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.

3rd 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[1], Marguerite Chartois[1], Pauline Farigoule[1,2], Martin Godefroid[1], Xavier Mesmin[1], Ileana Quiquerez[3], Sabrina Borgomano[3], François Casabianca[4], Guénaëlle Genson[1], Anne-Alicia Gonzalez[1], Laetitia Hugot[3], Maxime Lambert[1], Éric Pierre[1], Sylvain Santoni[5], Jean-Claude Streito[1], Jean-Pierre Rossi[1] and Jean-Yves Rasplus[1]

[1] CBGP, INRAE, Montferrier-sur-Lez, France; [2] AgroParisTech, Paris, France; [3] CBNC, OEC, Corte, France. [4] LRDE, INRAE, Corte, France; [5] 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

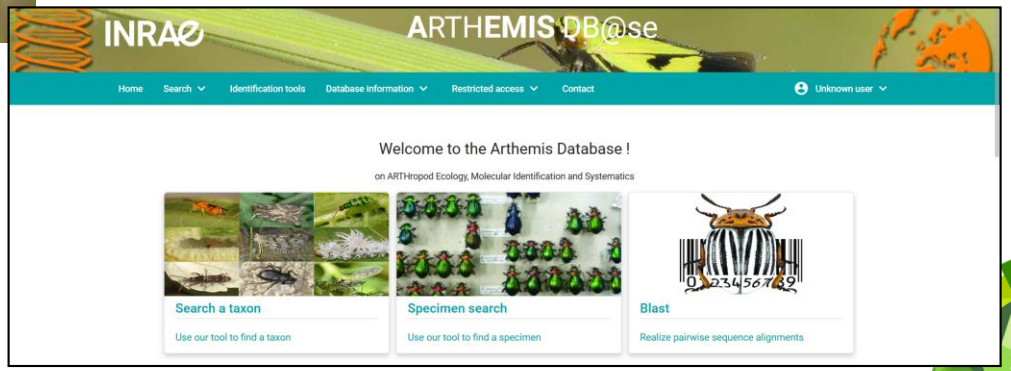
- Identification based on morphological characters can be difficult, sometimes impossible (eggs, nymphs) and always time consuming

- Massive molecular survey of communities of vectors (e.g. through metabarcoding) will be possible only with a reference database built by taxonomists

- 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)

Cruaud et al. 2017. *Scientific reports* 7:41948

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

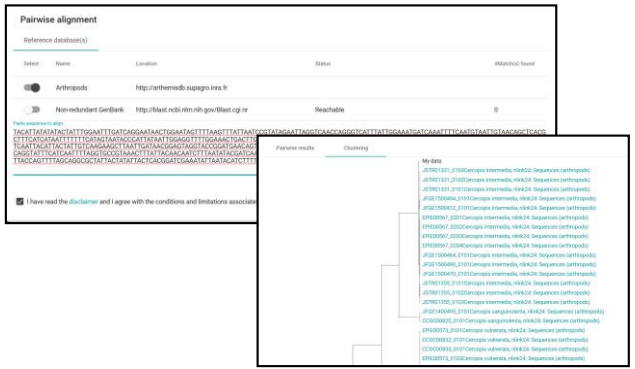


A CURATED *COI* DATABASE FOR VECTORS

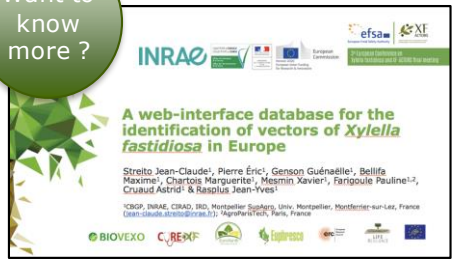
- All frequent EU species (#74) have been barcoded + 32 non-EU species
- Pictures of habitus and genitalia
- Identification of a query sequence by BLAST
- Reliable identification for 80% of the species

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

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



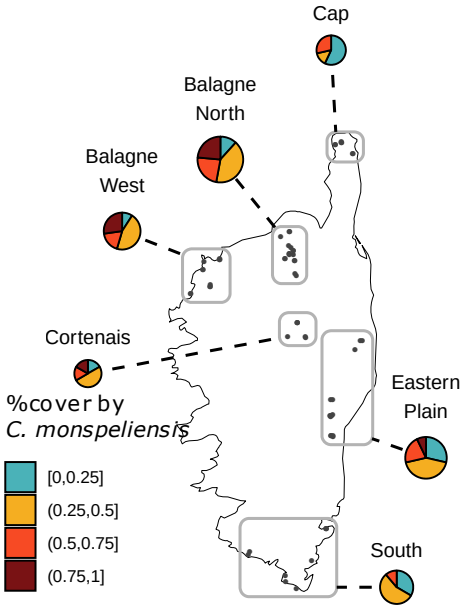
Want to know more ?



