The metallic wood-boring beetles of Canada and Alaska. Coleoptera: Buprestidae. Insects and Arachnids of Canada 15(12). • Kelnarova, I., Jendek, E., Grebennikov, V.V., Bocak, L. (2019) First molecular phylogeny of Agrilus (Coleoptera: Buprestidae), the largest genus on Earth, with DNA barcode database for forestry pest diagnostics. Bull Entomol Res., 109 (2), 200-211 (LINK)	
		• Chamorro, M. L., Jendek, E., Haack, R. A.,	

- A website has been implemented. It includes useful information for the NRLs network:
  - The work programme
  - Main European regulation in Plant Health
  - A network map of NRLs with contacts details
  - Information about the planned, ongoing and completed Proficiency Tests, Training sessions and Workshops
  - A review of the available methods is published on the webpage of the EURL
  - A list of useful bibliography for the identification of the main taxonomic groups of insects and mites is available on the website



- A website has been implemented. It includes useful information for the NRLs network:
  - The work programme
  - Main European regulation in Plant Health
  - A network map of NRLs with contacts details
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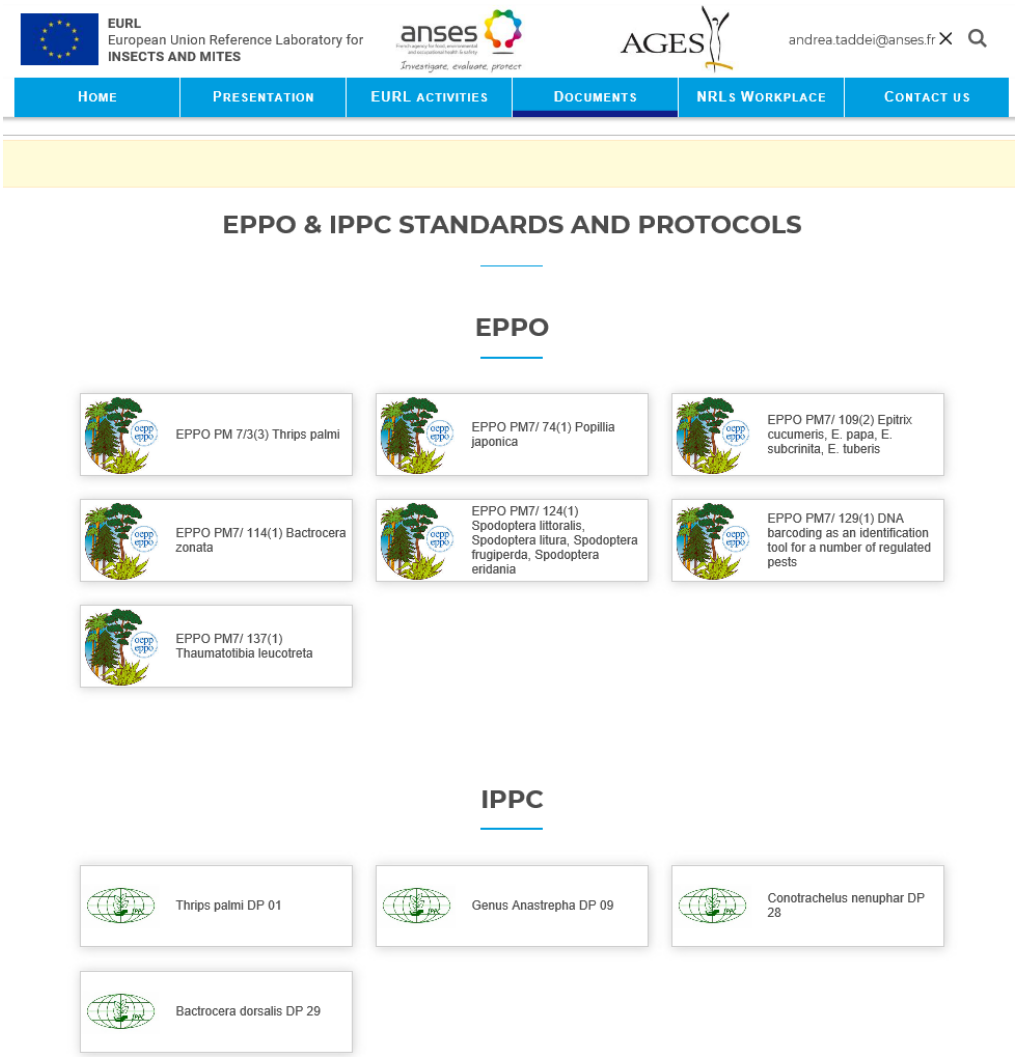

