The distribution and phenology of five potential vectors of *Xylella fastidiosa* in Belgium

Séverine Hasbroucq$^{1,2}$, Claude Bragard$^2$, Noemi Casarin$^2$, Ewelina Czwienckez$^{1,2}$, Jean-Claude Grégoire$^1$

$^1$Université libre de Bruxelles, Belgium  
$^2$Université catholique de Louvain, Belgium
The **Xyleris** research project (2016-2018), funded by the Federal Public Service Health, Food Chain Safety and Environment, gathers the *Université libre de Bruxelles* (ULB), the *Université catholique de Louvain* (UCL) and the *Institute for Agricultural and Fisheries Research* (ILVO).

One of its tasks is to investigate the presence and distribution of possible insect vectors in Belgium, their life cycle and their capacity to transmit the bacterium (UCL-ULB).

Another task is testing the potential use of the vectors as "Spy Insects".
EFSA opinion (2015):
- All xylem sap-feeders are potential vectors
- Fauna Europaea + faunistic publications
- *Philaeus spumarius* already identified as a vector in Apulia
- In addition: four species identified as very common and widespread

**Context** - starting from the EFSA opinion (2025)

[Diagram showing the number of countries or smaller areas each species is found in]

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European Conference on Xylella fastidiosa 2017: finding answers to a global problem
Context - starting from the EFSA opinion (2025)

- Philaenus spumarius
- Aphrophora alni
- Aphrophora salicina
- Cicadella viridis
- Cercopis vulnerata
Mapping the distribution of potential vectors

- Inventory of the collections of the Royal Belgian Institute of Natural Sciences (RBINS) (1893-2000)
- Searching the Observations.be database (2015-2016)
- Field surveys (June - October 2016; June 2017)
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**Cercopis vulnerata**: Distribution in Belgium - all available data

Legend:
- Field captures (summer 2016-spring 2017)
- Observations.be data (2010-2016)
- RBINS data (1893-2000)

Regions_Naturelles:
- Ardenne
- Campine
- Condroz et sillon Sambre-Mosan
- Dunes
- Fagne-Famenne-Caletienne
- Lorraine
- Meuse moyenne
- Polders
- Région limonneuse
- Région sablo-limonneuse
Phenology of the potential vectors – field data
Phenology & life history of the potential vectors
laboratory observations
Phenology & life history of the potential vectors
laboratory observations

- Oviposition patterns of *Aphrophora salicina*
  (inside the end of *Salix* shoots)
Phenology & life history of the potential vectors

- Oviposition patterns of *Philaenus spumarius* (at the base of grass blades, protected by a layer of foam)
Phenology & life history of the potential vectors
laboratory observations

- Oviposition patterns of *Cicadella viridis*
  (inside *Juncus* stems)
Phenology & life history of the potential vectors laboratory observations

- Parasitism of the *Cicadella viridis* eggs (inside *Juncus* stems)

Parasitism rate: **6.67%** (700 field-collected eggs)

Identification pending. Probably *Anagrus incarnatus* Hal. (Mymaridae)
Phenology & life history of the potential vectors
laboratory observations

- Immature development of *Cicadella viridis*
  - Five instars
  - Nymphs mobiles on grasses
  - No spittle
  - Cephalic capsule width: 0.5 - 1.6 mm according to nymphal instar
  - Nymphs 1.4 - 5 mm long, according to nymphal instar
**Phenology & life history of the potential vectors**

**laboratory observations**

- Immature development of *Aphrophora salicina*
  - Five instars
  - Nymphs remain on *Salix* stems
  - Spittle protection
  - Cephalic capsule width: 0.5 - 2.0 mm according to nymphal instar
  - Nymphs 2 – 6.5 mm long, according to nymphal instar
### Phenology & life history of the potential vectors

**laboratory observations**

- **Overwintering**

<table>
<thead>
<tr>
<th>Species</th>
<th>stage</th>
<th>Winter diapause</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Philaenus spumarius</em></td>
<td>eggs</td>
<td>yes</td>
</tr>
<tr>
<td><em>Aphrophora salicina</em></td>
<td>eggs</td>
<td>yes</td>
</tr>
<tr>
<td><em>Cicadella viridis</em></td>
<td>eggs</td>
<td>no</td>
</tr>
</tbody>
</table>
Distribution, phenology & life history of the potential vectors - conclusions

- All species are widely distributed throughout the Belgian territory
- All species are univoltine, except *Cicadella viridis*
- *Cercopis vulnerata* adults are only present a few weeks (May-June)
- All are polyphagous, except *Aphrophora salicina* (only on *Salix*)
- All overwinter as eggs, except *C. vulnerata* (as nymphs)
Testing for "Spy insects"

- Collection of large numbers of insects over the whole Belgian territory;
- Storage at -20° C;
- DNA extraction and PCR;
- Design of a multiplex PCR for detection of both X. fastidiosa and targeted insect vectors (Cytochrome oxidase I, ITS non-conserved rDNA);
- High throughput screening of the collected insects.
Testing for "Spy insects"

Both *X. fastidiosa* and insects detected

Multiplex PCR & quantitative PCR
Testing for "Spy insects"

- Exciting results pending confirmation …
Conclusions and perspectives

- Potential vectors present throughout the Belgian territory;
- *P. spumarius*, *A. salicina*, *A. alni* and *C. viridis* have a protracted adult life;
- *C. vulnerata* has a short adult life, but is locally very abundant;
- *C. viridis* is multivoltine; the other species are univoltine;
- *C. viridis* locally very abundant;
- Multiplex PCR for detecting both *X. fastidiosa* and insect vectors;
- High throughput screening of the collected insects.
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