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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  
European Union funding  
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>

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# WHY VECTORS ?

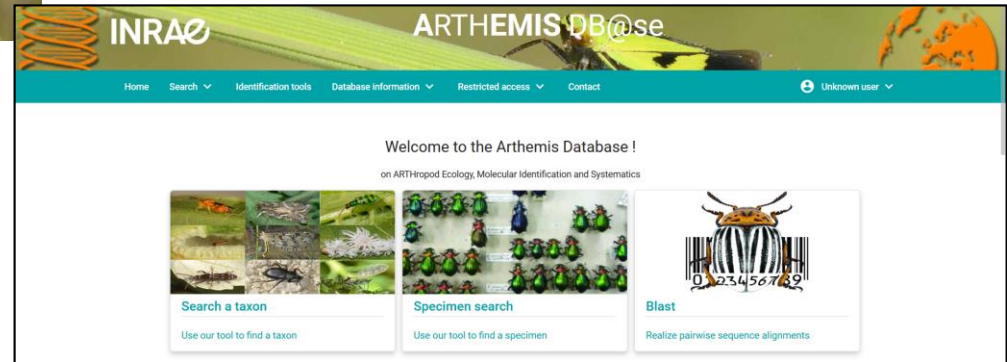
- Because nobody will ever study malaria or dengue without studying mosquitoes..
- Studies on the biology /ecology /distribution of vectors are crucial to understand and control the spread of *Xf*
- Virtually nothing was known for EU vectors before XF-ACTORS
- Significant progresses have been made
- 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 checked="" 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 (Clustering)

**My hits**

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- *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** **efsa** **EU**

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

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

<sup>1</sup>INRAE, UR1464, CIRAD, IRD, Montpellier Supagro, Univ. Montpellier, Montpellier-sur-Les, France  
<sup>2</sup>UMR 1066, INRAE, UR1464, CIRAD, IRD, Montpellier Supagro, Univ. Montpellier, Montpellier-sur-Les, France

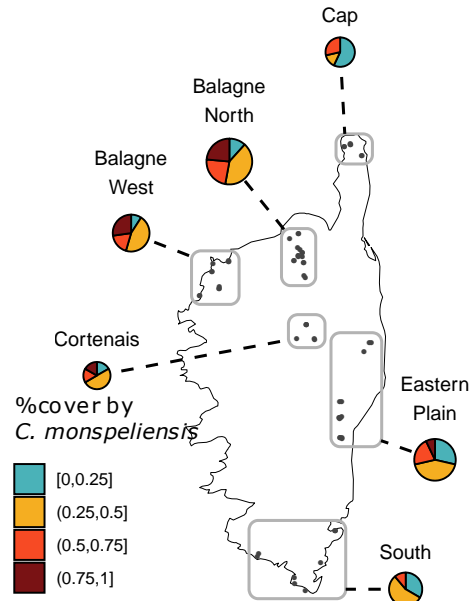
© BIOVEOX C-REFP **INRAE** **efsa** **EU**

DOI:10.5281/zenodo.4680659

# PHILAEANUS SPUMARIUS : KNOW YOUR ENEMY

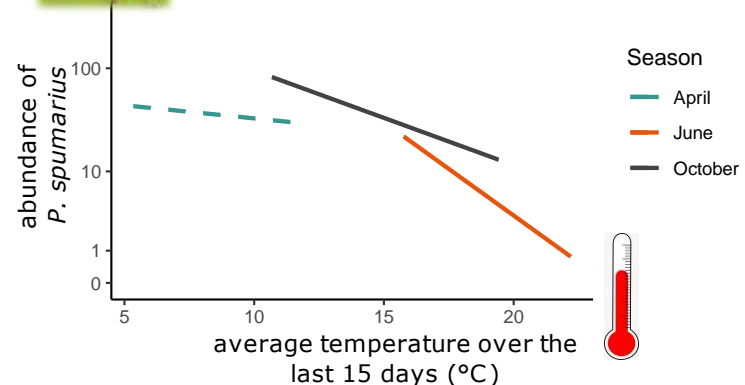
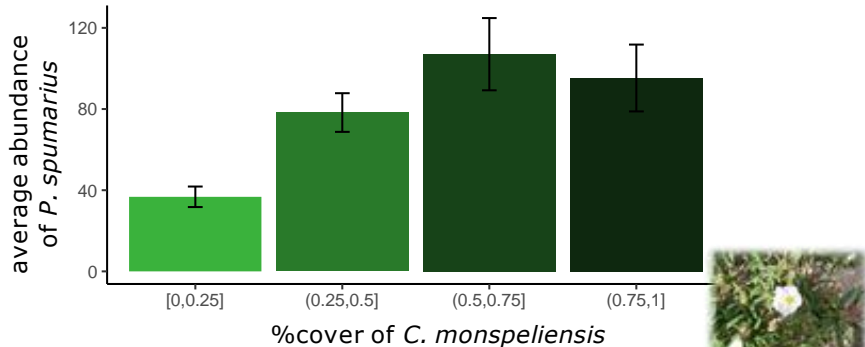
- Extensive research on *Ps* in different countries in the course of XF-ACTORS.
- Climate and vegetation drivers influencing its abundance still poorly known, esp. in semi-natural habitats.