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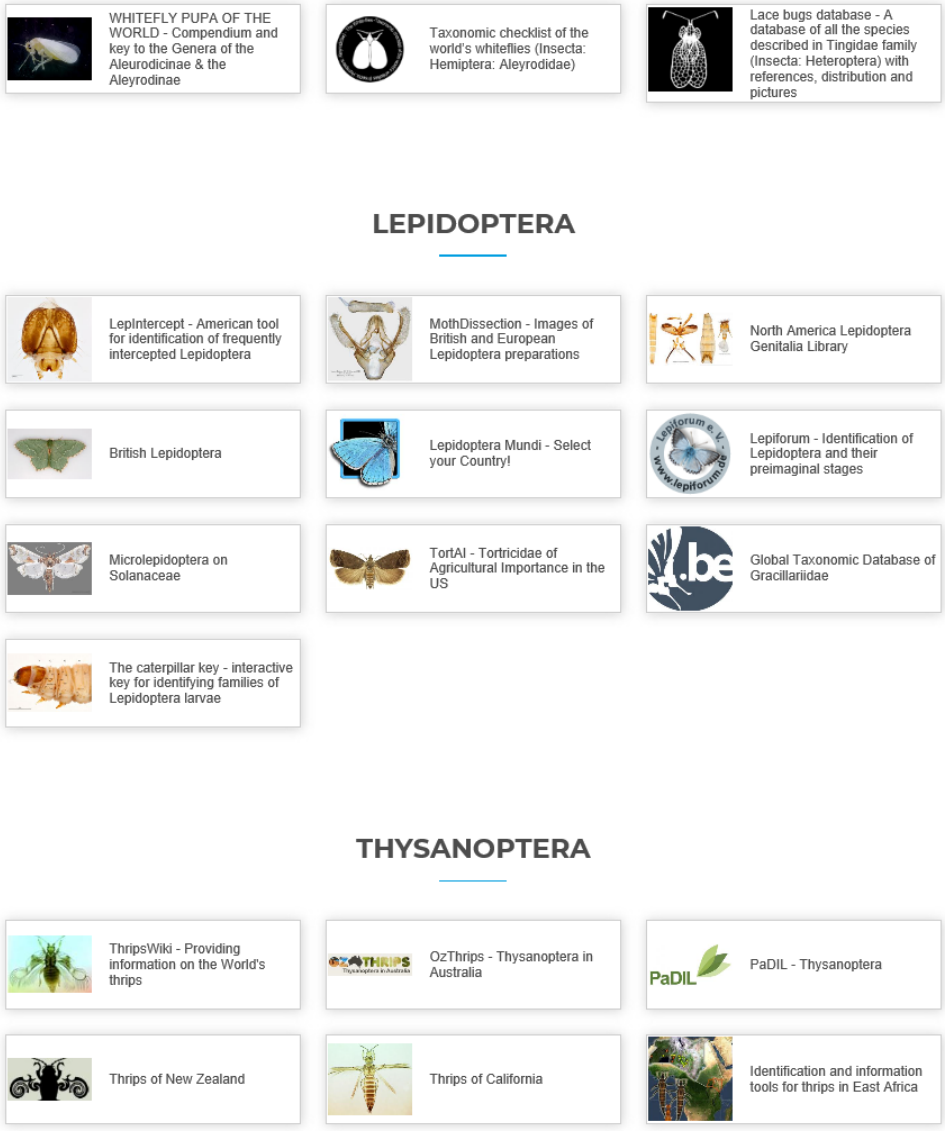

