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

Séverine Hasbroucq[1,2], Claude Bragard[2], Noemi Casarin[2], Ewelina Czwienczek[1,2], Jean-Claude Grégoire[1]

[1]*Université libre de Bruxelles, Belgium*
[2]*Université catholique de Louvain, Belgium*

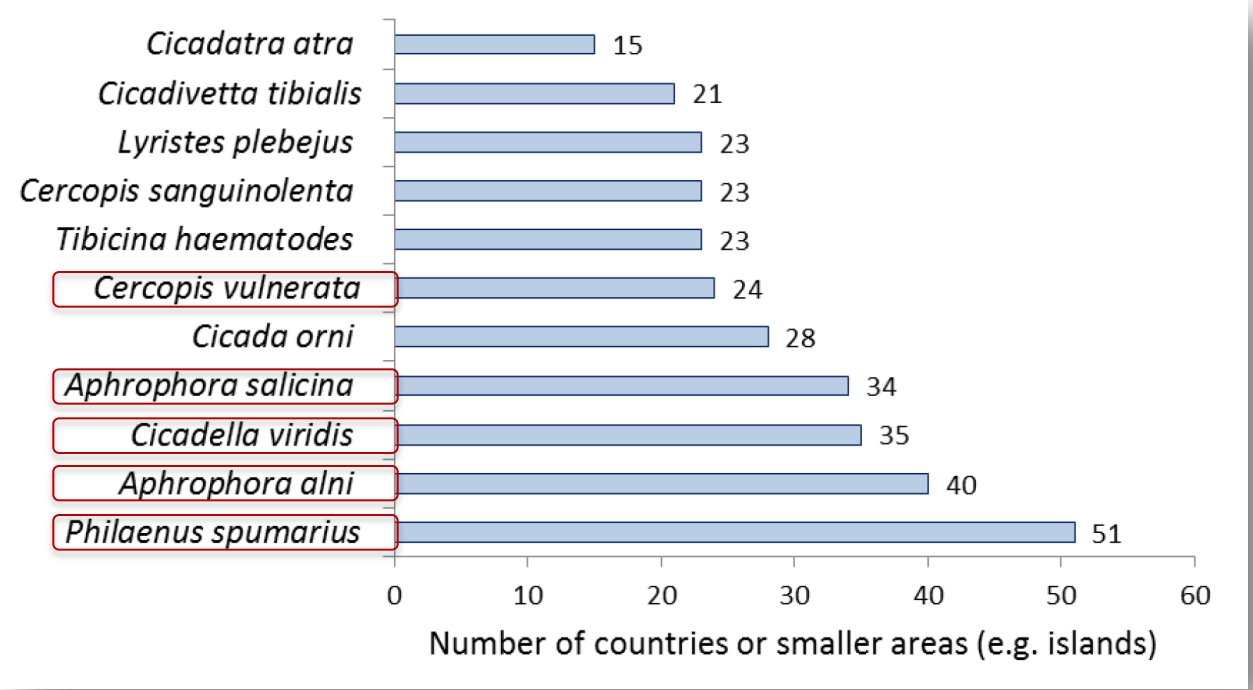
Context – A Belgian collaborative research project

- 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*".

Context - starting from the EFSA opinion (2025)

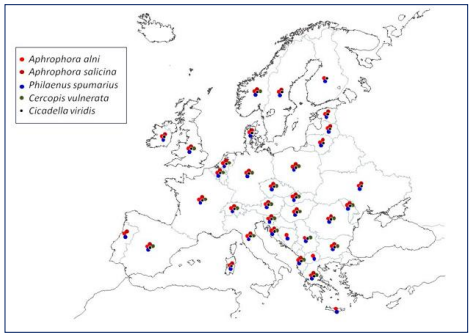
EFSA opinion (2015):

- All xylem sap-feeders are potential vectors
- *Fauna Europaea* + faunistic publications
- *Philaenus spumarius* already identified as a vector in Apulia
- In addition: four species identified as very common and widespread



EFSA Panel on Plant Health, 2015. Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory. *EFSA Journal* 2015;13(1): 3989, 262 pp.

