ورشة عمل خطط الطوارئ كتمرين محاكاة عملي

Contingency Exercise Workshop

Xylella fastidiosa

Hammamet, Tunisia, 26 – 28 May 2025

Aphrophoridae candidate vectors of *Xylella fastidiosa* in Tunisia

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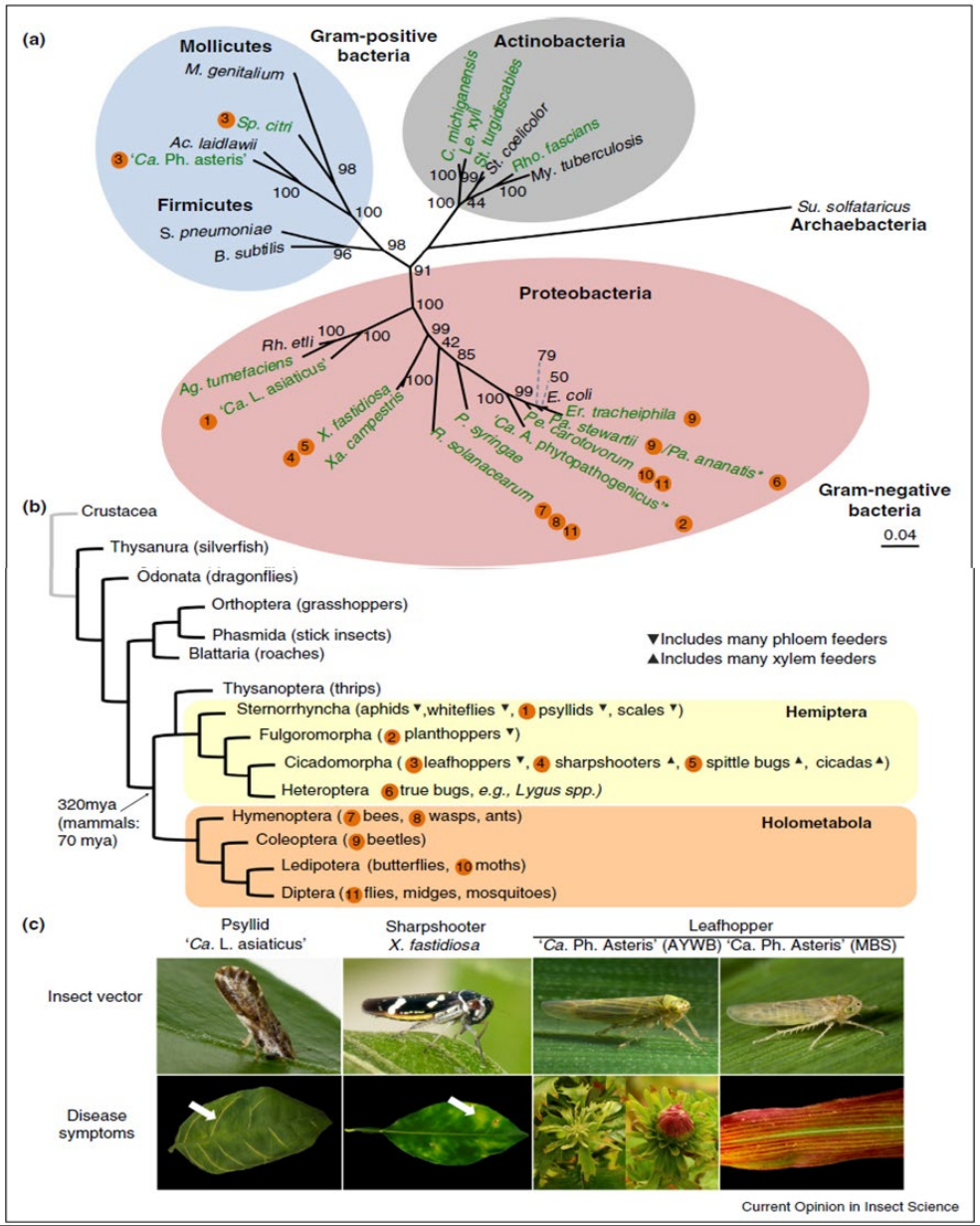
Vectors/pathogens

Several bacteria are transmitted by insect vectors (psyllids, thrips, leafhoppers, spittlebugs, butterflies, beetles, etc.)

Xylella fastidiosa
- Gram-negative bacterium
- Transmitted by spittlebugs and leafhoppers

