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## On the importance of multidisciplinary studies on insect vectors to better understand vector-borne plant diseases

Astrid Cruaud, Marguerite Chartois, Pauline Farigoule, Martin Godefroid, Xavier Mesmin, Ileana Quiquerez, Sabrina Borgomano, François Casabianca, Guénaëlle Genson, Anne-Alicia Gonzalez, et al.

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INRAE



European Commission

Horizon 2020  
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for Research & Innovation



3<sup>rd</sup> European Conference on  
Xylella fastidiosa and XF-ACTORS final meeting

# On the importance of multidisciplinary studies on insect vectors to better understand vector-borne plant diseases

Astrid Cruaud<sup>1</sup>, Marguerite Chartois<sup>1</sup>, Pauline Farigoule<sup>1,2</sup>, Martin Godefroid<sup>1</sup>, Xavier Mesmin<sup>1</sup>, Ileana Quiquerez<sup>3</sup>, Sabrina Borgomano<sup>3</sup>, François Casabianca<sup>4</sup>, Guénaëlle Genson<sup>1</sup>, Anne-Alicia Gonzalez<sup>1</sup>, Laetitia Hugot<sup>3</sup>, Maxime Lambert<sup>1</sup>, Eric Pierre<sup>1</sup>, Sylvain Santoni<sup>5</sup>, Jean-Claude Streito<sup>1</sup>, Jean-Pierre Rossi<sup>1</sup> and Jean-Yves Rasplus<sup>1</sup>

<sup>1</sup> CBGP, INRAE, Montferrier-sur-Lez, France; <sup>2</sup> AgroParisTech, Paris, France; <sup>3</sup> CBNC, OEC, Corte, France. <sup>4</sup> LRDE, INRAE, Corte, France; <sup>5</sup> AGAP, INRAE, Montpellier, France

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# WHY VECTORS ?

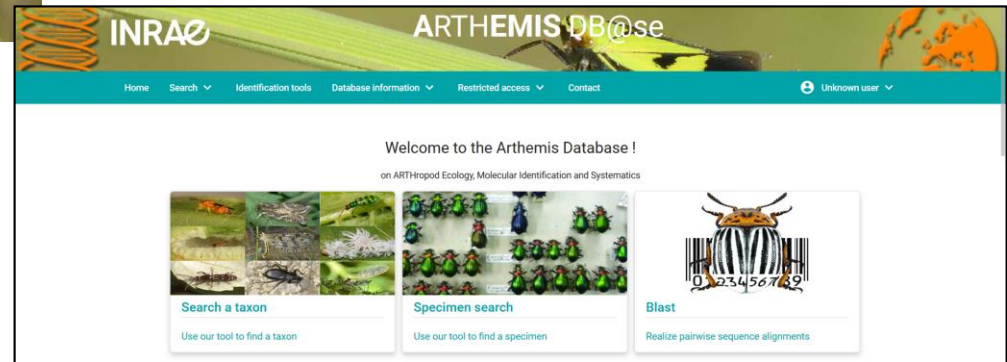
- Because nobody will ever study malaria or dengue without studying mosquitoes..
- Studies on the biology /ecology /distribution of vectors are crucial to understand and control the spread of *Xf*
- Virtually nothing was known for EU vectors before XF-ACTORS
- Significant progresses have been made
- I will report on studies conducted by my research group in the framework of XF-ACTORS and other national (french) projects.

# A CURATED COI DATABASE FOR VECTORS

- Identification based on morphological characters can be difficult, sometimes impossible (eggs, nymphs) and always time consuming
- Massive molecular survey of communities of vectors (e.g. through metabarcoding) will be possible only with a reference database built by taxonomists

<https://arthemisdb.supagro.inra.fr/>

- Several specimens/species
- Different locations when possible
- Non destructive DNA extraction
- 2 step PCR + MiSeq sequencing (Cruaud et al. 2017)
- Bioinformatic workflow with quality controls (contaminants; NUMTs; heteroplasms; introgression)



# A CURATED COI DATABASE FOR VECTORS

- All frequent EU species (#74) have been barcoded + 32 non-EU species
- Pictures of habitus and genitalia
- Identification of a query sequence by BLAST
- Reliable identification for 80% of the species



<https://arthemisdb.supagro.inra.fr/>

Pairwise alignment

Reference database(s)