Context - starting from the EFSA opinion (2025)



Cicadella viridis

Philaenus spumarius

Cercopis vulnerata

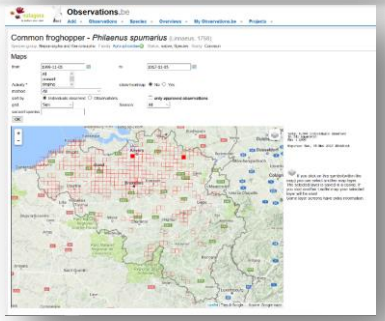
Aphrophora alni

Aphrophora salicina

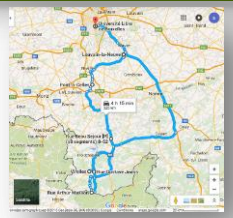
Mapping the distribution of potential vectors

- 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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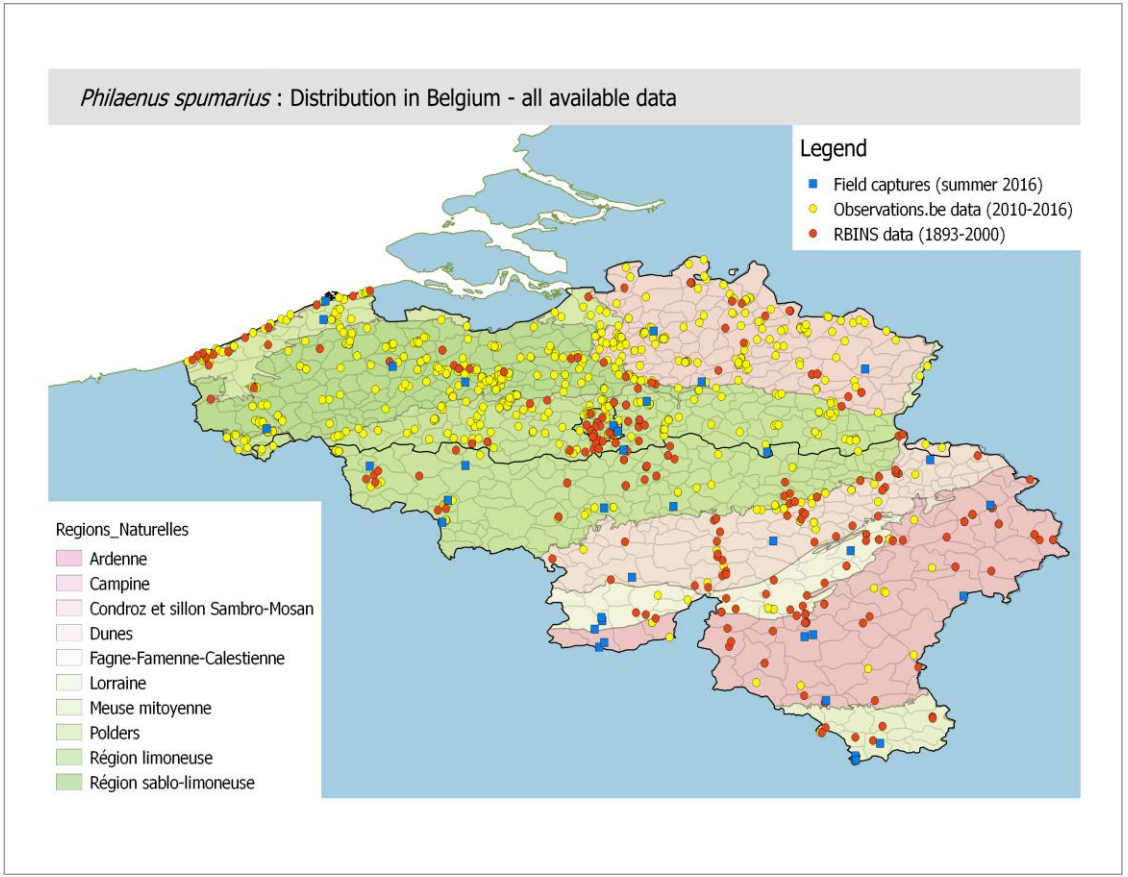