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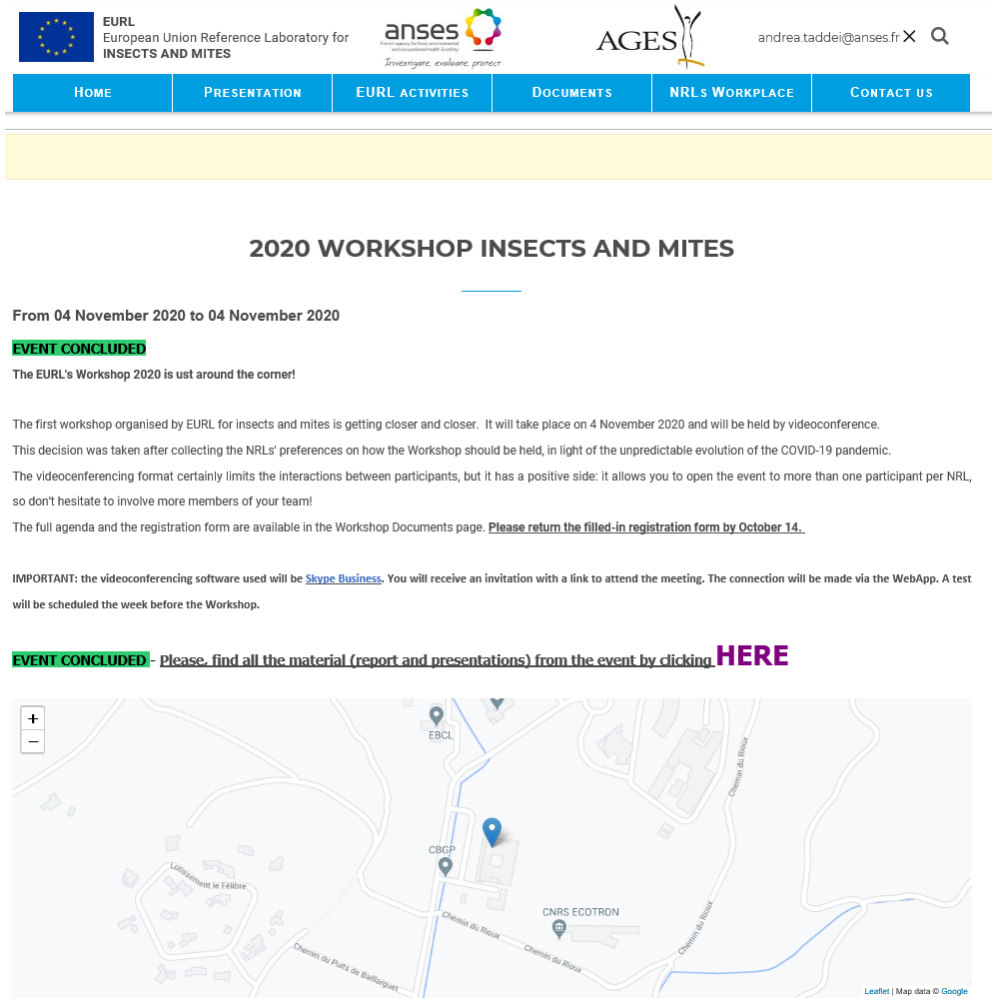
- Annual Workshop
  - November 4, 2020: 25 National Reference Laboratories (UE and Switzerland), 93 registered members and up to 76 workstations connected at the same time

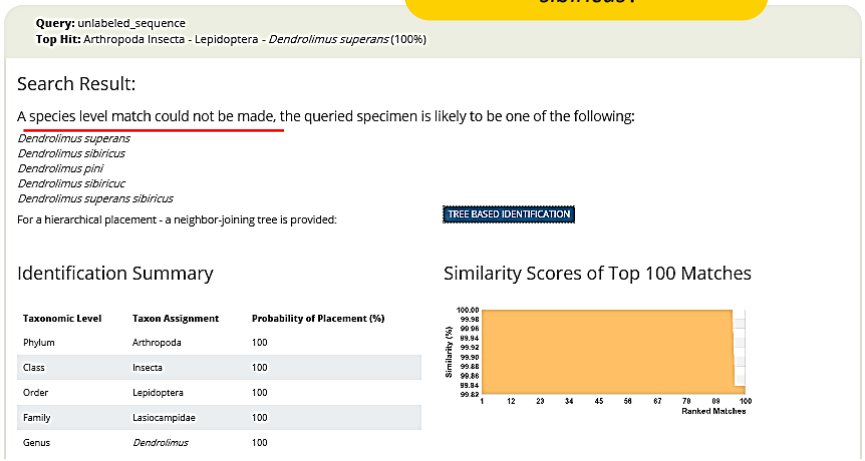


- Providing **scientific assistance to the Commission**
  - Example: availability of methods for identification of non-EU Fruit flies to species level (or genus level) at larval stage.