Select	Name	Location	Status	Matches found
<input type="checkbox"/>	Arthropods	<a href="http://arthemisdb.supagro.inra.fr">http://arthemisdb.supagro.inra.fr</a>		
<input type="checkbox"/>	Non-redundant GenBank	<a href="http://blast.ncbi.nlm.nih.gov/Blast.cgi">http://blast.ncbi.nlm.nih.gov/Blast.cgi</a>	Reachable	0

Pairwise results

My hits

- EF092521.1|100Carpus inermis|18424| Sequences (arthropods)
- ... (other hits)

- *P. spumarius* and *P. tessellatus* same COI and morphologically close → synonyms?
- Possible species complexes within *Neophilaenus* and *Aphrophora* → clarification in progress

Want to know more?

**INRAE** **efsae** **XL**

**A web-interface database for the identification of vectors of *Xylella fastidiosa* in Europe**

Strelto Jean-Claude<sup>1</sup>, Pierre Eric<sup>1</sup>, Genson Guénaëlle<sup>1</sup>, Bellifa Maxime<sup>1</sup>, Charlata Marguerite<sup>1</sup>, Mestrin Xavier<sup>1</sup>, Farigoule Pauline<sup>1,2</sup>, Cruaud Astrid<sup>1</sup> & Raspius Jean-Yves<sup>1</sup>

<sup>1</sup>CIRP, INRAE, CIRAD, IRD, Montpellier Supagro, Univ. Montpellier, Montpellier-sur-Les, France  
<sup>2</sup>UFR Sciences de la Vie et de l'Environnement, Université de Bourgogne, Dijon, France

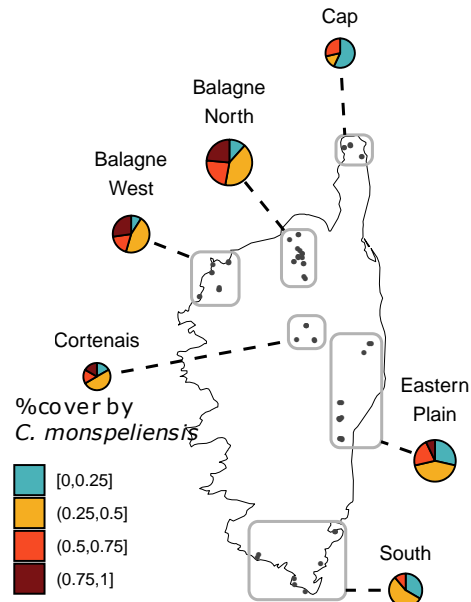
**BIOVEOX** **C-REFP** **INRAE** **EFSA** **XL**

DOI:10.5281/zenodo.4680659

# PHILAENUS SPUMARIUS : KNOW YOUR ENEMY

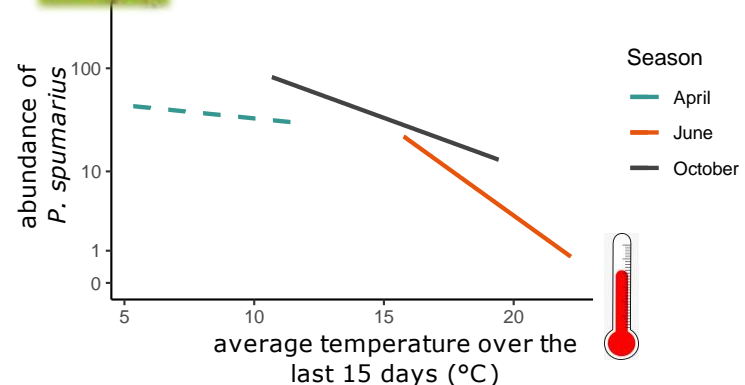
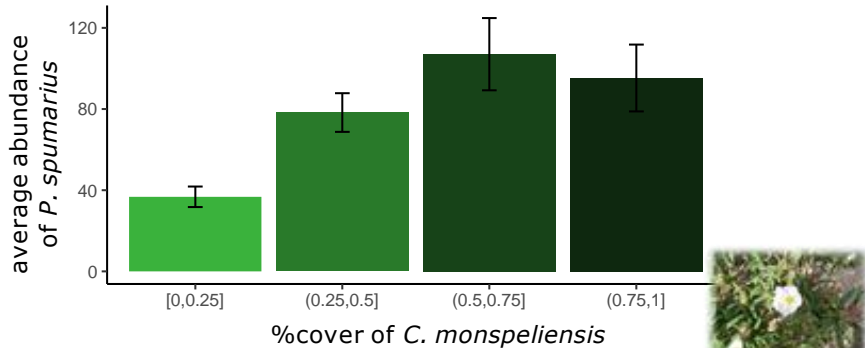
- Extensive research on *Ps* in different countries in the course of XF-ACTORS.
- Climate and vegetation drivers influencing its abundance still poorly known, esp. in semi-natural habitats.