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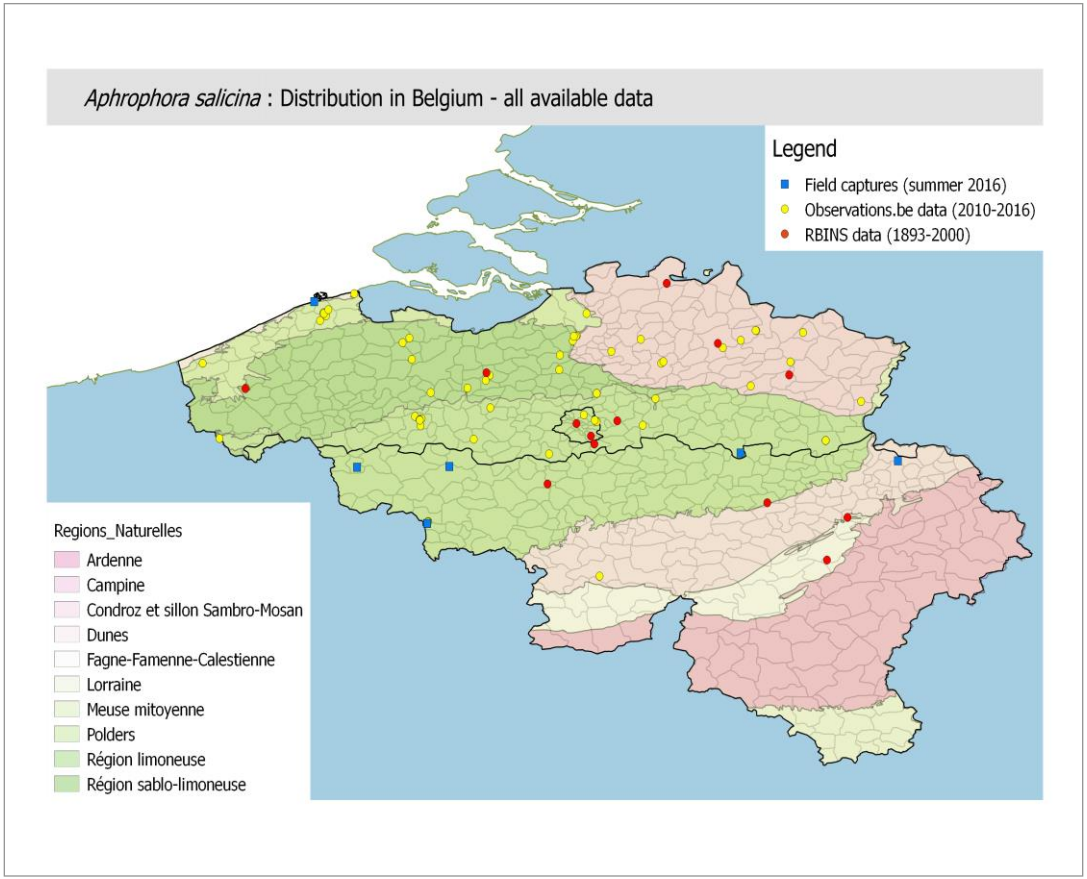

