Molecular identification of *Hyalesthes* and *Reptalus* species (Hemiptera: Cixiidae) allows monitoring of vector species throughout the year

Sabrina Bertin¹, Luca Picciau¹, Zoltán Ács², Alberto Alma¹ and Domenico Bosco¹

¹ DIVAPRA - Entomology and Zoology, University of Turin, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy
² Fitolab Plant Pest Diagnostic and Advisory Ltd. Istenhegyi út 29., Budapest H-1125, Hungary
Hyalesthes and Reptalus spp: as vectors

Both *Hyalesthes* and *Reptalus* species are reported as vectors of STOLBUR phytoplasmas (*Candidatus Phytoplasma solani*, 16SrXII-A genetic group)

- **Hyalesthes obsoletus**: vector of stolbur diseases affecting several horticultural crops and grapevine → i.e. Bois Noir (BN), a grapevine yellow (Maixner et al., 1994).

- **Hyalesthes luteipes**: found positive to BN (Trivellone et al., 2005).

- **Reptalus quinquecostatus**: can successfully inoculate BN into an artificial feeding medium through parafilm (Pinzauti et al., 2008).

- **Reptalus panzeri**: found positive to BN (Palermo et al., 2004), transmits the maize redness (Jović et al., 2007).
Hyalesthes and Reptalus: species identification

Currently based on morphological features, mainly concerning the male genitalia

The identification is restricted to specialist entomologists

These morphological features hamper the identification of juveniles and females

DNA-based approaches can help:
- mitochondrial COI sequence
- ITS2 ribosomal sequence
Hyalesthes and Reptalus: samples

✓ Hyalesthes obsoletus
✓ Hyalesthes scotti
✓ Hyalesthes luteipes
✓ Reptalus cuspidatus
✓ Reptalus quinquecostatus
✓ Reptalus panzeri
✓ Reptalus melanochaetus
### Hyalesthes species - COI analysis

<table>
<thead>
<tr>
<th></th>
<th>H. obsoletus</th>
<th>H. luteipes</th>
<th>H. scotti</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>600bp</td>
<td>300bp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PCR-RLFP, TaqI restriction enzyme**
Hyalesthes species - ITS2 analysis

H. s.    H. l.    H. o.    M

PCR assay

1600bp
500bp
Hyalesthes species – ITS2 analysis

PCR-RLFP, TaqI restriction enzyme

H. o.  H. s.  M

800bp  200bp
Reptalus species - COI analyses

PCR-RLFP, AluI restriction enzyme
**Reptalus species - ITS2 analyses**


PCR assay
Reptalus species - ITS2 analyses

PCR-RLFP, TaqI restriction enzyme
Discrimination among *Hyalesthes* and *Reptalus* species

- both COI and ITS2 markers are species-specific (no within-species polymorphisms have been recorded)

- no polymorphisms ascribable to different geographic origin and/or different host plants have been noticed within each considered species

Given their specificity and conservation both COI and ITS markers can be considered new reliable identification tools
New identification tools: advantages

• The new tools require rapid and handy procedures

  They contribute to broaden the current handful of competent scientists

• The molecular assays can be applied to the species identification of juveniles and females

  They contribute to faunal studies and insect vectors monitoring:
  i) extending the collection period to the whole year
  ii) associating cixiids with their actual host plants
The project was supported by the Piemonte Region under the grant “Studi su fitoplasmi della vite e loro vettori” and benefited from Italian-Hungarian Bilateral grant.

Thanks for your attention!