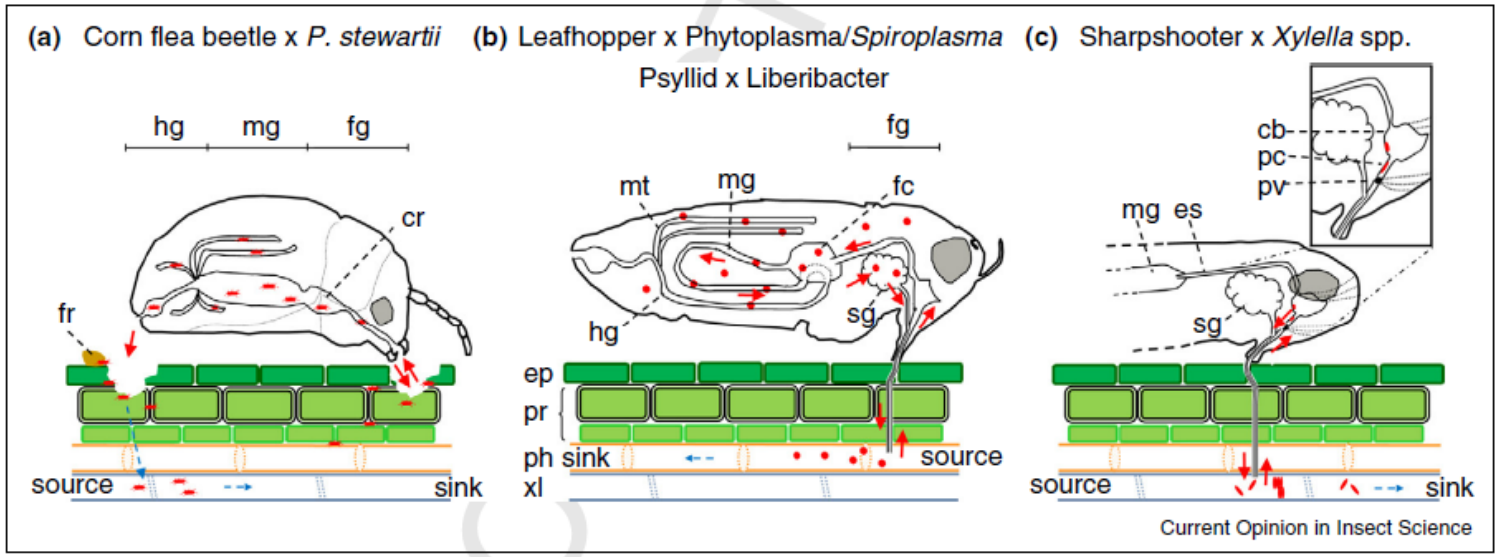


3 strategies of bacterial transmission by insects

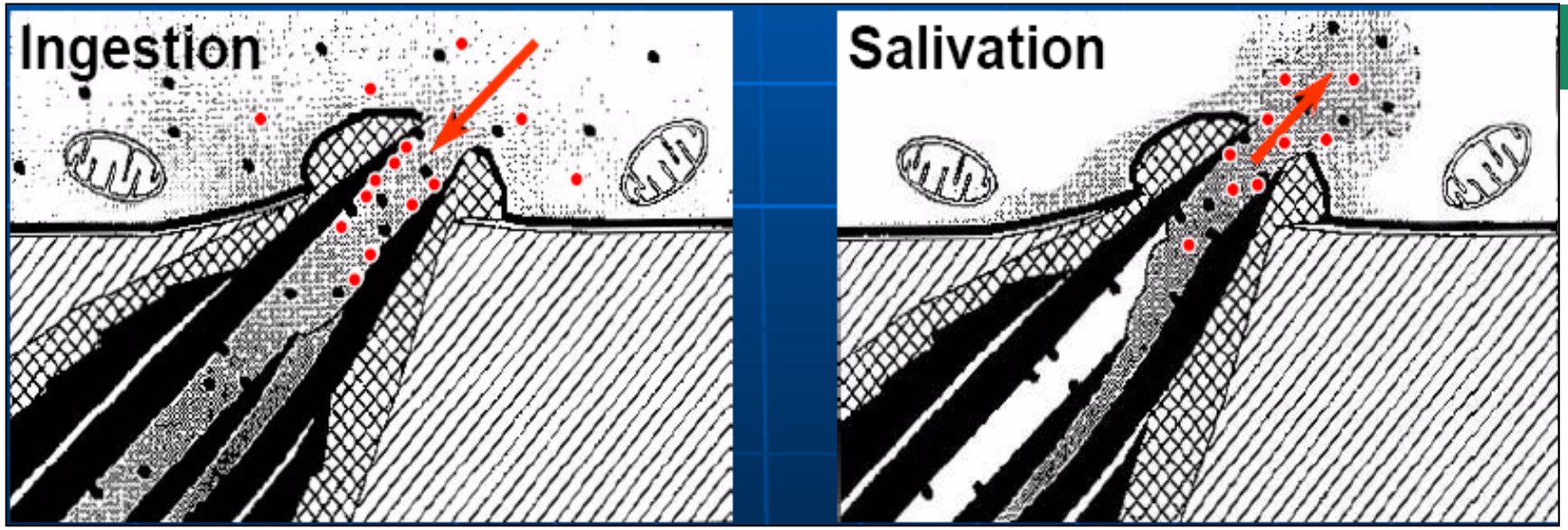
Xylem, phloem, parenchyme feeders

- *X. fastidiosa* non-circulating but persistent in the vector

- Bacteria attached to the cuticle in the cibarium, multiplies and is inoculated by egestion



Xylella fastidiosa acquisition/transmission



The adult, by feeding on the sap of an infected plant, becomes immediately capable of transmitting the disease (Rodas, 1994).

As the bacterium multiplies in the insect's oral cavity, the insect remains infectious for several months (Purcell and Finlay, 1979; Hill and Purcell, 1995).

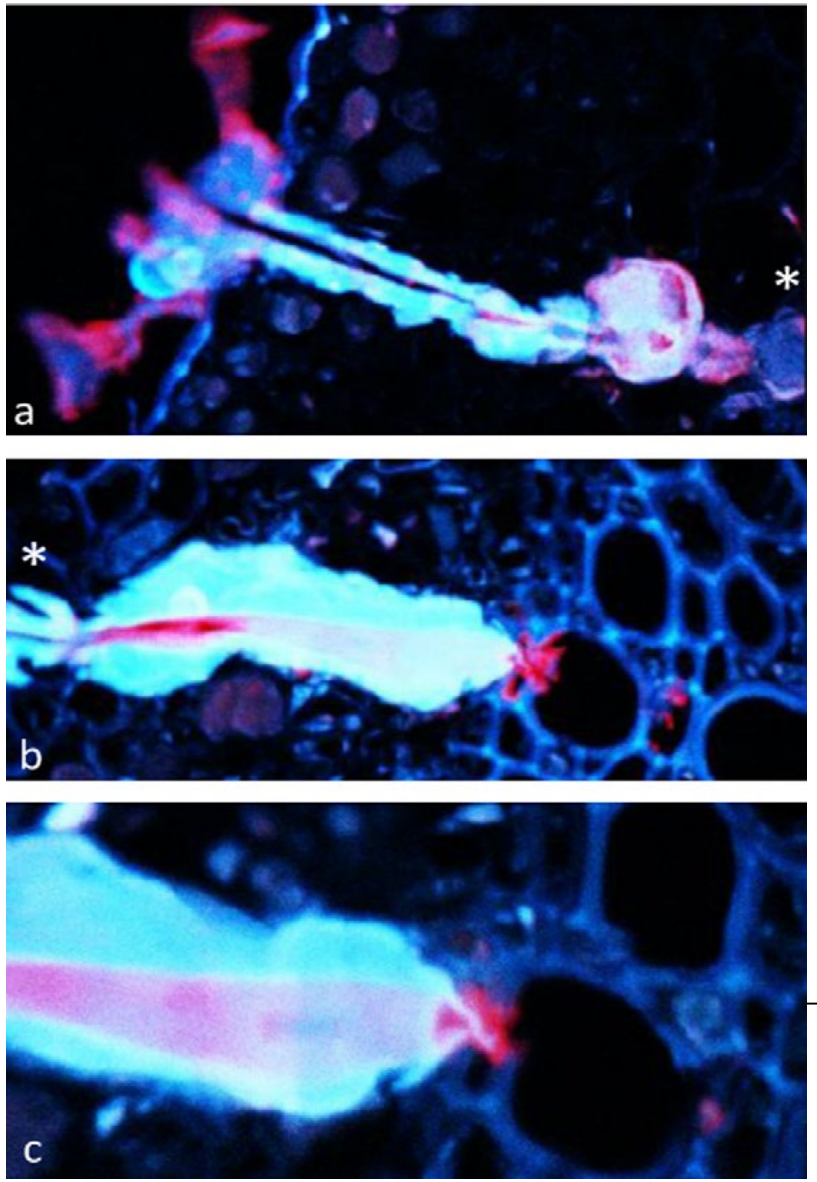
The presence of a few bacterial cells is enough for the insect to transmit the disease.

Bacterial transmission

X. fastidiosa

- The threshold value for bacterial acquisition by the insect is around 106 to 107 bacterial cells per gram of tissue (Hill and Purcell, 1997).
- Acquisition efficiency is positively correlated with the feeding duration on the host plant (Almeida and Purcell, 2003; Daugherty and Almeida, 2009).
- Transmission rates of *X. fastidiosa* is positively correlated with the number of vectors (Costa et al., 2000).

Homalodisca vitripennis (Germar)/ *Xylella fastidiosa*



A xylem-limited bacterium that causes Pierce's disease (grapevine)

inoculation of *X. fastidiosa* during vector feeding

Scanning microscopy showing a salivary sheath of *H. vitripennis* on a vine petiole. (a) Distal half of the salivary sheath on the surface of the petiole. (b) and (c) adjacent sections showing the proximal half of the sheath within the petiole as its tip enters a xylem vessel.