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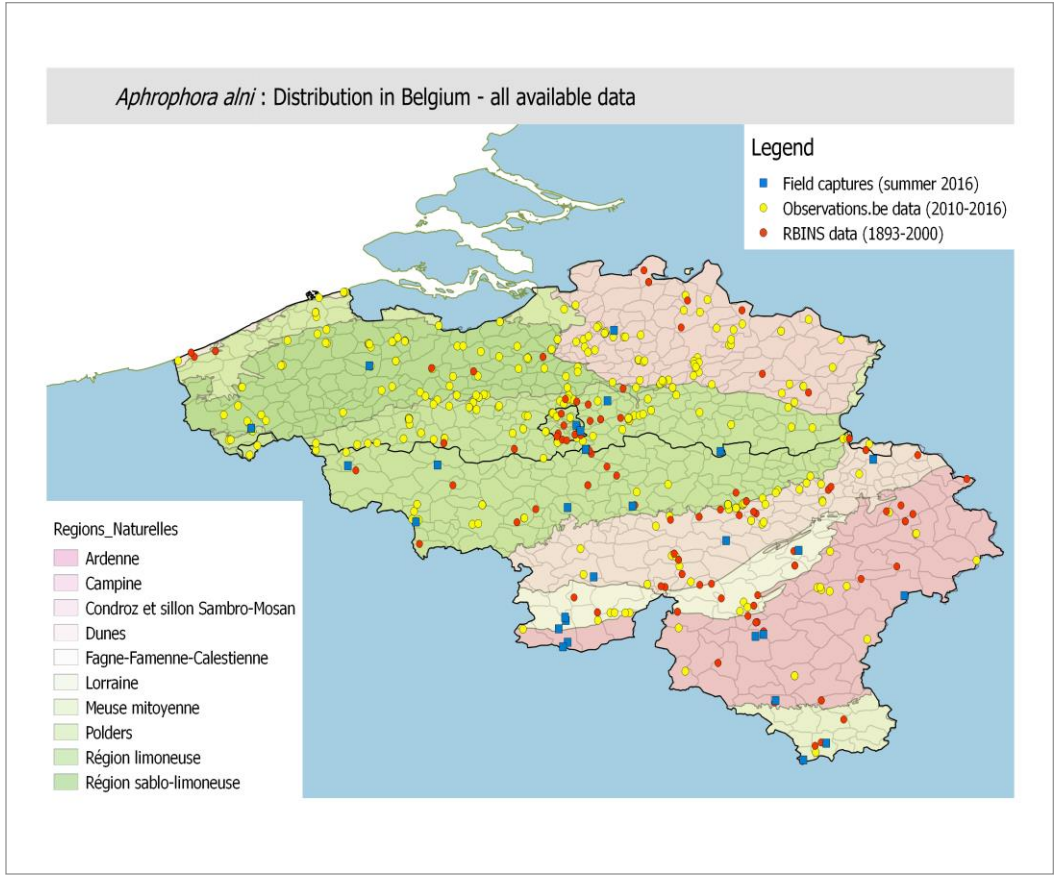

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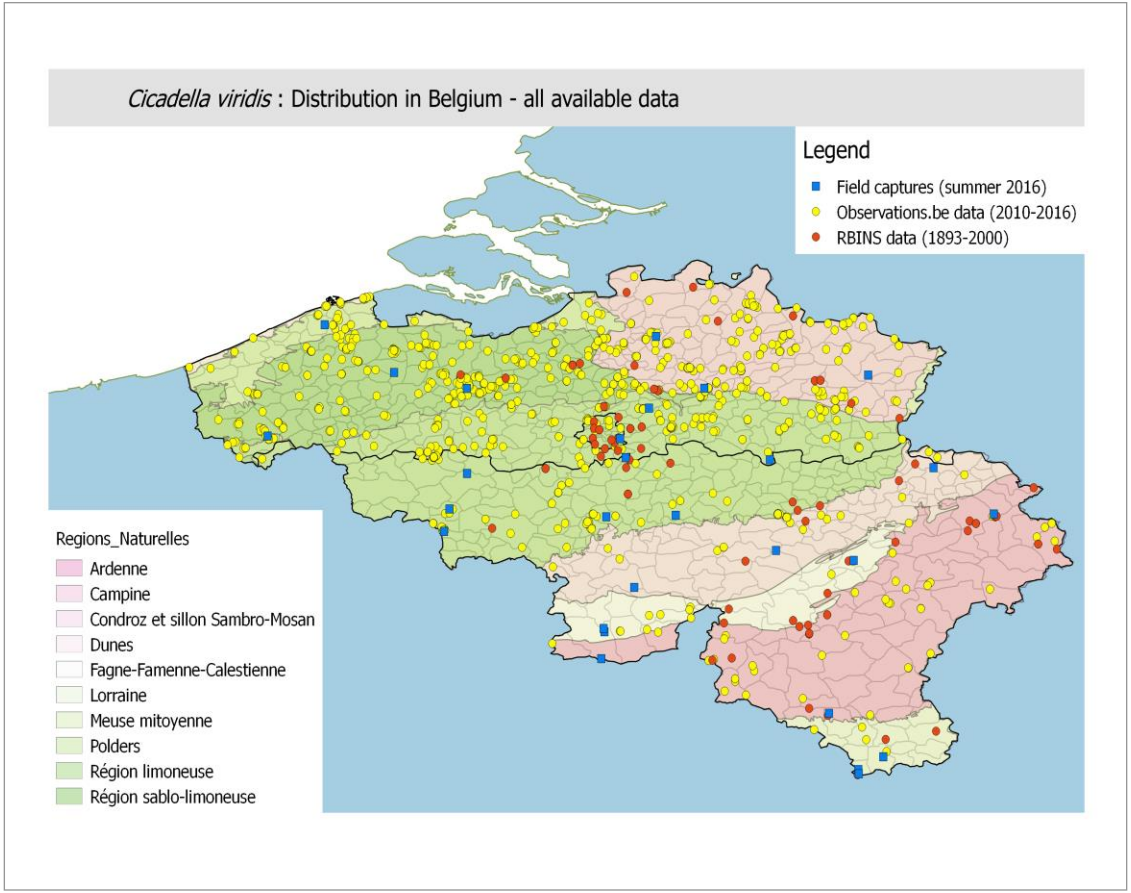