- 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



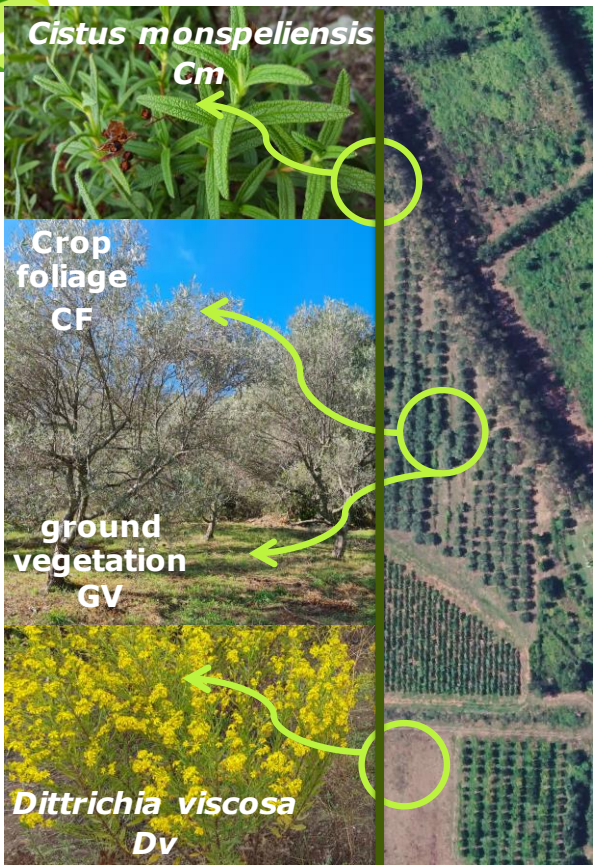
# PHILAENUS SPUMARIUS : THE CORSICAN EXCEPTION ?

- 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 ?)