Backus et al., 2012

Auchenorrhyncha potential vectors in the world

Currently, the group includes 70 Hemiptera species

- Aphrophoridae
- Cercopidae
- Clastopteridae
- Cicadellidae
- Cicada
- Membracidae

Auchenorrhyncha potential vectors in the world

- 70 species in Americas
- 5 in Asia
- 11 in Europe
- 7 in Mediterranean region

North Africa

- *Philaenus tesselatus**
- *Philaenus maghresignus*
- *Neophilaenus campestris**
- *Neophilaenus linneatus*
- *Lepeyronia coleoptrata*
- *Aphrophora alni*
- *Graphocephala hirsuta*

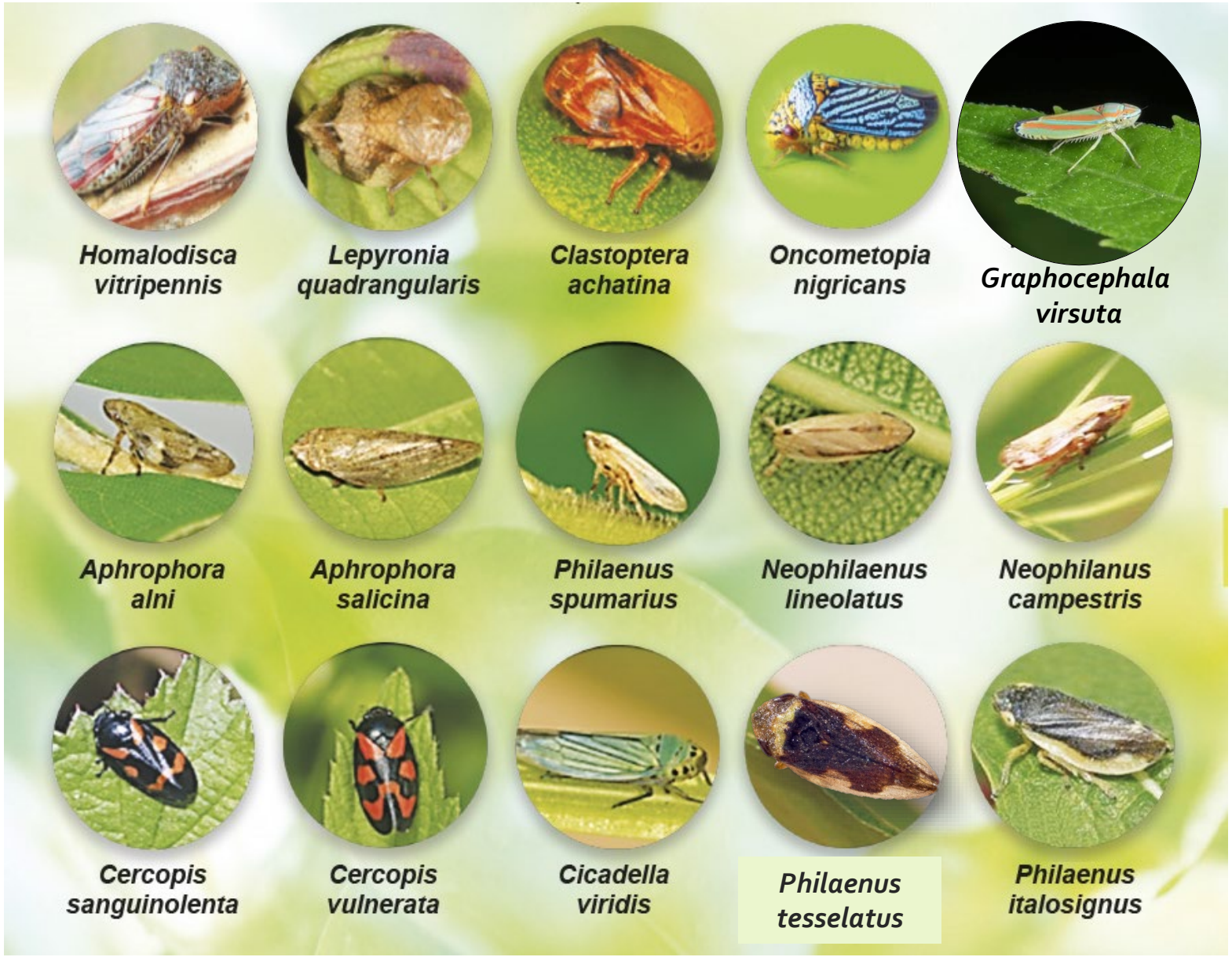
Main Vectors feeding on xylem

17 suspected species

- **Cicadellidae** : *Cicadella viridis, Graphocephala virsuta, Homalodisca vitripennis, Oncometopia nigricans*
- **Clastopteridae** : *Clastoptera achatina*
- **Aphrophoridae** : *Aphrophora alni, Aphrophora salicina, Lepyronia coleoptrata, Neophilaenus campestris, N. lineatus, Philaenus spumarius, P. italosignus, P. tesselatus, P. maghresignus, Cercopis vulnerata, C. sanguinolenta*

Confirmed vectors in mediterranean region *(Italy)*:
Philaenus spumarius, Neophilaenus campestris, P. italosignus

Auchenorrhyncha vectors and potential vectors in the world



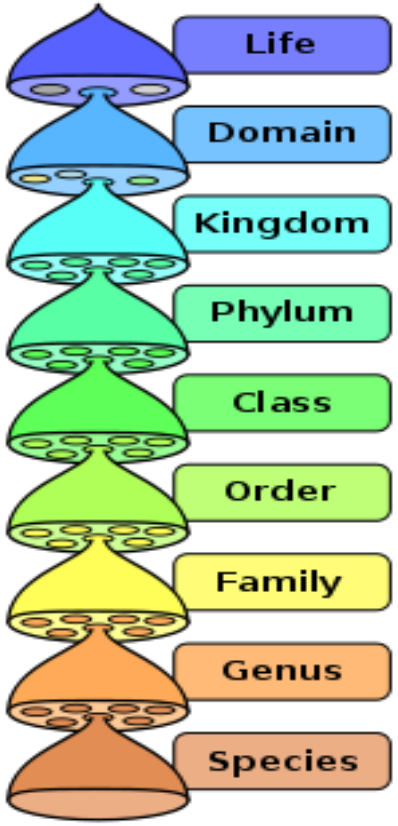
More than 17 species are vectors or potential vectors

Philaenus spumarius is a well-known vector, extremely polyphagous species (nearly 1000 plant species) and can be found in large numbers in many environments (hedges, meadows, crops, gardens, river banks, high-altitude meadows, forests).

In America, the most important vectors of *X. fastidiosa* are *G. virsuta*, *O. nigricans*, *P. spumarius* and *H. vitripennis*

***Graphocephala virsuta* reported from Algeria**

Taxonomy of the most efficient vector



Animalia

Arthropoda

Insecta

Hemiptera/Auchenorrhyncha

Aphrophoridae

Philaenus **Neophilaenus**

Philaenus spumarius ***Neophilaenus campestris***

(Spittlebugs 5,3 à 6,9 mm)

Spittlebugs and Leafhoppers Collection Methods

- Sweep net
- Threshing with a Japanese umbrella
- Mechanical vacuum cleaners (D-Vac)
- Sticky yellow chromo-attractant panels (glue traps)
- Traps embedded in the ground (Barber traps).
- Light traps, Malaise tent type traps are also available
- Sticky Wire Traps

Each technique produces different results regarding the species captured and the combination of several techniques is necessary to exhaustively identify and evaluate Auchenorrhyncha populations.