- density of *Ps* monitored 3 times /year during 3 years in 64 plots throughout Corsica
- Visual counting of white foam blobs / sweep netting of adults
- Description of the vegetation + daily temperature retrieved from weather databases
- GLMM



# PHILAEANUS SPUMARIUS : THE CORSICAN EXCEPTION ?

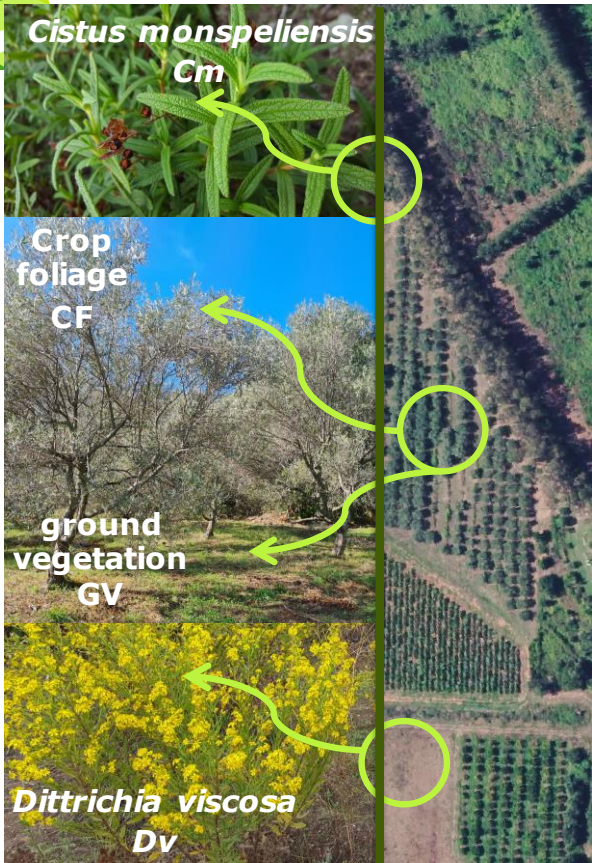
- Abundance of *Ps* positively correlated with density of *Cistus monspeliensis*
  - ➔ contrast with mainland France and other countries in EU, more polyphagous
  - ➔ *Xf* expansion facilitated by disturbed habitats / firebreaks ?
- Abundance of *Ps* negatively correlated with increasing temperatures
  - ➔ Role of *Ps* as vector in summer is reduced (other vectors ?)



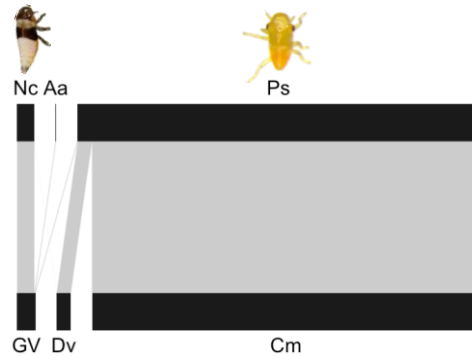


# SPITTLEBUGS IN CROPS: NETWORK OF INTERACTIONS

Survey of spittlebugs in the vicinity of 16 organic clementine and olive groves (2 years)