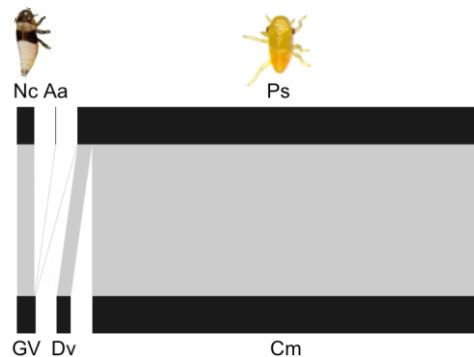


# SPITTLEBUGS IN CROPS: NETWORK OF INTERACTIONS

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

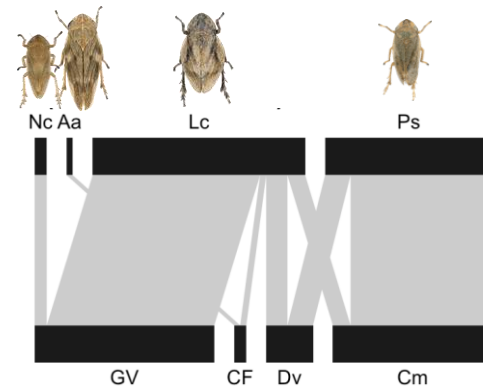


Spring 6647 spittles



- Strong host preferences
- No nymph found on crop foliage

Summer 119 adults

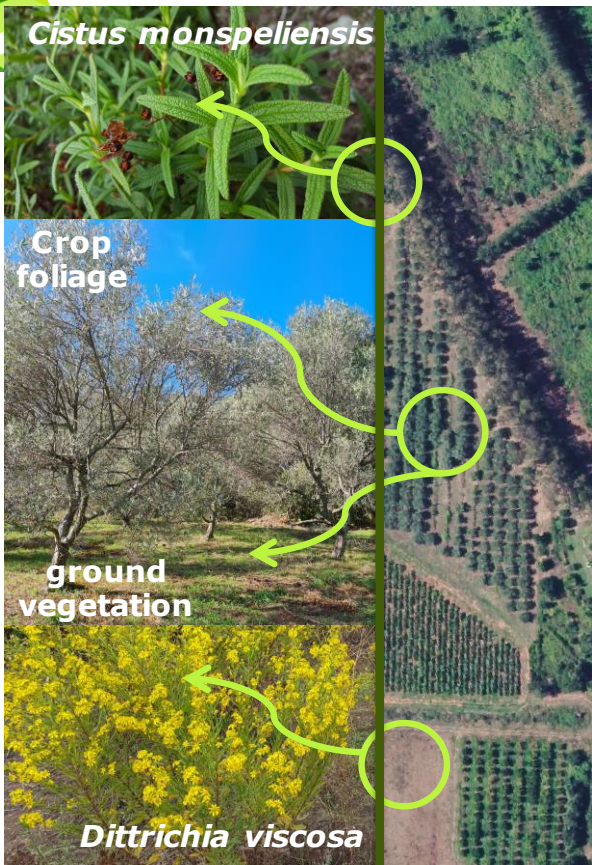


- Few spittlebugs
- Only *A. alni* and *L. coleoptrata* on crop foliage.
- **No summer migration of *Ps* to crop foliage as observed in Italy**

