Quick identification of commonly intercepted Tephritidae in Europe:

How does molecular identification help the morphology?

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Introduction

Before

Entomological identification = morphological methods
but no keys or incomplete keys => it’s a challenge

Now!

Molecular analysis provides a complementary approach
1 – Statute of Tephritid

- Family of Diptera named « Fruit fly »
- All species phytophagous
- Important worldwide pest

⇒ All non-European Tephritid on the EU quarantine list
Annex IA1 and IA2
2 – Biology

- Adult female
- Pupa
- Egg

Bactrocera sp.

Adult female

Pupa

Egg

Ceratitis cosyra

Larva
3 instars
named L1, L2 and L3

ovipositor
3 – Detection and usual species found in import

Host plants:
- Mangifera indica
- Annona
- Psidium guajava
- Syzydium
- Ziziphus
- Capsicum
- Momordica
- Citrus ...

- **Genus**: Anastrepha sp.
- **Species**: Bactrocera dorsalis, Ceratitis capitata, Dacus ciliatus, Bactrocera correcta, Ceratitis cosyra, Bactrocera cucurbitae, Ceratitis rosa, Bactrocera latifrons, Bactrocera zonata

10 / 2,500 worldwide species of Tephritidae
4 – Distortion of sampling and rearing

A - Larvae → rearing → adults

B - 2 Larvae
   - 1 for morphology
   - 1 for molecular

C - 1 larva
   - $\frac{1}{2}$ for morphology
   - $\frac{1}{2}$ for molecular

- failure of emergence
- duration

If two or more species?
5 – Morphology of larvae

- **anterior spiracle**
- **tubules**
- **Head**
- **mouthhook**
- **dental sclerite**

**Part of cephalopharyngeal skeleton**
6 – Preparing and mounting larva for analysis
7 – Morphological analysis

Mouth hook

- Presence or absence of preapical tooth
- Size of this tooth

Anterior spiracle

- Shape
- Number of tubules
8 – Molecular analysis

Workflow for molecular identification

DNA extraction → PCR amplification COI → Sanger sequencing

Blast → Sequence editing

Molecular ID

Bactrocera dorsalis
Ceratitis capitata
Ceratitis cosyra
Ceratitis rosa
Bactrocera correcta
Bactrocera cucurbitae
Bactrocera latifrons
Bactrocera zonata
Dacus ciliatus
...

Plant health laboratory – Entomology and invasive plants unit
9 – Results

• 180 specimen analysed so far
• For each specimen, we obtain:

  1 Slide & 1 Morphological ID
  1 COI sequence & 1 Molecular ID

• Possibility to assess performance criteria of the key by comparing the morphological ID with the molecular ID
• Work under way…
10 - Presentation of the key

**Key to third instar larvae for species intercepted in Europe:**

1. Preapical tooth present.
   - Preapical tooth absent.

2. Preapical tooth large and visible.
   - Preapical tooth small.

3. Anterior spiracle with more than 14 tubules (on Cucurbitaceae).
   - Anterior spiracle with less than 14 tubules (on Mangifera -Africa).

4. On Capsicum spp. and anterior spiracle with at least 14 tubules.
   - On host plant different or other number of tubules.

5. Anterior spiracle concave centrally and dental sclerite absent.
   - Anterior spiracle not concave centrally. Dental sclerite present.

6. Ventral apodeme not projecting posteriorly and anterior spiracles with 9-10 tubules.
   - Ventral apodem projecting posteriorly.

7. At least 13 tubules on one of the two anterior spiracles.
   - Anterior spiracles with less than 13 tubules.

8. Anterior spiracles with 9-11 tubules (average 10.1).
   - Anterior spiracles with 10-12 tubules (average 10.5).

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- **Dacus ciliatus**
- **Bactrocera cucurbitae**
- **Ceratitis capscyra** or **Ceratitis rosa**
- **Bactrocera latifrons**
- **Anastrepha sp.**
- **Ceratitis capitata**
- **Bactrocera zonata**
- **Bactrocera correcta**
- **Bactrocera dorsalis** or inv. dens

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Important: knowing pathway of import and pests situation
### 11 – Strengths and limitations of the 2 methods:

**Morphological:**

**+:**
- low cost
- rapidity
- few materials
- + or – « fresh » larva
- « field » use

**−:**
- only with L3
- poor reliability/ some species
- invalid / new species
- invalid / morphological anomalies
- knowledges in entomology

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**Molecular:**

**+:**
- all instar
- high reliability
- valid / new species
- valid / non-normal form
- no specific knowledges

**−:**
- high cost
- duration
- expensive materials
- need « good » DNA
- requires specific laboratory
12 – Use and other applications

- Imported control or control in focus area
- IPM Integrate pest management

Application to other larvae of invasive or regulated pests:

ex: Diptera, Drosophilidae (Zaprionus indianus/Drosophila suzukii etc…)

ex: Lepidoptera, Tortricidae (Thaumatotibia leucotreta/other Tortricidae)
Thank you

♩ ♪ All you need is larvae ♪ ♩