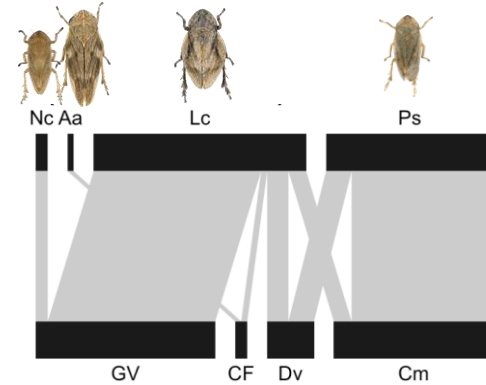


Spring 6647 spittles



- Strong host preferences
- No nymph found on crop foliage

Summer 119 adults

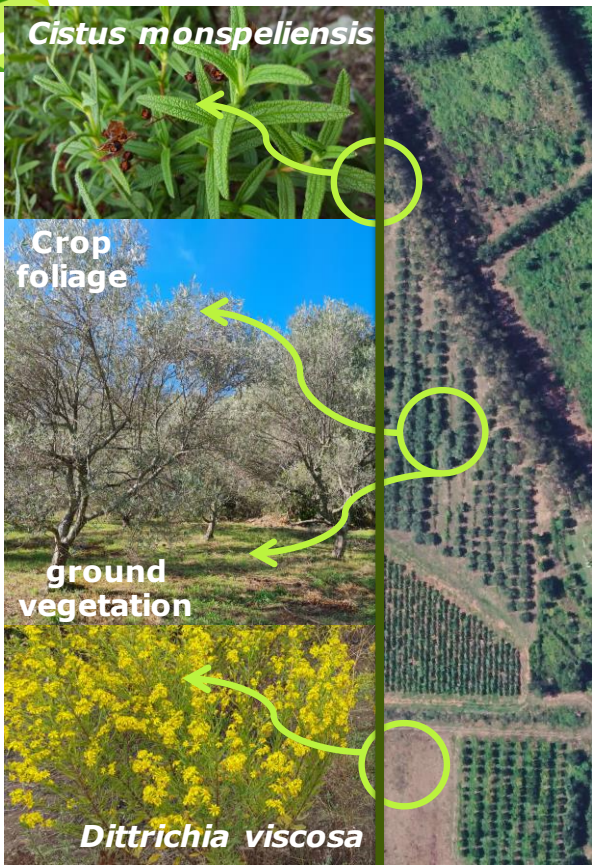


- Few spittlebugs
- Only *A. alni* and *L. coleoptrata* on crop foliage.
- **No summer migration of *Ps* to crop foliage as observed in Italy**

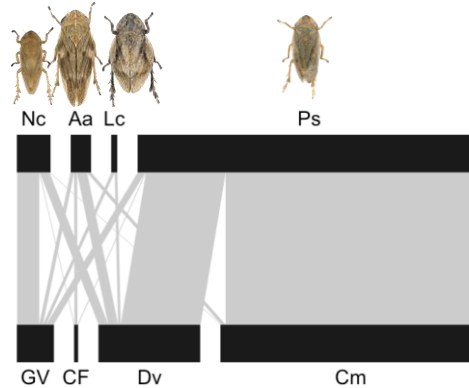


# PHILAEANUS SPUMARIUS: THE CORSICAN EXCEPTION ?

- Survey of spittlebugs in the vicinity of clementine and olive groves (2 years)



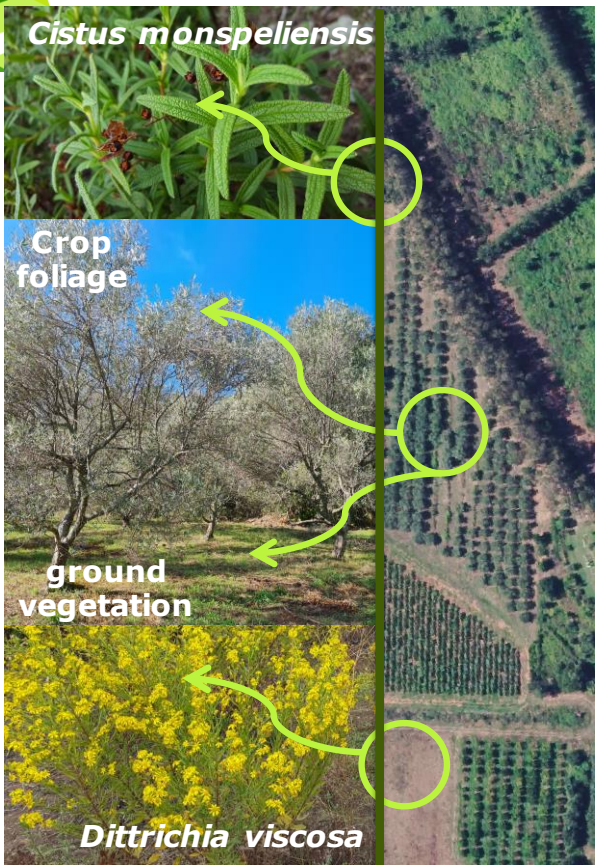
Fall 1595 adults



- complex network
- *Ps* - *Cm* major component
- All species but *Lc* found on crop foliage.
- **Possible role of other vectors need to be investigated**