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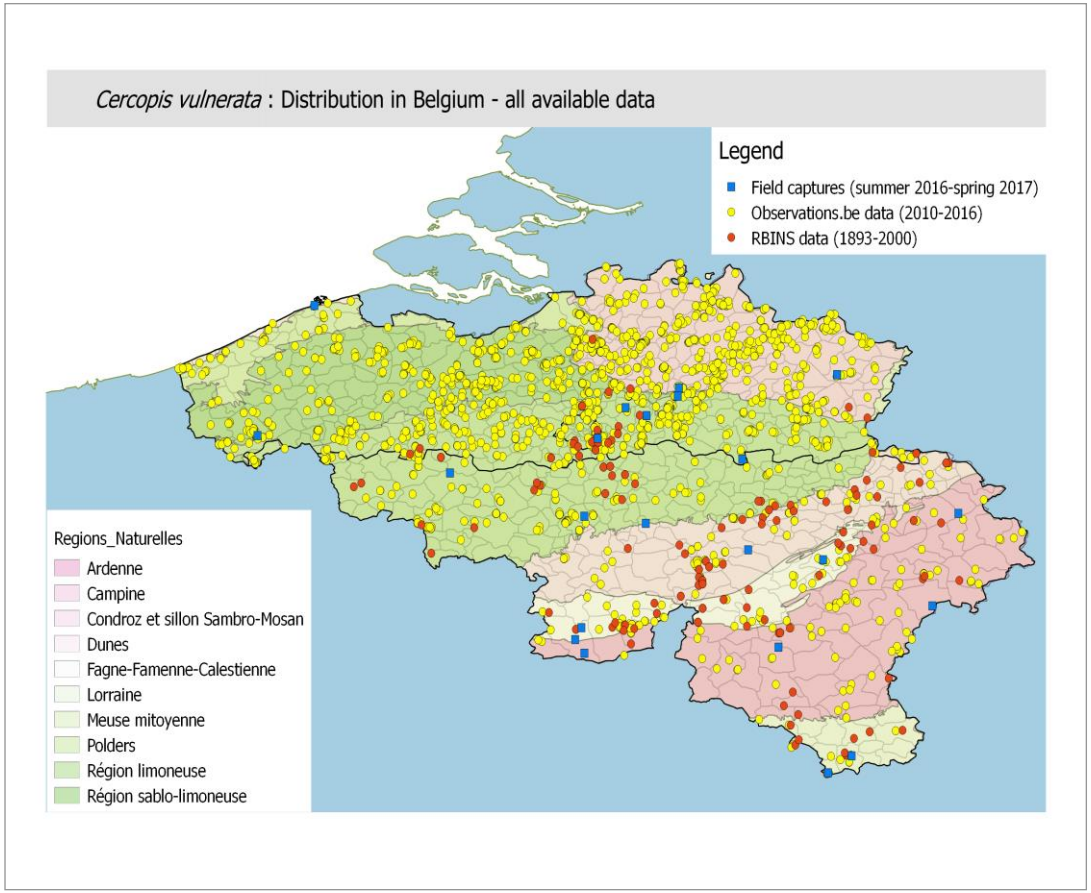
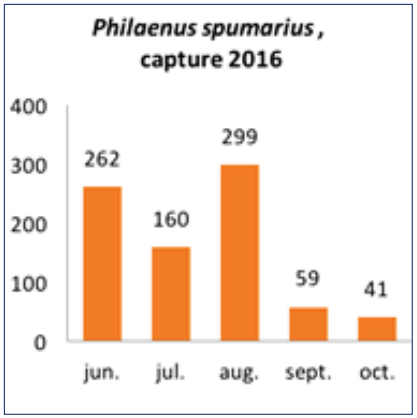
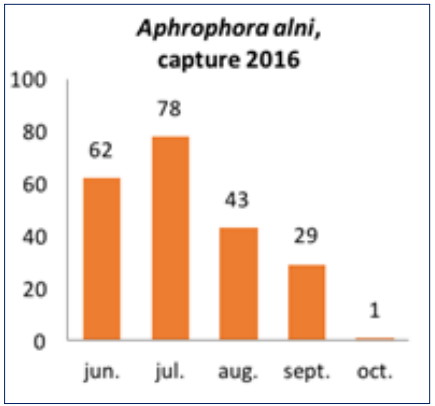
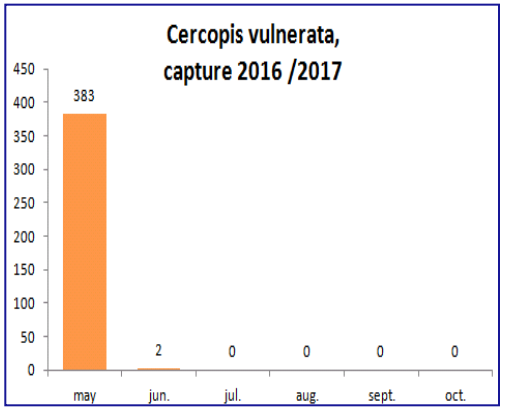
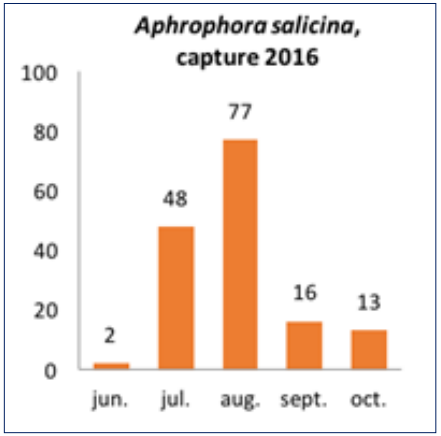