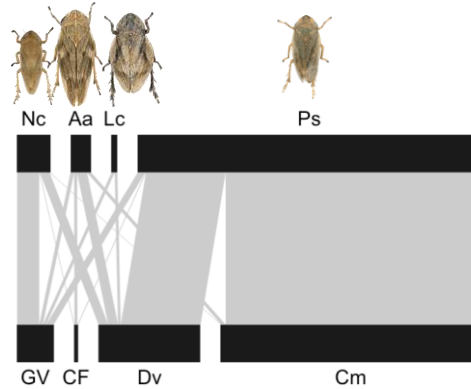


# PHILAEANUS SPUMARIUS: THE CORSICAN EXCEPTION ?

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



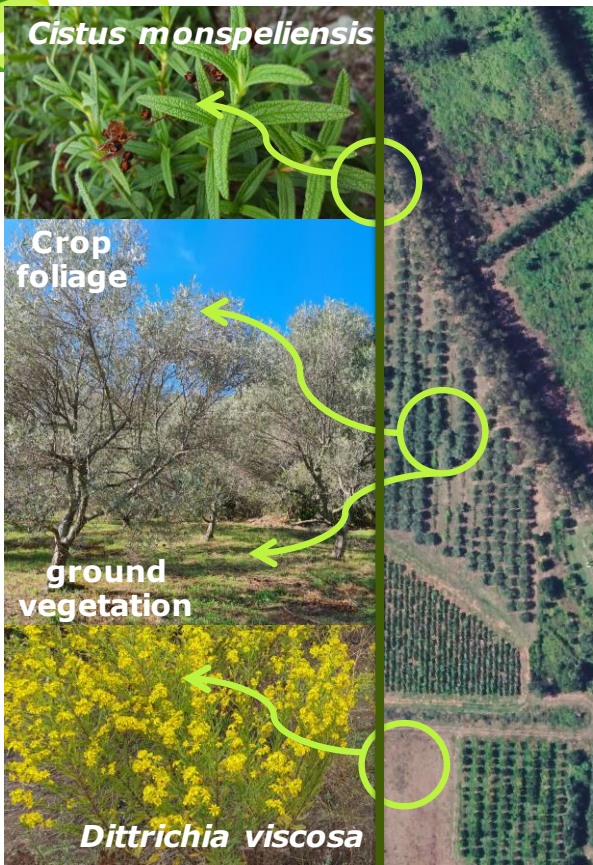
Fall 1595 adults



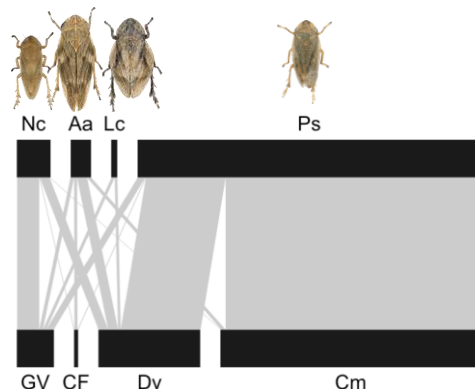
- complex network
- *Ps* - *Cm* major component
- All species but *Lc* found on crop foliage.
- **Possible role of other vectors need to be investigated**

# PHILAENUS SPUMARIUS: THE CORSICAN EXCEPTION ?

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



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 ?

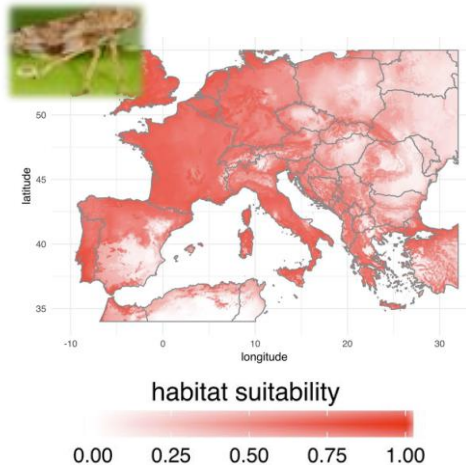
DOI: [10.5281/zenodo.4680115](https://doi.org/10.5281/zenodo.4680115)



DOI: [10.5281/zenodo.4680290](https://doi.org/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

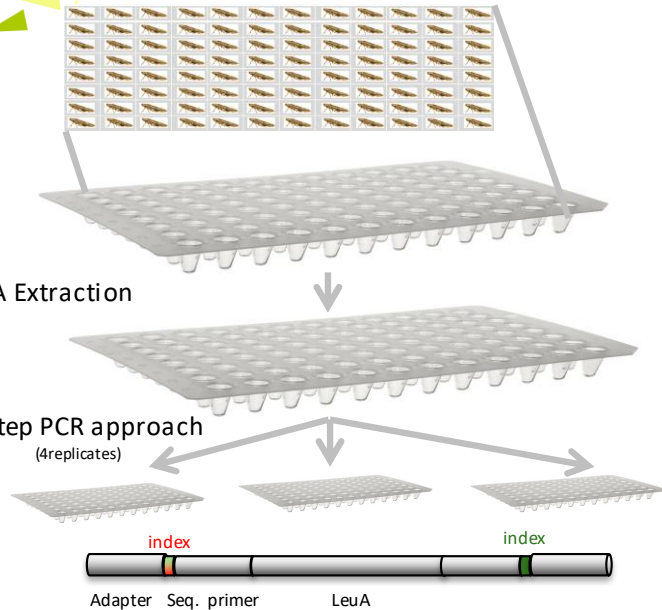
Astrid Cruaud<sup>1</sup>, Anne-Alicia Gonzalez<sup>1,2</sup>, Martin Godefroid<sup>1</sup>, Sabine Nidelet<sup>1</sup>, Jean-Claude Streito<sup>3</sup>, Jean-Marc Thuillier<sup>3</sup>, Jean-Pierre Rossi<sup>3</sup>, Sylvain Santoni<sup>2</sup> & Jean-Yves Rasplus<sup>1</sup>

# PHILAEANUS SPUMARIUS : THE PERFECT SENTINEL

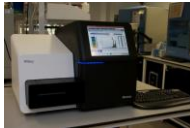
- DNA extraction (by specimen)
- 2 step PCR approach
- first PCR : targeted region is amplified using specific primers flanked by tails
- These tails allow for a second PCR reaction to add Illumina adaptors and indexes to multiplex samples
- MiSeq sequencing + Bioinformatics to get id.
- monitore the 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