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

PHILAENUS SPUMARIUS : THE CORSICAN EXCEPTION ?

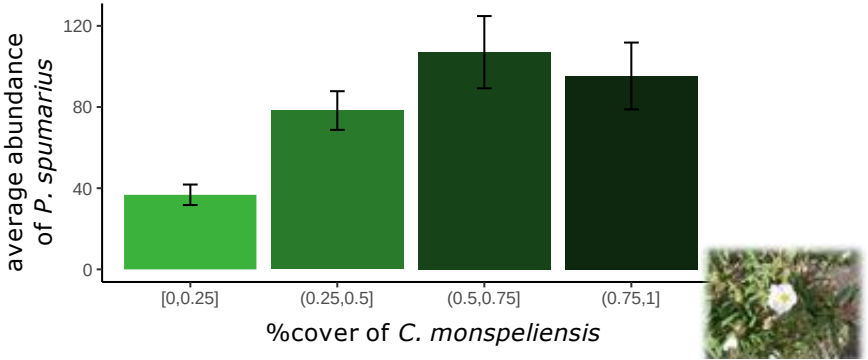
- Abundance of *Ps* positively correlated with density of *Cistus monspeliensis*

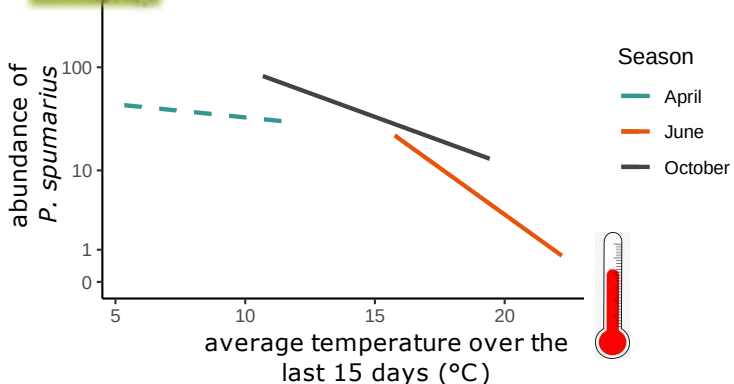
➔ contrast with mainland France and other countries in EU, more polyphagous

➔ *Xf* expansion facilitated by disturbed habitats / firebreaks ?

- Abundance of *Ps* negatively correlated with increasing temperatures

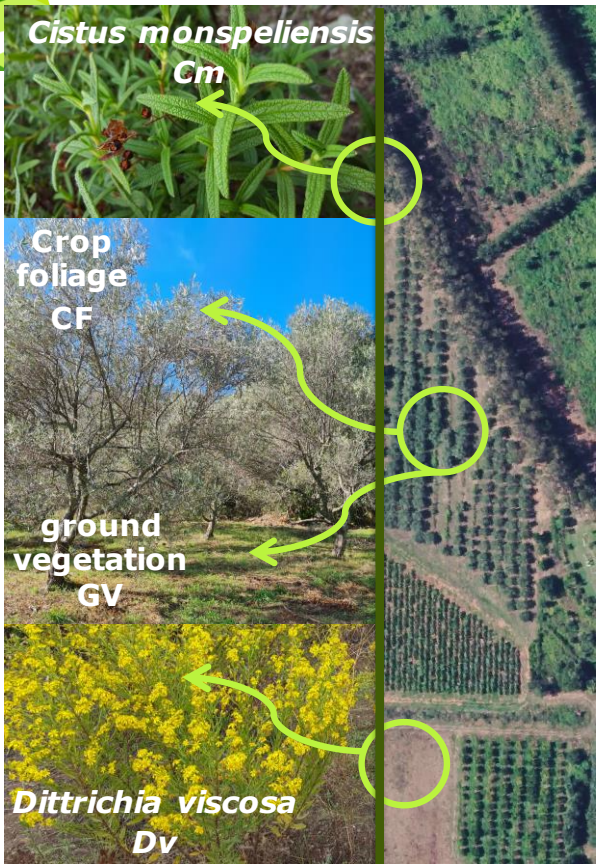
➔ Role of Ps as vector is summer is reduced (other vectors ?)