Trap types


Malaise tent


Sweep net


D-Vac


Malaise trap


Japanese umbrella


Sticky yellow traps


Light traps

Sticky Trap

Important Notations

Collection monitoring data must include:

1) A unique collection number;

2) geographic coordinates;

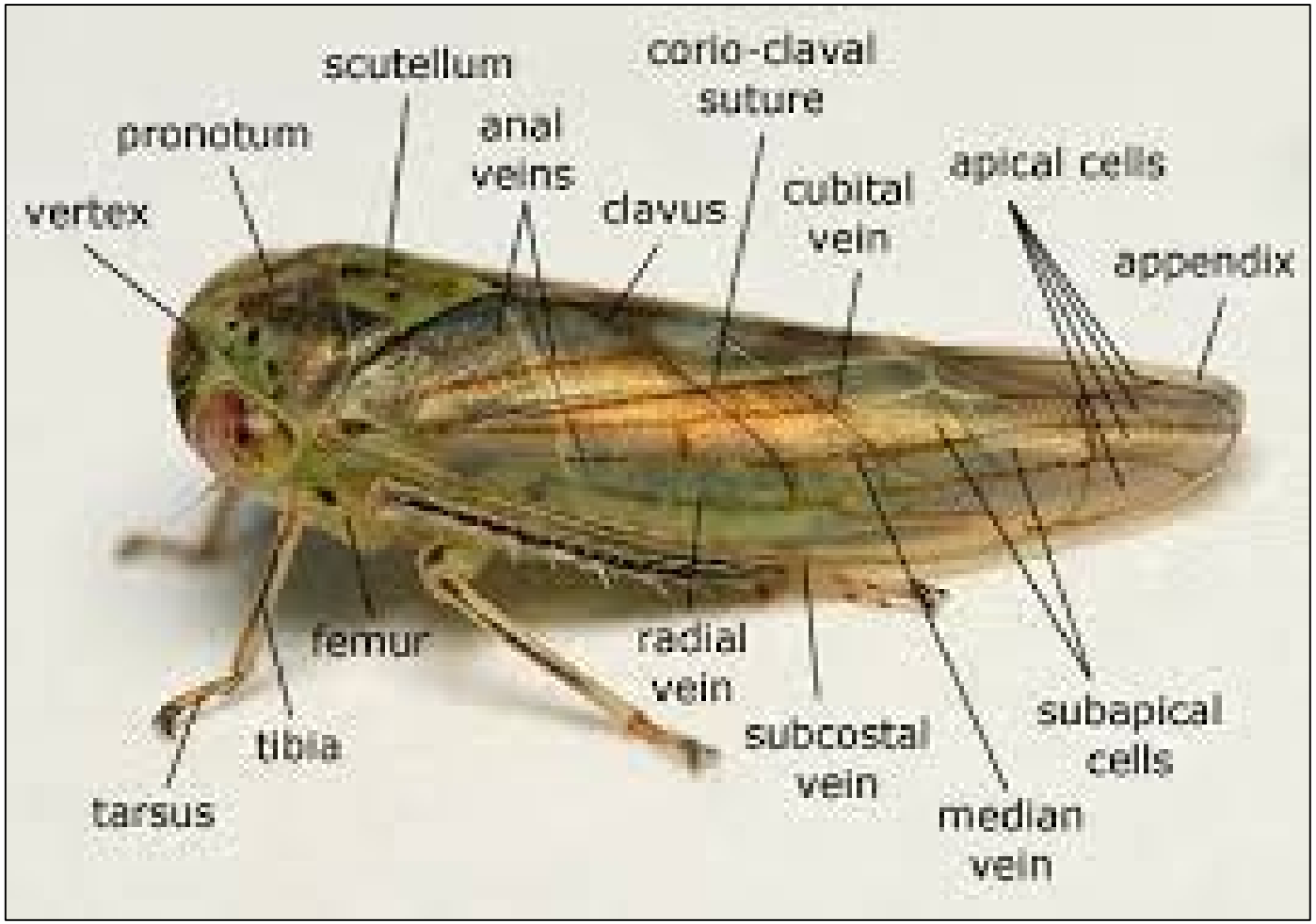
3) The date and name of the person who collected samples;

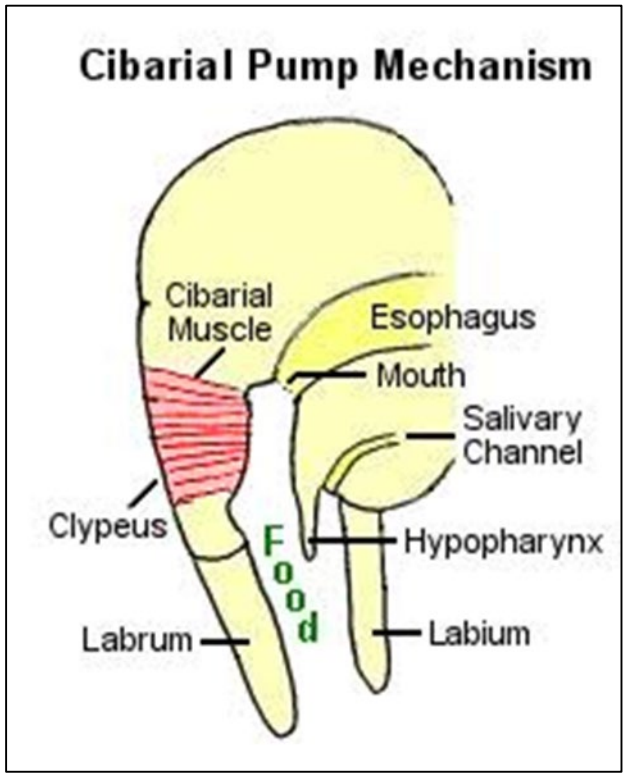
4) The name of the host plant;

5) A list of plants; a typology of plant density showing symptoms. ;

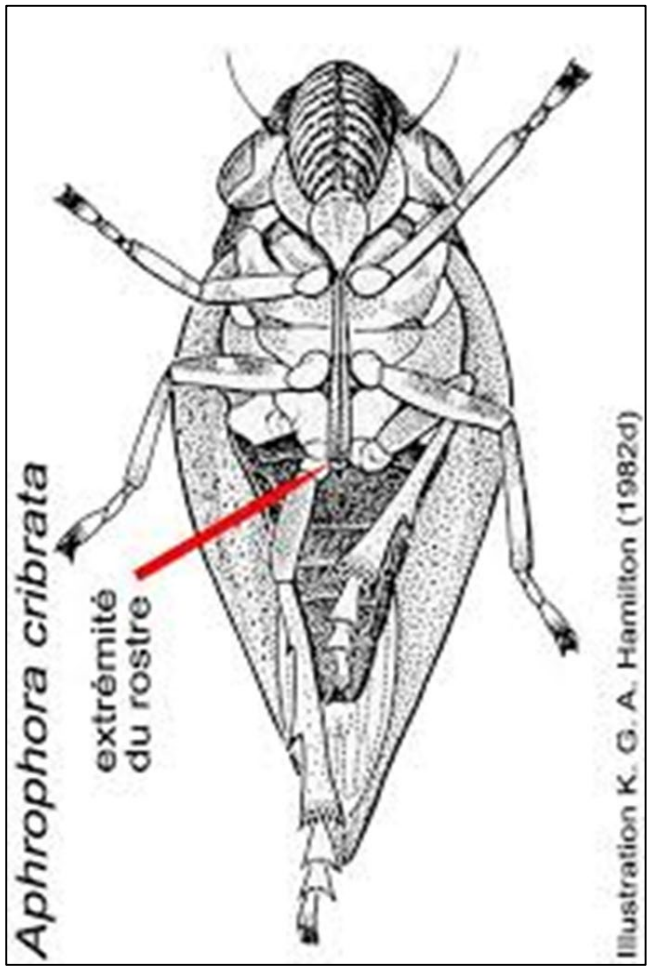
collection of insect vectors by sweep net.