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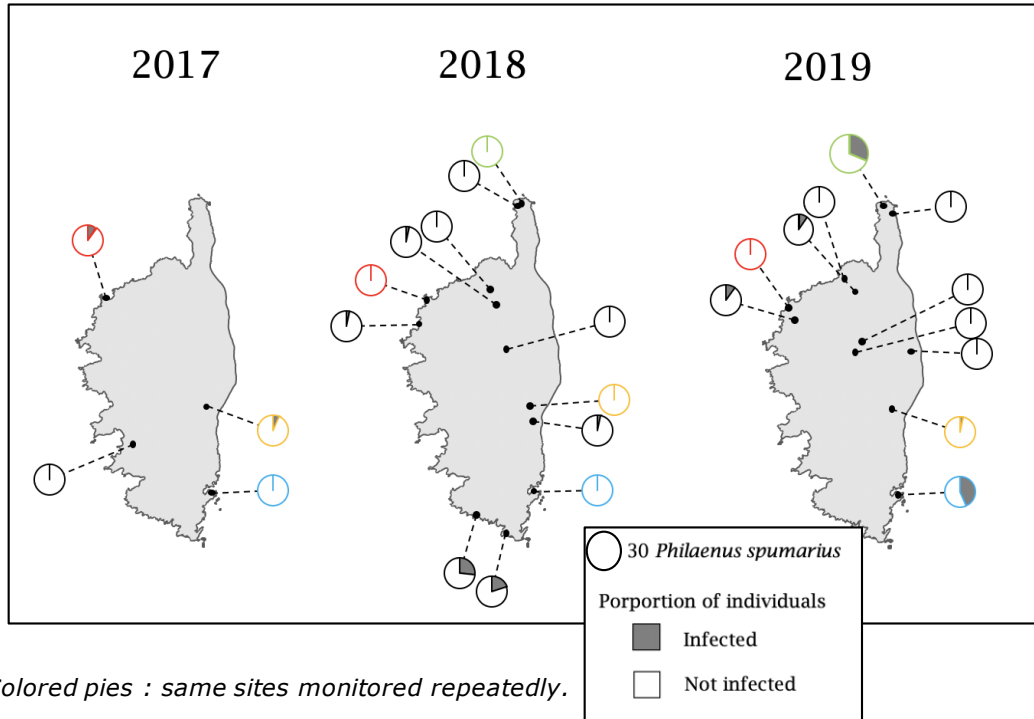
High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy

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

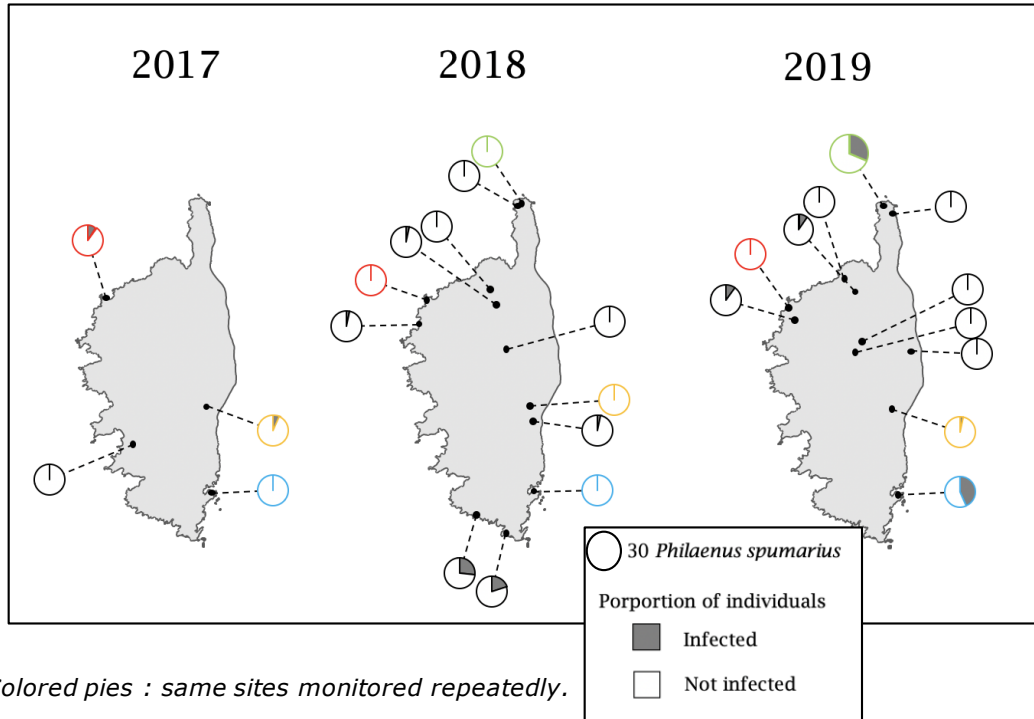
## Preliminary results



- *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



Colored pies : same sites monitored repeatedly.