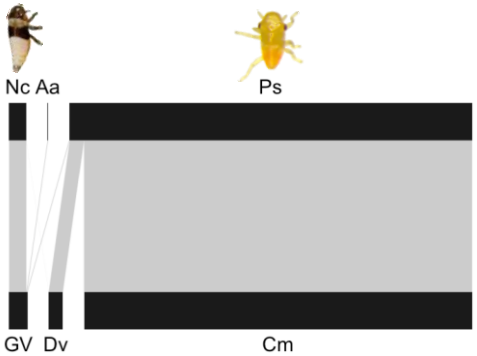


SPITTLEBUGS IN CROPS: NETWORK OF INTERACTIONS

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

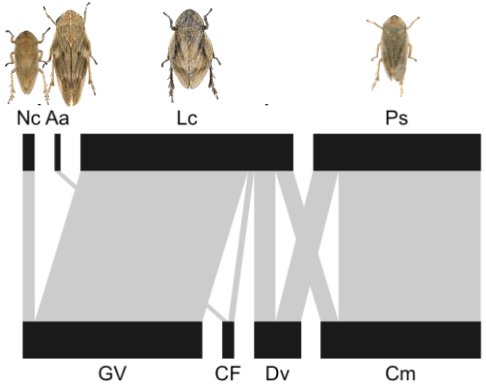


Spring 6647 spittles



- Strong host preferences
- No nymph found on crop foliage

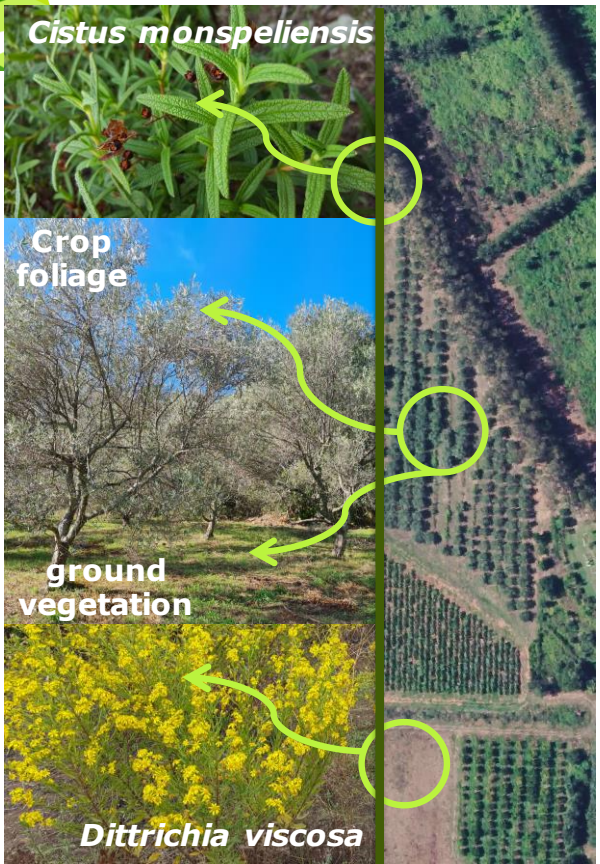
Summer 119 adults



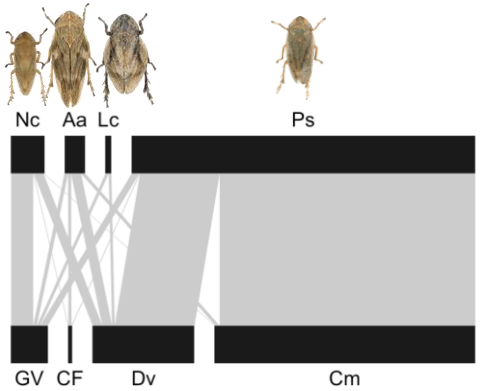
- Few spittlebugs
- Only *A. alni* and *L. coleoptrata* on crop foliage.
- **No summer migration of *Ps* to crop foliage as observed in Italy**

PHILAENUS SPUMARIUS : THE CORSICAN EXCEPTION ?