Identification criteria (dorsal view)

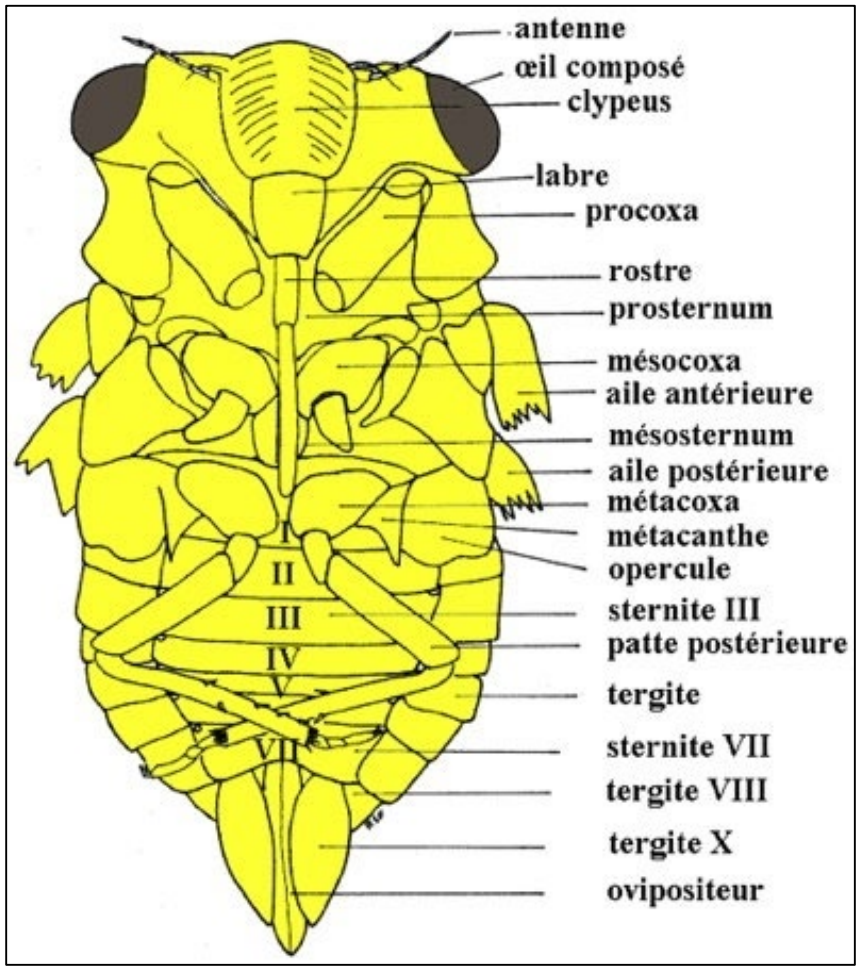




Morphological features (ventral view)



Male

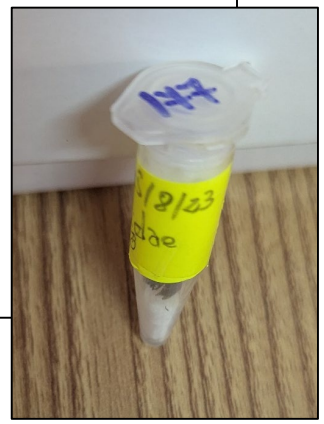


Female

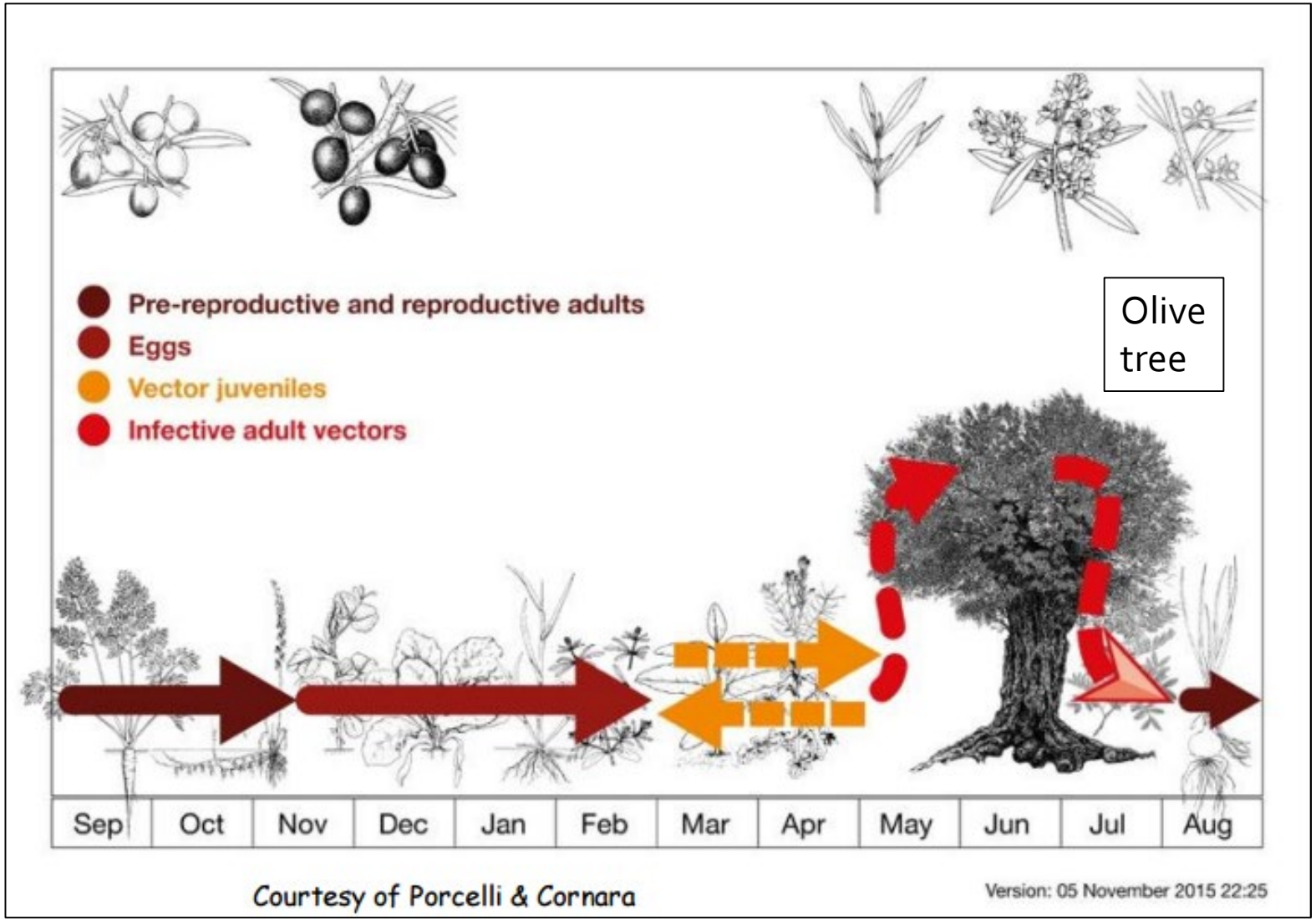
Male genitalia used for species identification

Insect Conservation

- The captured insects must be placed in ethanol 75 then transferred to ethanol 96 in containers which prevent evaporation of the solvent.
- The volume of insects should not exceed one third of the volume of ethanol.
- The storage can be done at 4°C for a short period or at -20°C for a longer period
- The tubes must be legibly and precisely labeled.