- *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 ?

**Sentinel insects to anticipate, detect and monitor *Xylella fastidiosa* outbreaks**

Pauline Farigoule<sup>1</sup>, Maxime Lambert<sup>1</sup>, Anne-Alicia Gonzalez<sup>2</sup>, Xavier Mesmin<sup>1</sup>, Sabine Nidelet<sup>1</sup>, Marguerite Chartois<sup>1</sup>, Jean-Pierre Rossi<sup>1</sup>, Sylvain Santoni<sup>1</sup>, Jean-Yves Rasplus<sup>3</sup> & Adrien Coussol<sup>1</sup>

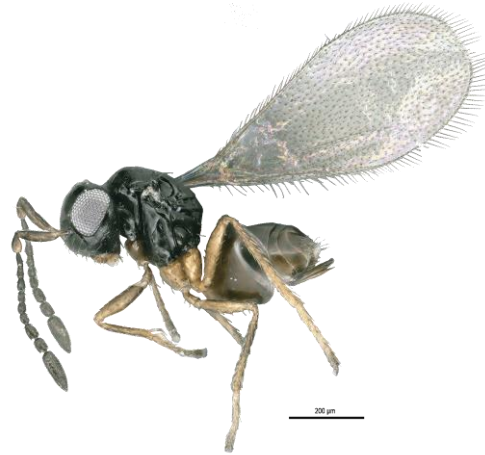
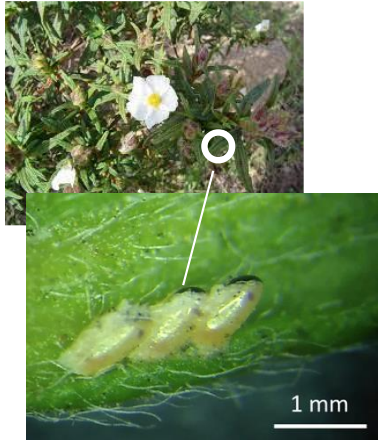
<sup>1</sup>AgropolisToulon, Paris, France  
<sup>2</sup>CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ. Montpellier, Montpellier-sur-Lez, France  
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BIOVEOXO C-REXIF Eupresca

DOI: 10.5281/zenodo.4682793

# PHILAEONUS SPUMARIUS : POSSIBLE CONTROL ?

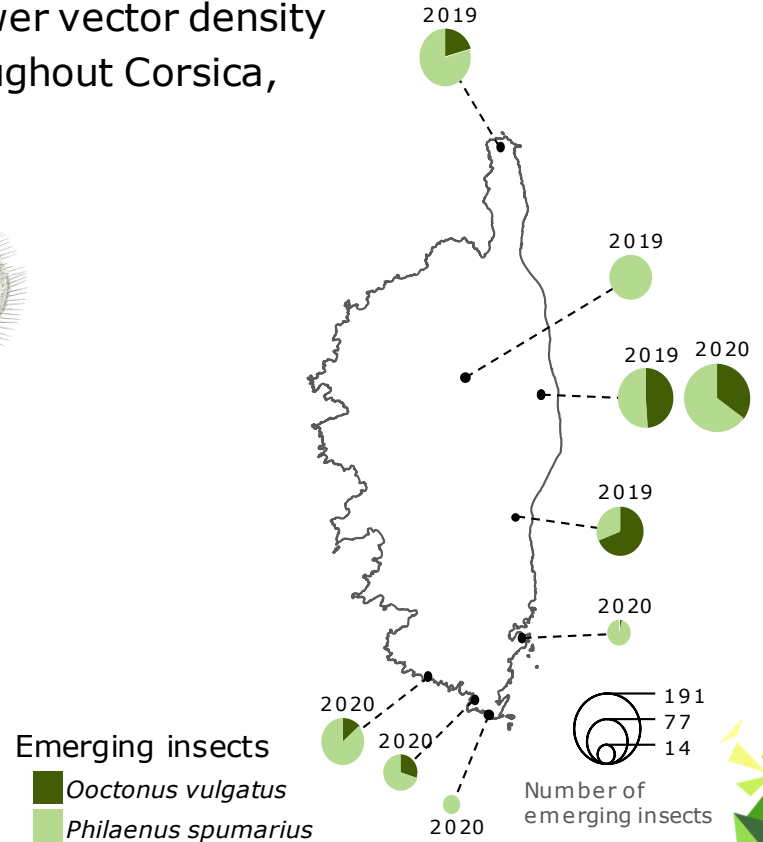
- Overlooked research field: biological control of *Ps* could be an environmental friendly lever to help lower vector density
- Collection of (ca 1100) eggs of *Ps* throughout Corsica,
- Daily monitoring of emergence
- Variable parasitism rates (0-69%)