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



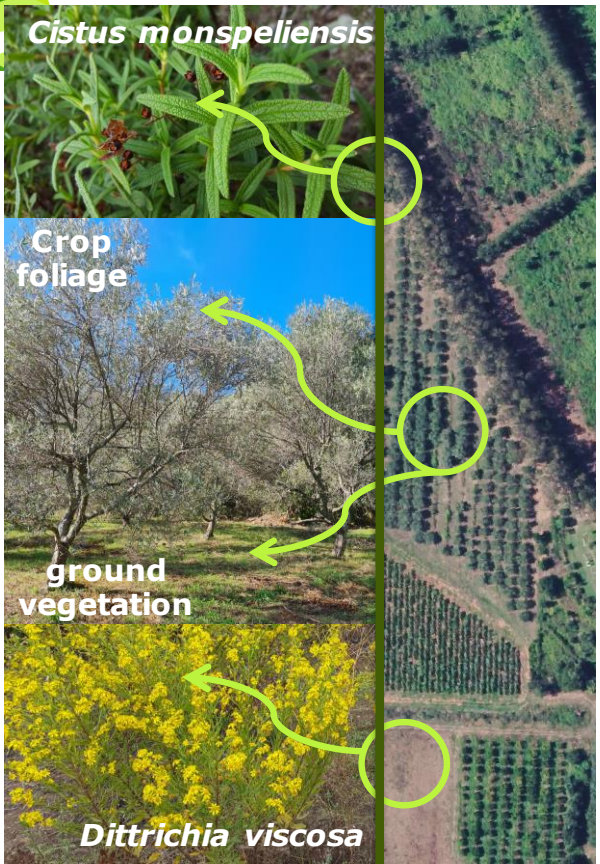
Fall 1595 adults



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

PHILAENUS SPUMARIUS : THE CORSICAN EXCEPTION ?

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



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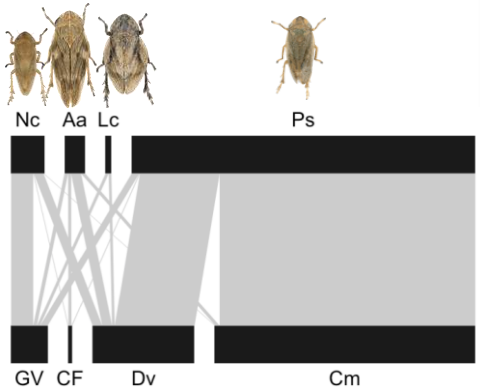


- complex network
- *Ps* - *Cm* major component
- All species but *Lc* found on crop foliage.
- ***Possible role of other vectors needs to be investigated***

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

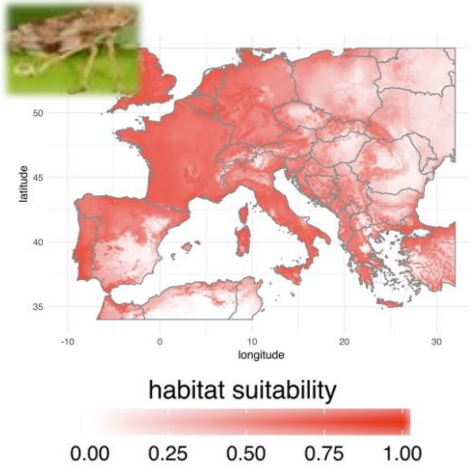
Want to know more ?

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

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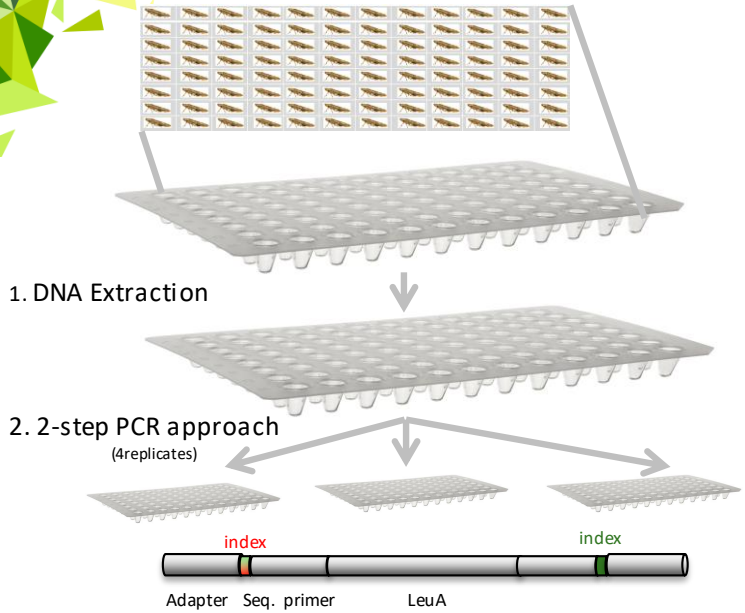
Published online: 23 October 2018