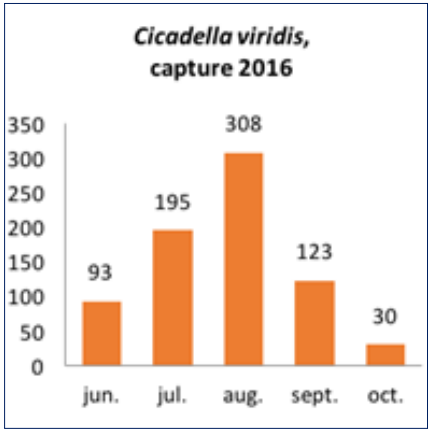
Phenology of the potential vectors – field data



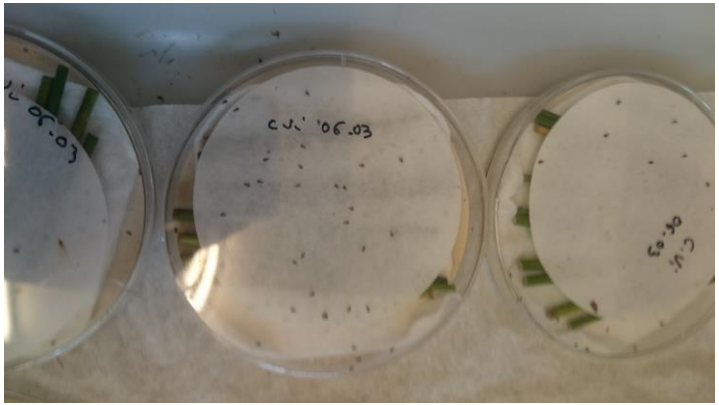






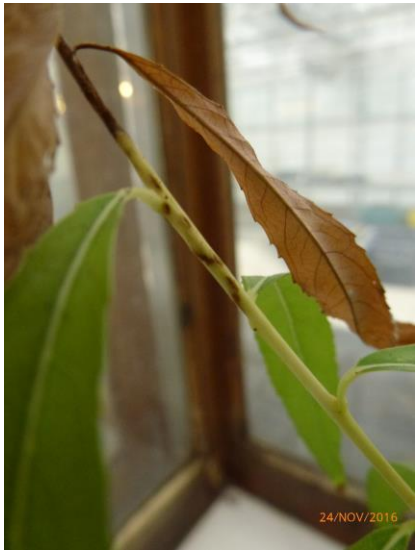


Phenology & life history of the potential vectors
laboratory observations



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
laboratory observations

- 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)