- Collaborating with laboratories in **third countries** and with the **European Food Safety Authority** (EFSA)

- Provide **confirmation of identification** at request of EC or EU NRLs. Some examples:
  - Germany: *Eotetranychus lewisi*
  - Luxembourg: Larvae of Tephritidae
  - Estonia: *Dendrolimus pini*

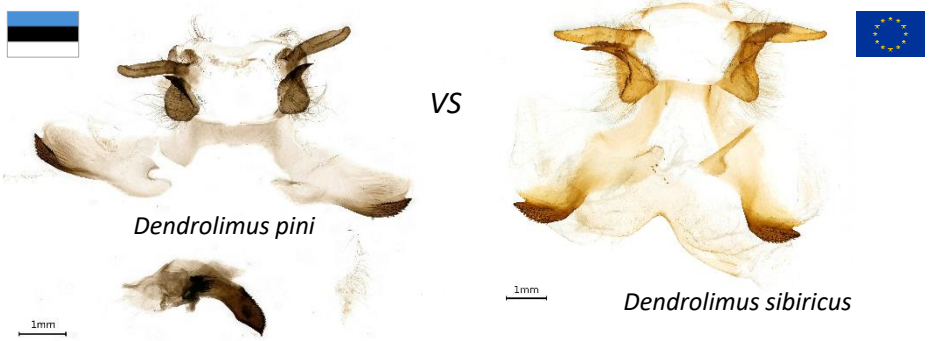
Dendrolimus specimens from NRL Estonia, molecular results ambiguous. Possible *D. sibiricus*?



Morphological and molecular taxonomy of *Dendrolimus sibiricus* Chetverikov stat.rev. and allied lappet moths (Lepidoptera: Lasiocampidae), with description of a new species

Kauri Mikkola & Gunilla Ståhls

Comparison with EURL *Dendrolimus sibiricus* reference material and available bibliography



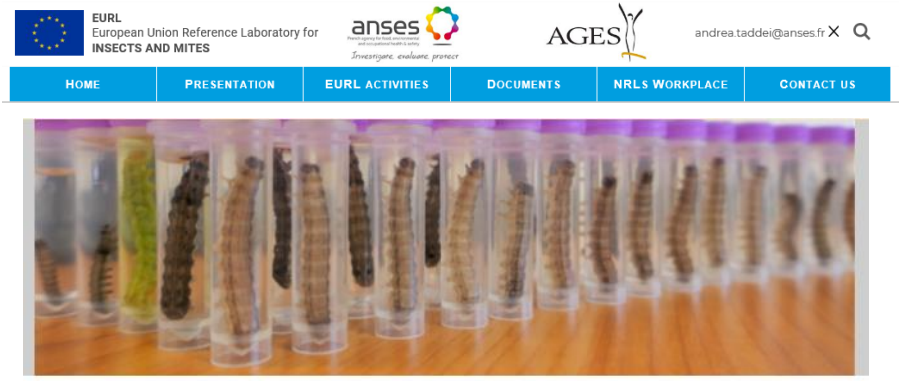
4. Reagents and Reference Collection

- Coordinating or performing test for the verification of the **quality of reagents**

- Create and maintain a **Reference material collection**
  - Testing of the Specify® database to manage the EURL reference collection under progress
  - Quality assured framework conditions under consideration
  - A "three levels" reference collection is planned
    - "EURL reference collection" (barcoded, internal EURL use, high resolution pics)
    - "Reference Collection for NRLs" (available to NRLs)
    - "Working collection" (for PTs, trainings, validation studies)

HIGH RESOLUTION PICTURES FOR EU PESTS

High resolution images for EU regulated species are available on this webpage. The images can be a valuable aid in identifying specimens, especially when reference specimens are missing, by highlighting the key morphological characteristics of each species, as indicated in diagnostic standards. The use of images alone, however, without consultation of identification protocols, should NEVER be done.