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

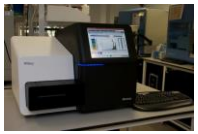
Astrid Cruaud[1], Anne-Alicia Gonzalez[1,2], Martin Godefroid[1], Sabine Nidelet[1], Jean-Claude Streito[1], Jean-Marc Thuillier[1], Jean-Pierre Rossi[1], Sylvain Santoni[2] & Jean-Yves Rasplus[1]

PHILAENUS 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

3. High-throughput sequencing



3. Bioinformatics


OPEN SCIENTIFIC REPORTS

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

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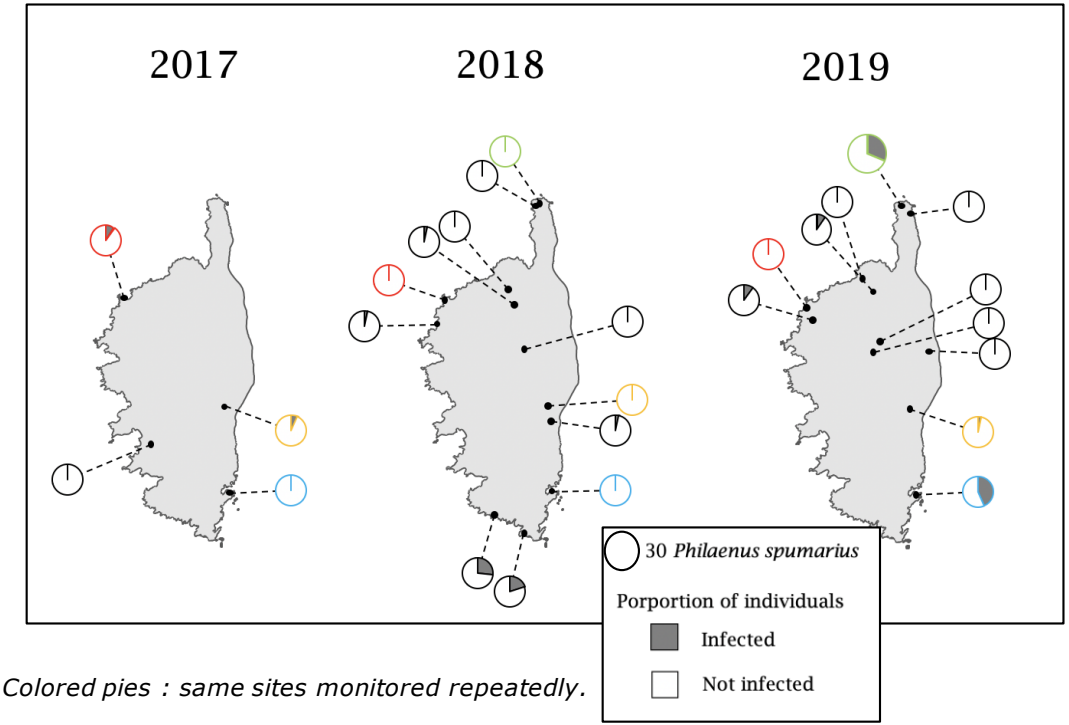
Published: 06 February 2017

Perrine Cruaud[1], Jean-Yves Rasplus[1], Lillian Jennifer Rodriguez[1,2] & Astrid Cruaud[1]