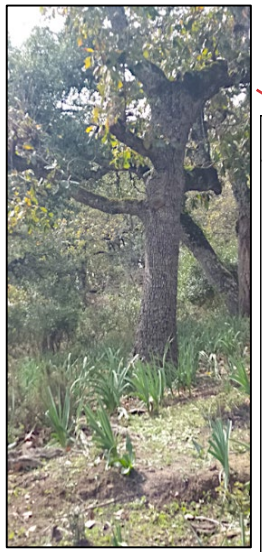
Philaenus cycle and Xylella dissemination



Tunisian experience

- Collection and Identification of potential vectors
- Forest/Dry grassland/Olive/Citrus/Peach/Almond propections
- Host plants of vectors
- Bar-coding and Xylella detection
- Natural ennemies identification

Spittlebugs collecting regions in Tunisia with the focus on two reservoir forests



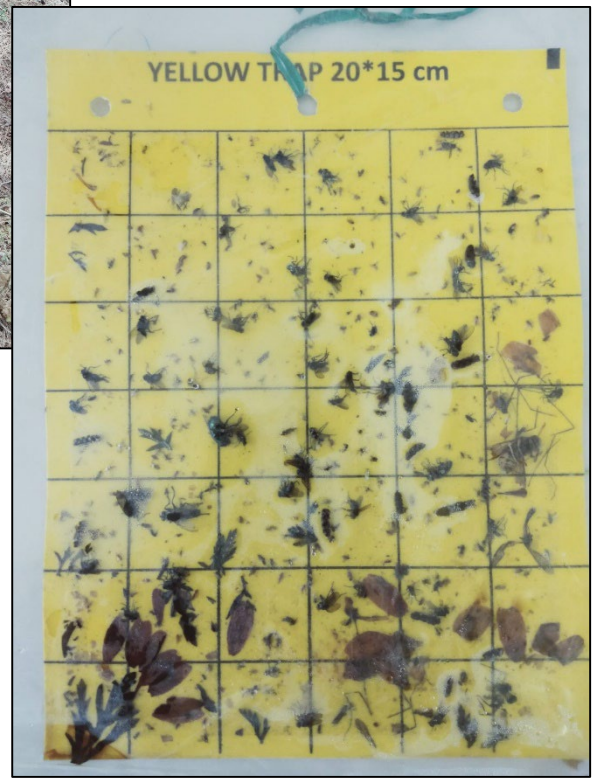
Feija forest

Dar Chichou forest

Collecting spittlebugs

- Sweep-net (50 sweeps in forests, 20 under fruit trees)
- Plant (20 plants of each species)

Trap used



- Limited numbers
- Insects adhered strongly to the glue, making removal difficult

- Good method

Identified material

More than 8,000 Auchenorrhyncha captured by sweep net

- 5010 Aphrophoridae (4 species identified)
- 3082 Cicadellidae (129), Typhlocibidae (5), Caliscelidae (1), Issidae (9), Delphacidae (3), Cixiidae (2), Dictyopharidae (2), Flattidae (1), Tettigometridae (6), Membracidae (2)

4 Aphrophoridae : Philaenus and *Neophilaenus* identification features

Species	Adult	Aedeagus	Hind leg
P. maghresignus			
P. tesselatus			
N. campestris			
N. lineatus			

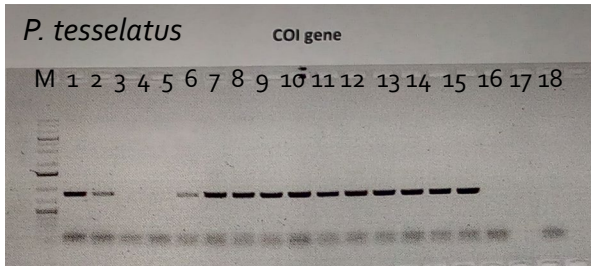
We did not identify *Philaenus spumarius* in Tunisia

Insect identification : *Philaenus tesselatus*

- Morphological: insects captured as males dissected to observe their genitalia

- Molecular: COI-based bar-coding

>16_COI-
FTTTGGGTATAATTTATGCTATAATAGCAATTGGTTTATTAGGTTTTGTGGTTTGAGCTCATCATATATTTACTGTAGGTAT
AGATGTTGATACACGTGCATATTTTACTTCAGCCACAATAATTATTGCTGTACCTACGGGTATCAAAATTTTTAGTTGATT
GGCTACAATACATGGAATACCATTCAAATTGNCTTCTCCTATTTTATGATCAATTGGATTTGTATTTNT

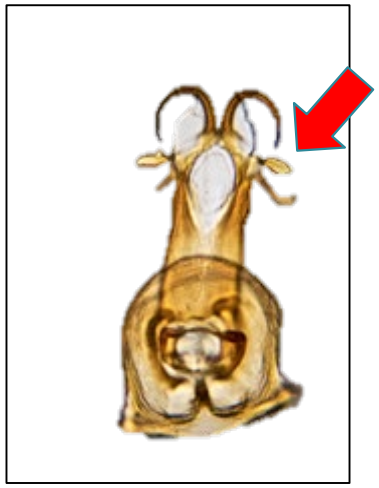


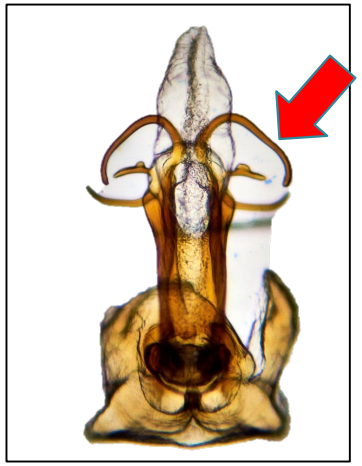
Sequence very close to GU446992.1 from NY Museum with a bootstrap value of 99,13% (NCBI bank)

Discrimination with Philaenus spumarius

Philaenus Spumarius (Linnaeus), A, Male posterior and anterior view; B, Hind tibia spines; C, forewing; D, hind wing; E, Male genital capsule lateral view; G, right style ventral-dorsal aspect; J, aedeagus, dorsal view;