*Ooctonus vulgatus*  
(Mymaridae)

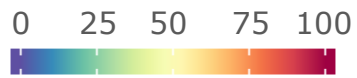
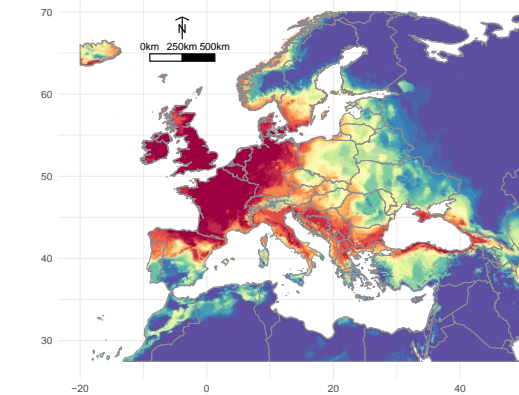
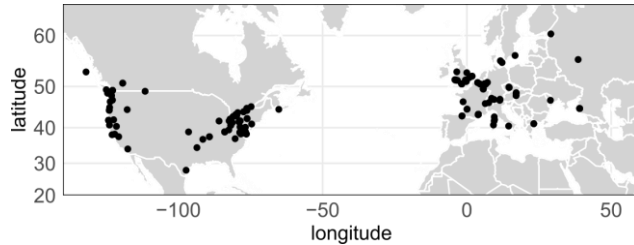


VS



# PHILAEENUS SPUMARIUS : POSSIBLE CONTROL ?

- Occurrences + SDM → *O. vulgatus* occurs or is likely to occur in many EU regions where *P. spumarius* also occurs



Proportion of models predicting *O. vulgatus* presence (%)

Provided that host specificity is confirmed and that mass rearing is possible, *O. vulgatus* could contribute to IPM of *P. spumarius*, and more generally, of Xf pathosystem

Want to know more ?

Wanted egg parasitoids: *Ooconus vulgatus* parasitizes *Philaenus spumarius* in Corsica and is probably widely distributed in Europe

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Logos: BIOVEOXO, C. RE Xf, INRAE, European Commission, efsa, Xf, Agence Nationale de Sécurité Sanitaire, Agence Française de Sécurité Alimentaire, Agence Française de Santé, Agence Française de Sécurité Chimique, Agence Française de Sécurité Nucléaire, Agence Française de Sécurité Industrielle, Agence Française de Sécurité Environnementale, Agence Française de Sécurité Sanitaire, Agence Française de Sécurité Alimentaire, Agence Française de Santé, Agence Française de Sécurité Chimique, Agence Française de Sécurité Nucléaire, Agence Française de Sécurité Industrielle, Agence Française de Sécurité Environnementale.

DOI: 10.5281/zenodo.4680103

**PeerU** *Ooconus vulgatus* (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) the main vector of *Xylella fastidiosa* in Europe

Xavier Mesmin<sup>1,2</sup>, Marguerite Chartois<sup>1</sup>, Guénaëlle Genson<sup>1</sup>, Jean-Pierre Rossi<sup>1</sup>, Astrid Cruaud<sup>1</sup> and Jean-Yves Rasplus<sup>2</sup>





**INRAE**



European  
Commission

Horizon 2020  
European Union funding  
for Research & Innovation



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

**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>,  
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>

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