PHILAENUS SPUMARIUS : THE PERFECT SENTINEL

Preliminary results

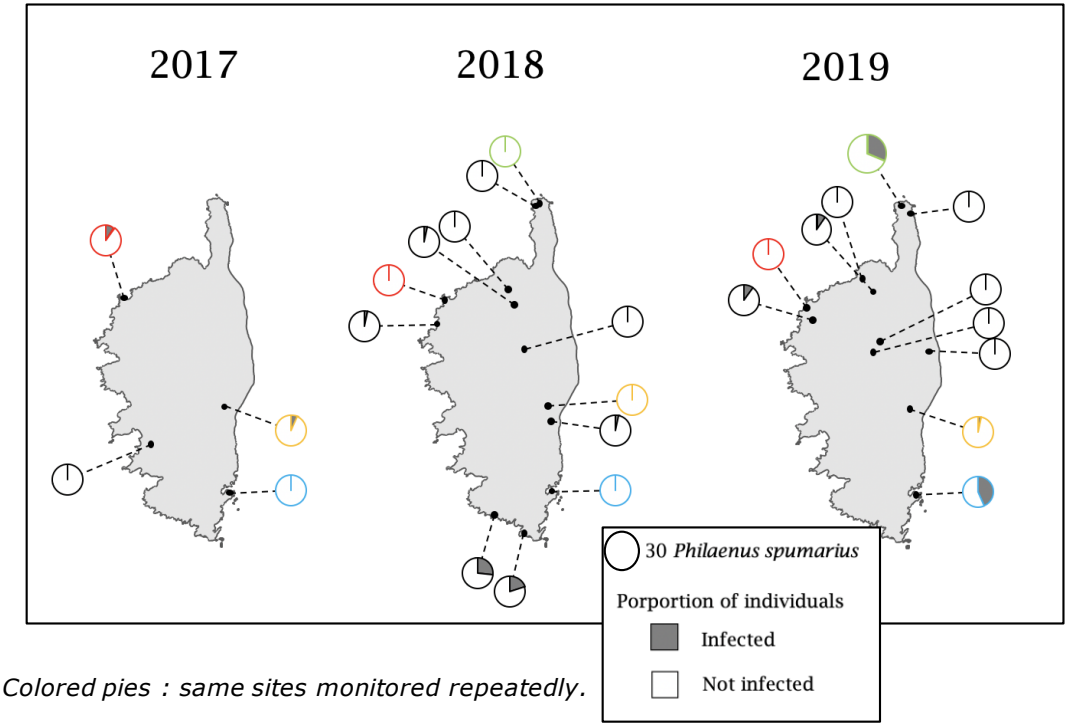


Colored pies : same sites monitored repeatedly.

- *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.

PHILAENUS SPUMARIUS : THE PERFECT SENTINEL

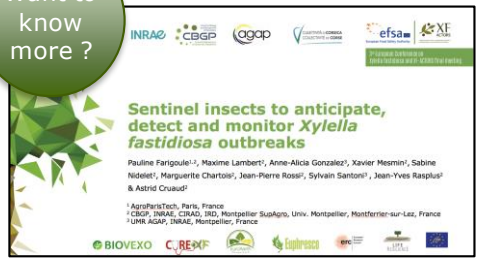
Preliminary results



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

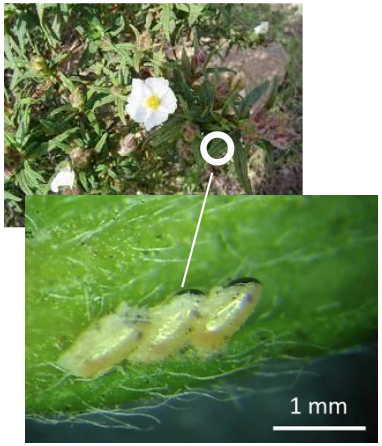
Want to know more ?

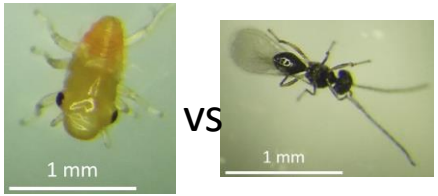


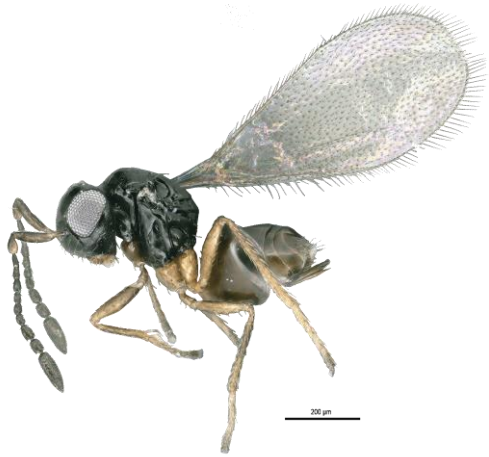
DOI: **10.5281/zenodo.4682793**

PHILAENUS SPUMARIUS : POSSIBLE CONTROL ?