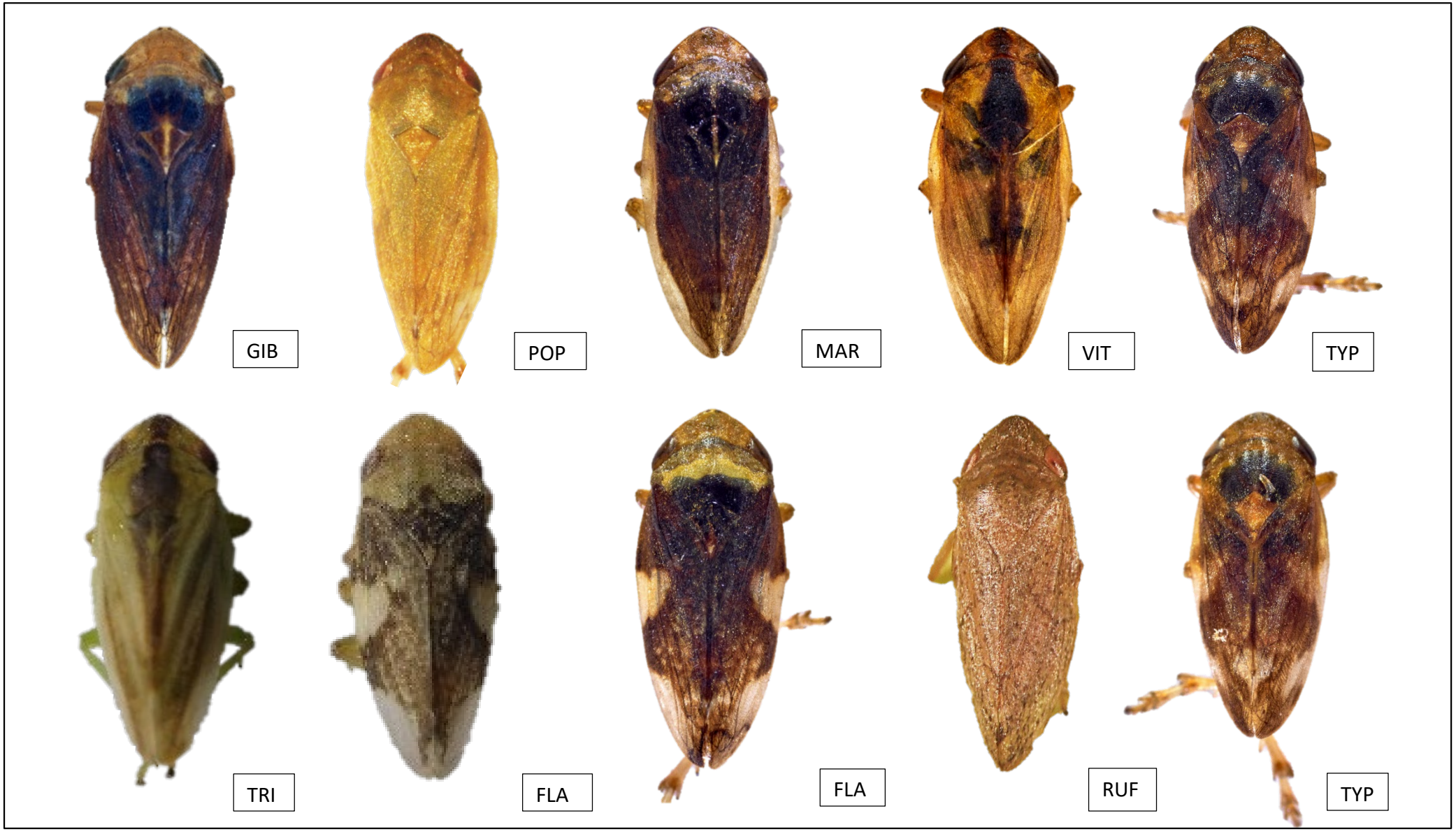
Philaenus Spumarius





Philaenus tesselatus

Morph diversity of *Philaenus tesselatus*



Morphs TRI, GIB, MAR, VIT, TYP, POP, FLA, RUF and TRI

Intermediate morphs

MAR /FLA

VAR/ TYP

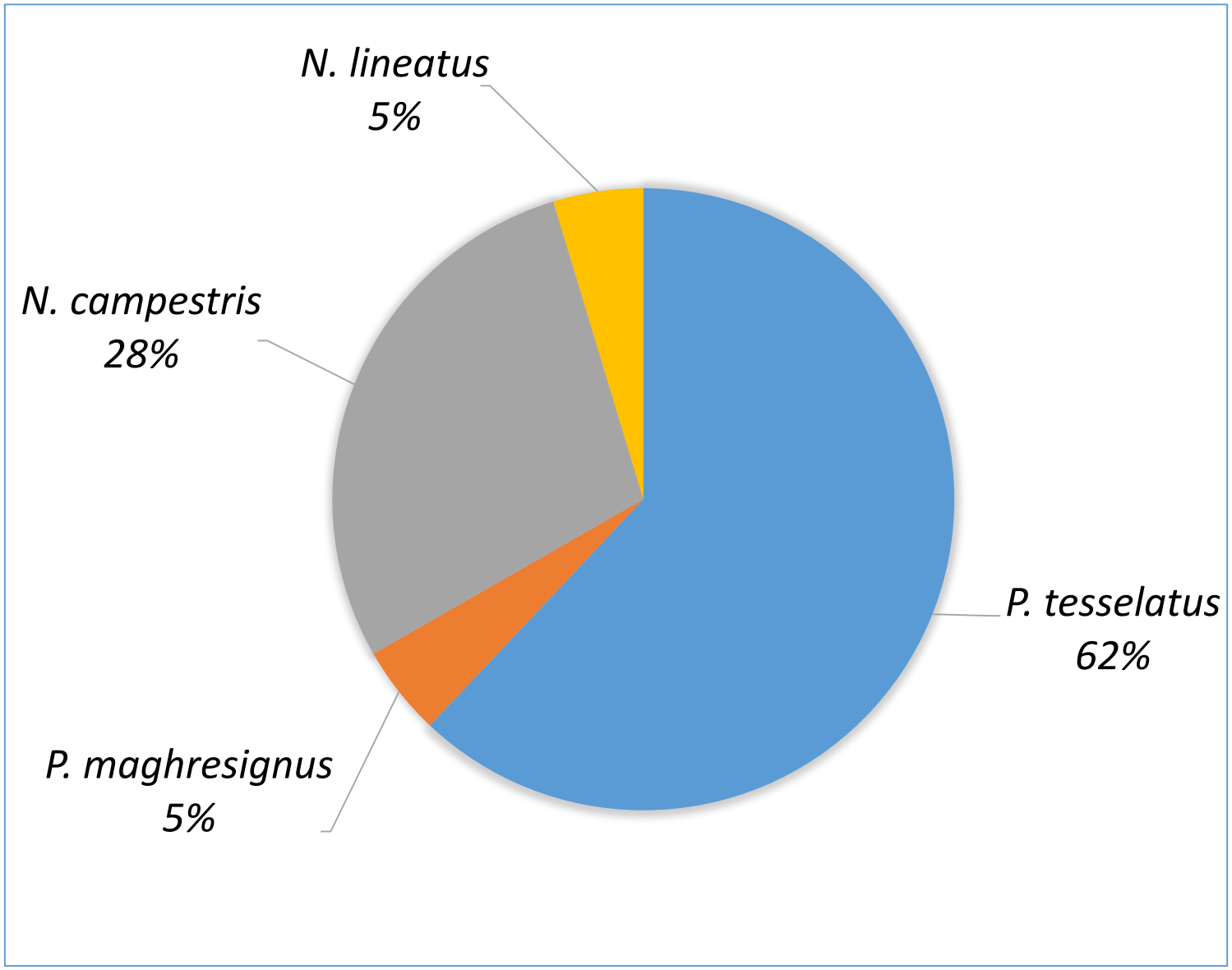
VIT/ TRI?