# PHILAENUS SPUMARIUS : THE CORSICAN EXCEPTION ?

- Survey of spittlebugs in the vicinity of clementine and olive groves (2 years)



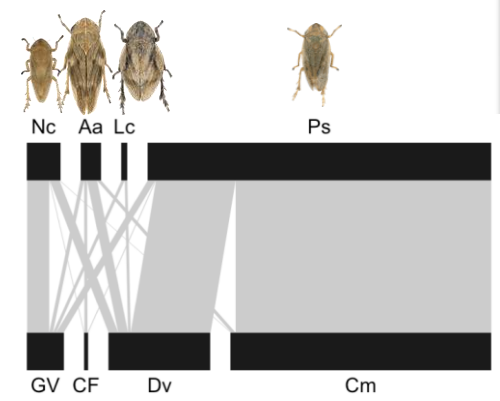
*Cistus monspeliensis*

Crop foliage

ground vegetation

*Dittrichia viscosa*

Fall 1595 adults



- complex network
- *Ps* - *Cm* major component
- All species but *Lc* found on crop foliage.
- **Possible role of other vectors needs to be investigated**

- Soil tillage against *Ps* probably less efficient than in Italy.
- Management of *C. monspeliensis* borders may decrease the threat posed by *Ps* to crops.

Want to know more ?



DOI: 10.5281/zenodo.4680115

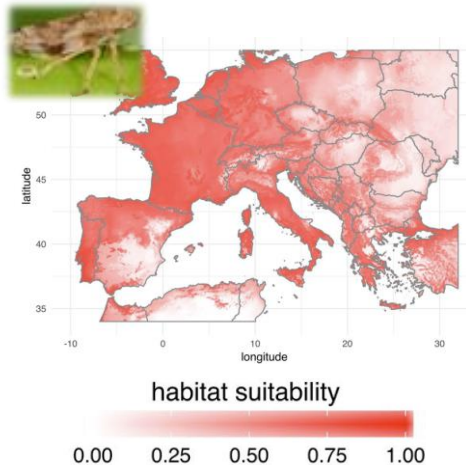


DOI: 10.5281/zenodo.4680290



# PHILAENUS SPUMARIUS : THE PERFECT SENTINEL

- Monitoring of *Xf* usually carried out by analyzing symptomatic plants.
- Plants are frequently asymptomatic
- Generalize the use of insect vectors as sentinels to complement plant survey.
- *Ps* is the perfect sentinel (largely distributed in EU acc. observations / SDMs)
- First test with nested PCR and Sanger sequencing; encouraging results
- High throughput approach needed



## OPEN SCIENTIFIC REPORTS

### Using insects to detect, monitor and predict the distribution of *Xylella fastidiosa*: a case study in Corsica

Received: 4 April 2018  
Accepted: 3 October 2018  
Published online: 23 October 2018