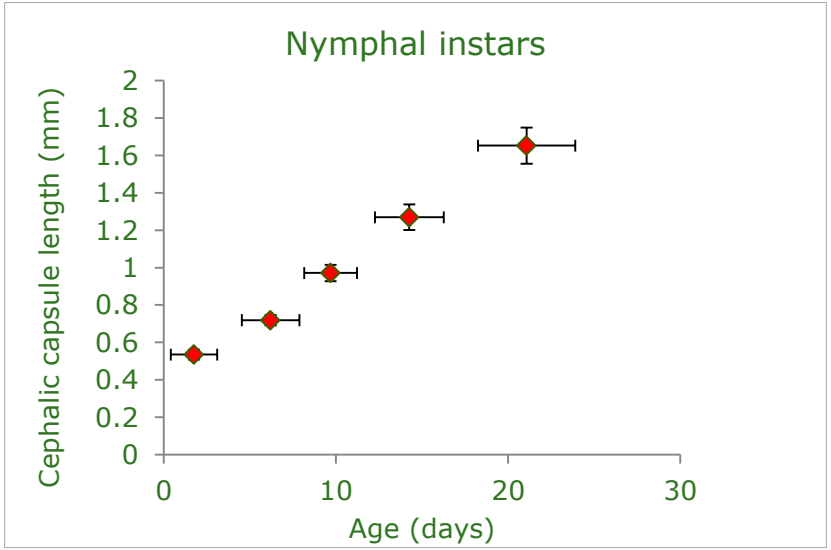
Parasitism rate: **6,67%** (700 field-collected eggs)

Identification pending. Probably *Anagrus incarnatus* Hal. (Mymaridae)

Phenology & life history of the potential vectors
laboratory observations

Immature development of *Cicadella viridis*

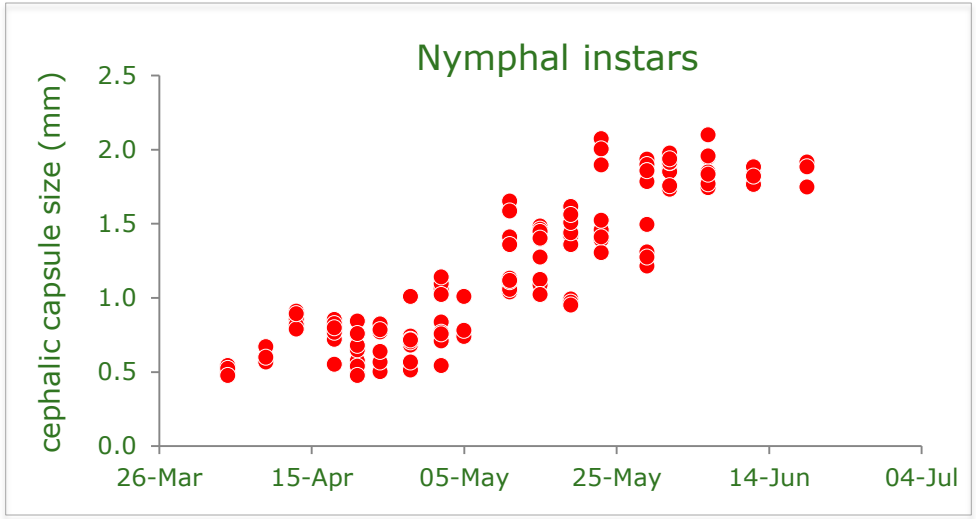
- Five instars
- Nymphs mobiles on grasses
- No spittle
- Cephalic capsule widtg: 0,5 - 1,6 mm according to nymphal instar
- Nymphs 1,4 - 5 mm long, according to nymphal instar



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 widtg: 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

Species	stage	Winter diapause
Philaenus spumarius	eggs	yes
Aphrophora salicina	eggs	yes
Cicadella viridis	eggs	no

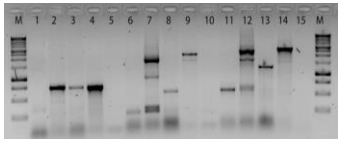
Distribution, phenology & life history of the potential vectors - conclusions

- 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)

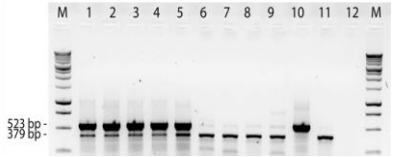
Testing for "*Spy insects*"

- 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. fastodiosa* and targeted insect vectors (Cytochrome oxidase I, ITS non-conserved rDNA);
- High throughput screening of the collected insects.