EURL REFERENCE COLLECTION

The Reference collection gathers morphological specimens and DNA to be used in the EURL activities and to be shared within the NRLs network

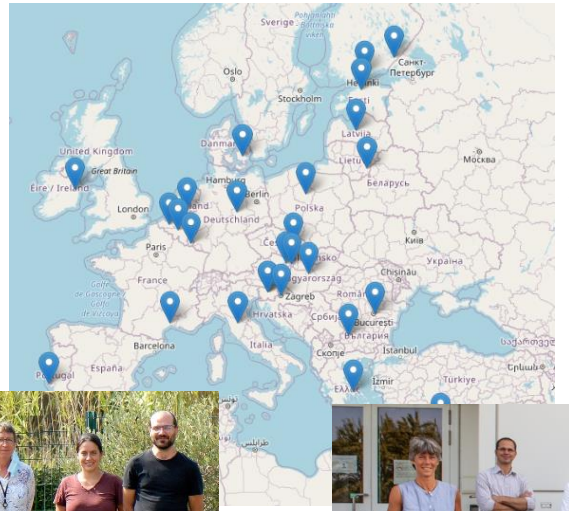
Reference material is of primary importance to serve as reference standards during the process of morphological and molecular identification of insects and mites.
One of the main tasks of EURL for Insects and Mites is to establish and maintain a high quality collection of reference material, called Reference collection (Regulation (EU) 2017/625 Art. 94 (2b)). The EURL's efforts focus on the recovery of specimens for the 16 species considered as priority for the Union territory (Priority pests, Annex of Regulation (EU) 2019/1702). In addition, the Reference collection includes other EU-regulated insects and mites species (Regulation (EU) 2019/2072), based on their availability. This Reference collection will allow the EURL to carry out activities such as training sessions and proficiency testing and will include specimens and DNA extracts to be transferred to NRLs upon request to serve in their diagnostic activities.

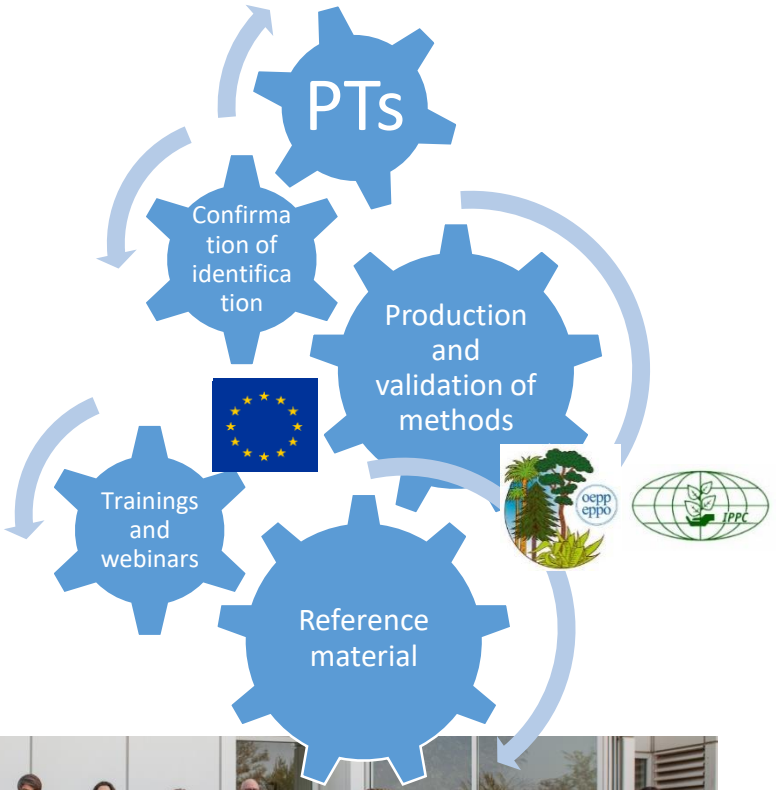
Reference material is available in two forms:
- **morphological specimens** (dry pinned material, preserved in vials with ethanol, slide-mounted)
- **DNA collections** (DNA extracts, artificial positive controls for selected pests (g-blocks) and sequence data)

A list of the available reference material will be uploaded soon at the bottom of this page, together with the instructions for submitting the request.

Work in progress, stay connected...

The main objective of EURL-Insects and Mites is to coordinate the network of EU National Reference Laboratories (EU NRLs) to ensure accurate identification of regulated or invasive insect and mite species. The provision of validated reference methods, reagents and technical information on pests, annual assessment tests and training of NRL staff should improve the quality of diagnosis and harmonise diagnostic procedures at European level.





Thank you

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The consortium between ANSES (France) and AGES (Austria) is the European Union Reference Laboratory for the identificationof regulated insects and mites

https://eurl-insects-mites.anses.fr/

eurl-insects-mites@anses.fr