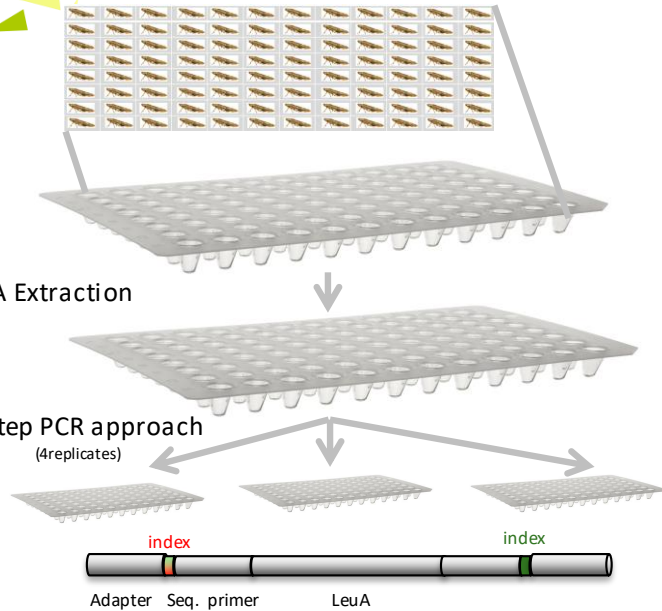
Astrid Cruaud<sup>1</sup>, Anne-Alicia Gonzalez<sup>1,2</sup>, Martin Godefroid<sup>1</sup>, Sabine Nidelet<sup>1</sup>, Jean-Claude Streito<sup>3</sup>, Jean-Marc Thuillier<sup>3</sup>, Jean-Pierre Rossi<sup>3</sup>, Sylvain Santoni<sup>2</sup> & Jean-Yves Rasplus<sup>1</sup>

# PHILAEANUS SPUMARIUS : THE PERFECT SENTINEL

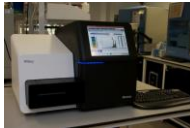
- DNA extraction (by specimen)
- 2 step PCR approach
- first PCR : targeted region is amplified using specific primers flanked by tails
- These tails allow for a second PCR reaction to add Illumina adaptors and indexes to multiplex samples
- MiSeq sequencing + Bioinformatics to get id.
- monitore the spatio-temporal prevalence of *Xf* within populations of *Ps* in Corsica
- 27 populations of 30 specimens each (more coming).
- 4 PCR1 replicates per insect to avoid false negatives

1. DNA Extraction

2. 2-step PCR approach  
(4 replicates)



3. High-throughput sequencing



3. Bioinformatics



OPEN SCIENTIFIC REPORTS

High-throughput sequencing of  
multiple amplicons for barcoding  
and integrative taxonomy

Received: 21 July 2016

Accepted: 03 January 2017

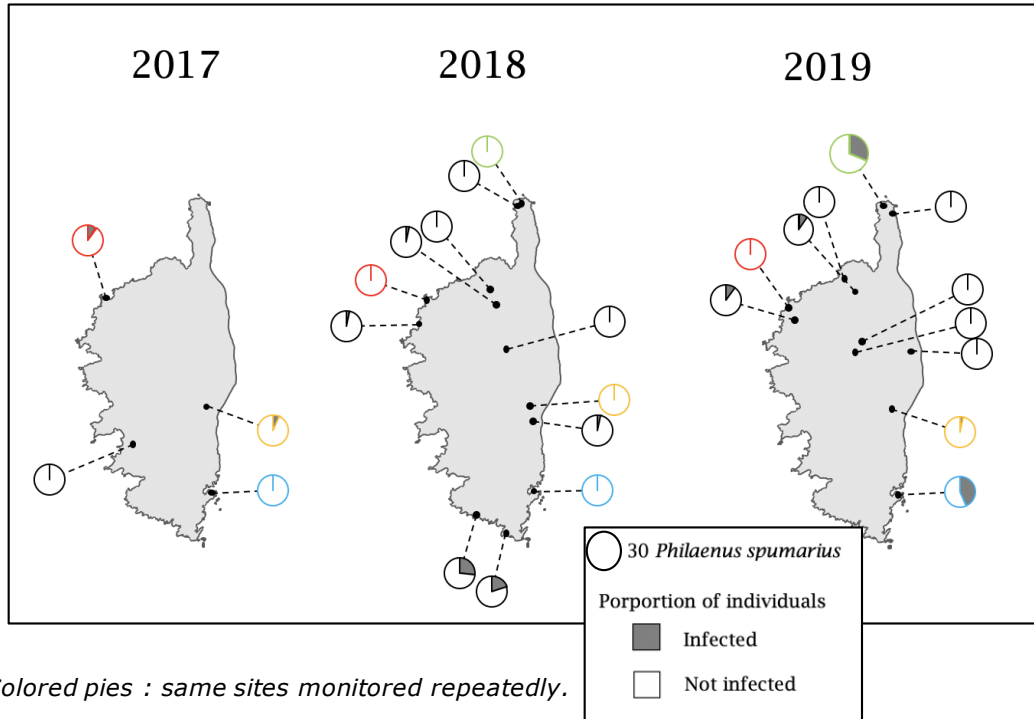
Published: 06 February 2017

Perrine Cruaud<sup>1</sup>, Jean-Yves Rasplus<sup>2</sup>, Lillian Jennifer Rodriguez<sup>1,2</sup> & Astrid Cruaud<sup>1</sup>