PCR amplification of the ITS regions of the putative vectors

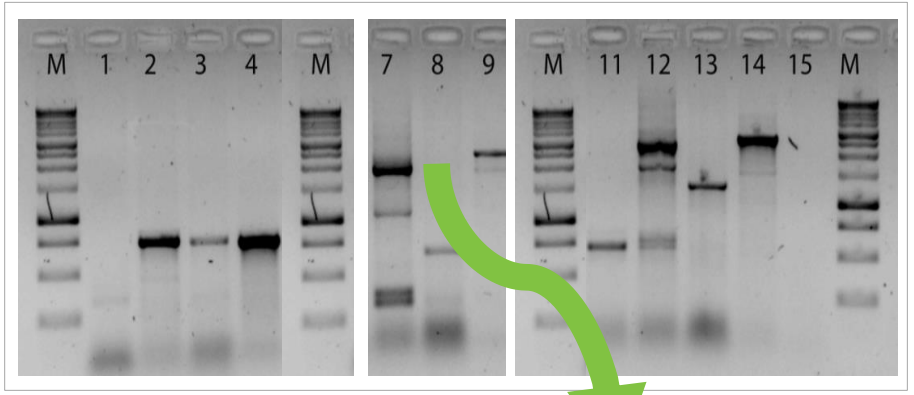


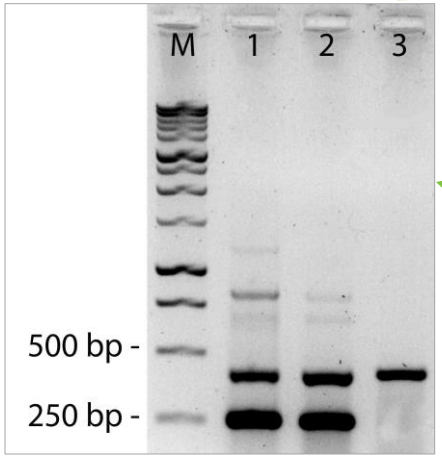
Multiplex PCR of insect DNA spiked with *X. fastidiosa* DNA



Testing for "*Spy insects*"

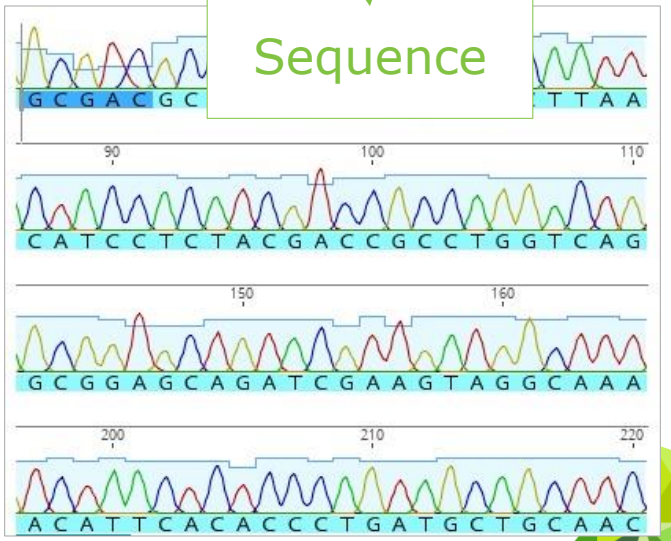
Both *X. fastidiosa*
and insects detected





Primer design

Sequence



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.

Acknowledgements

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- EFSA
- Domenico Bosco, Stefano Demichelis, Luca Picciau, Elisa Plazio, Matteo Ripamonti, Taca Vancheva, Rodrigo Almeida, Pierre Lhoir, Martine Maes, Johan Van Vaerenbergh, Marieke Lippens and many others