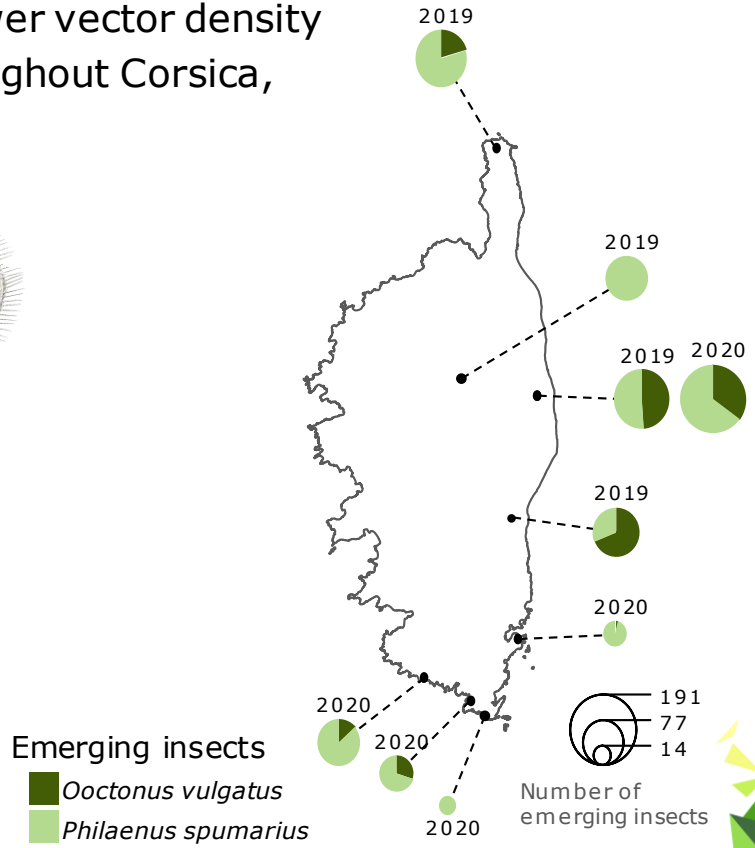
- Overlooked research field: biological control of *Ps* could be an environmental friendly lever to help lower vector density
- Collection of (ca 1100) eggs of *Ps* throughout Corsica,
- Daily monitoring of emergence
- Variable parasitism rates (0-69%)





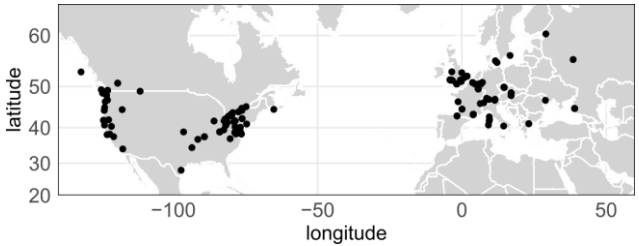


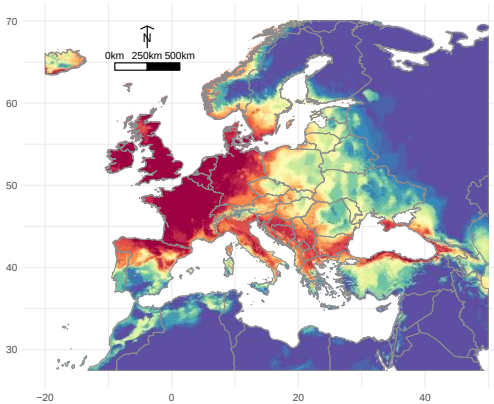
Ooctonus vulgatus
(Mymaridae)



PHILAENUS SPUMARIUS : POSSIBLE CONTROL ?

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





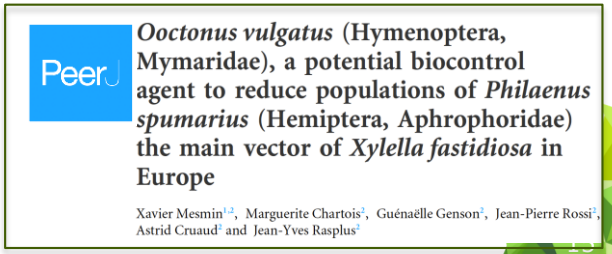
0 25 50 75 100

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 ?

DOI: **10.5281/zenodo.4680103**



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Thanks for your attention,

Keep morale up,
Big up for the organization & technical staff !

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