# PHILAENUS SPUMARIUS : THE PERFECT SENTINEL

## Preliminary results

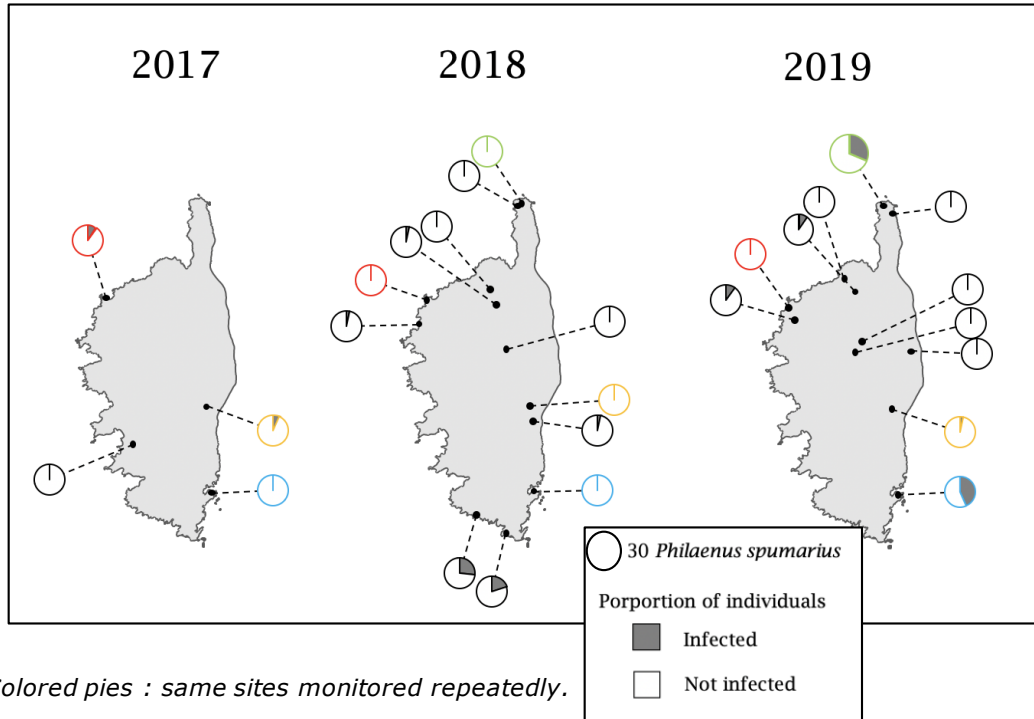


Colored pies : same sites monitored repeatedly.

- *Xf* throughout Corsica
- *Xf* present in presumably uncontaminated areas based on plant monitoring
- No spatial, temporal or vegetation-related pattern of prevalence emerge from our first results.

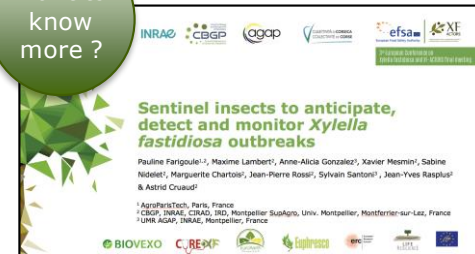
# PHILAEENUS SPUMARIUS : THE PERFECT SENTINEL

## Preliminary results



- *Xf* introduction likely predates 2015
- ecological resilience of Corsican ecosystems linked to plant diversity and lack of monoculture farming ?
- Sentinel insects are good complement to plant survey and their use could be generalized in EU

Want to know more ?



DOI: [10.5281/zenodo.4682793](https://doi.org/10.5281/zenodo.4682793)









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**Thanks for your attention,  
Keep morale up,  
Big up for the organization & technical staff !**

Astrid Cruaud<sup>1</sup>, Marguerite Chartois<sup>1</sup>, Pauline Farigoule<sup>1,2</sup>, Martin Godefroid<sup>1</sup>,  
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