TRI ?

VIT ?

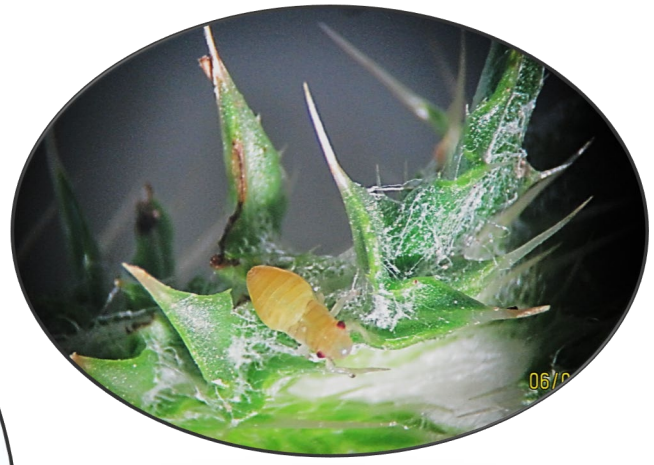
VIT ?

Composition of Aphrophoridae adults in tunisian regions, 2018-2021



Biological cycle of *P. tesselatus*

Fev.-Mar.



Nymphs

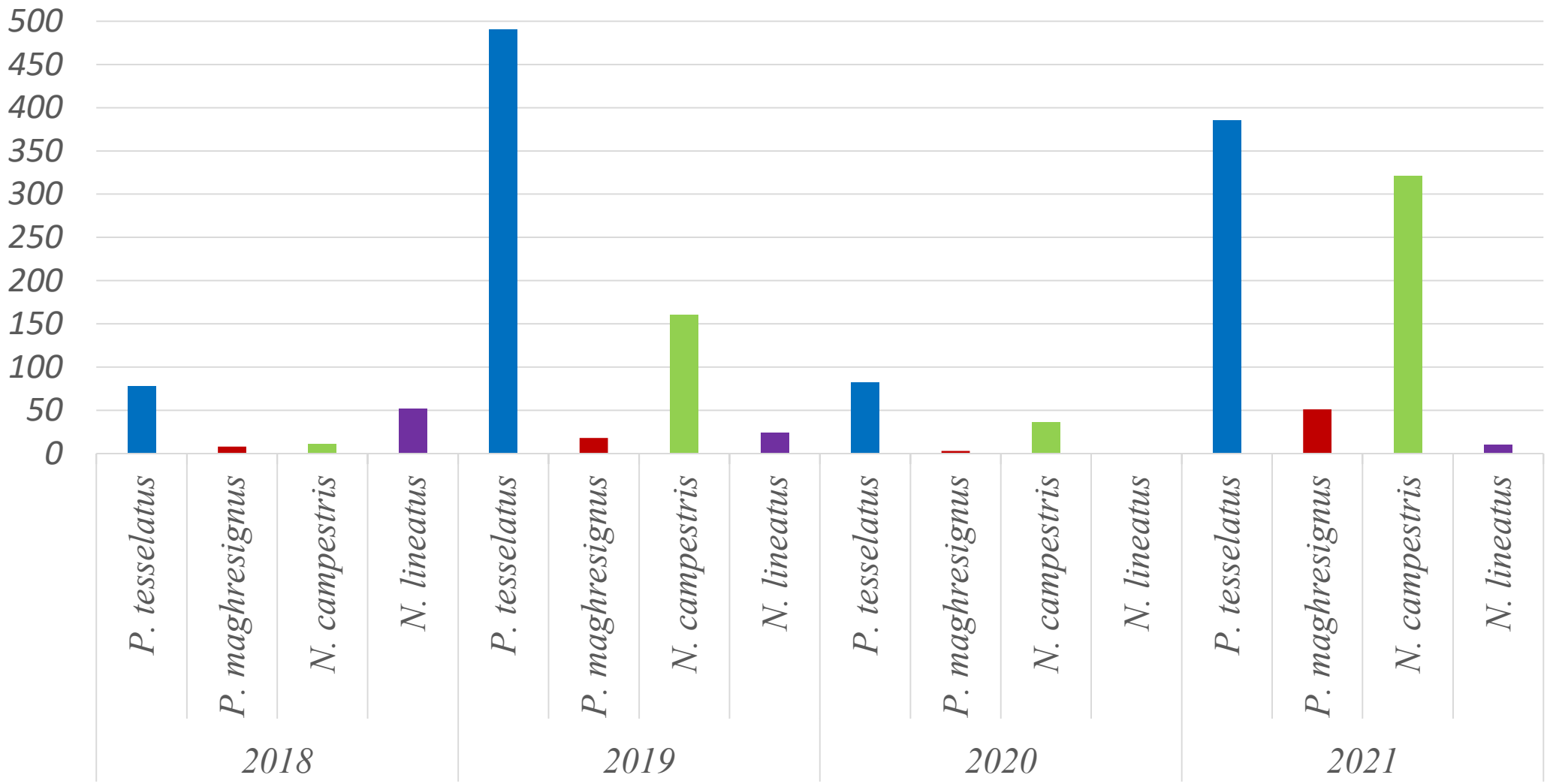
Adults

Late Apr.-May

Mar.-Apr.

molting Nymphs

Frequency variations of the four spittlebugs according to years



Philaenus tesselatus/Neophilaenus campestris/P. maghresignus

Polyphagous and well adapted at many plants and habitats

- *Sonchus oleraceus*
- *Smyrnium olusatrum*
- *Glebionis segetum*
- *Cirsium arvense*
- *Ranunculus repens*
- *Sonchus oleraceus*
- *Scolymus grandiflorus*

- *Rumex crispus*
- *Daucus carota*
- *Mercurialis annua*
- *Hedysarum coronarium…*

Oligophagous on Poaceae

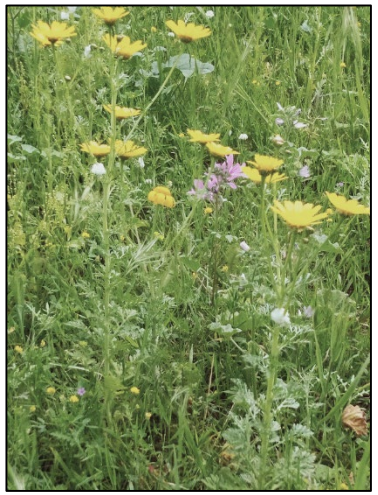
- *Festuca*
- *Avena sativa*
- *Bromus...*

Monophage
Asphodelus microcarpus

Host plants/Nymphs-Adult of ***P. tesselatus*** (Dar Chichou)

Scolymus grandiflorus

Glebionis segetum



Sonchus oleraceus

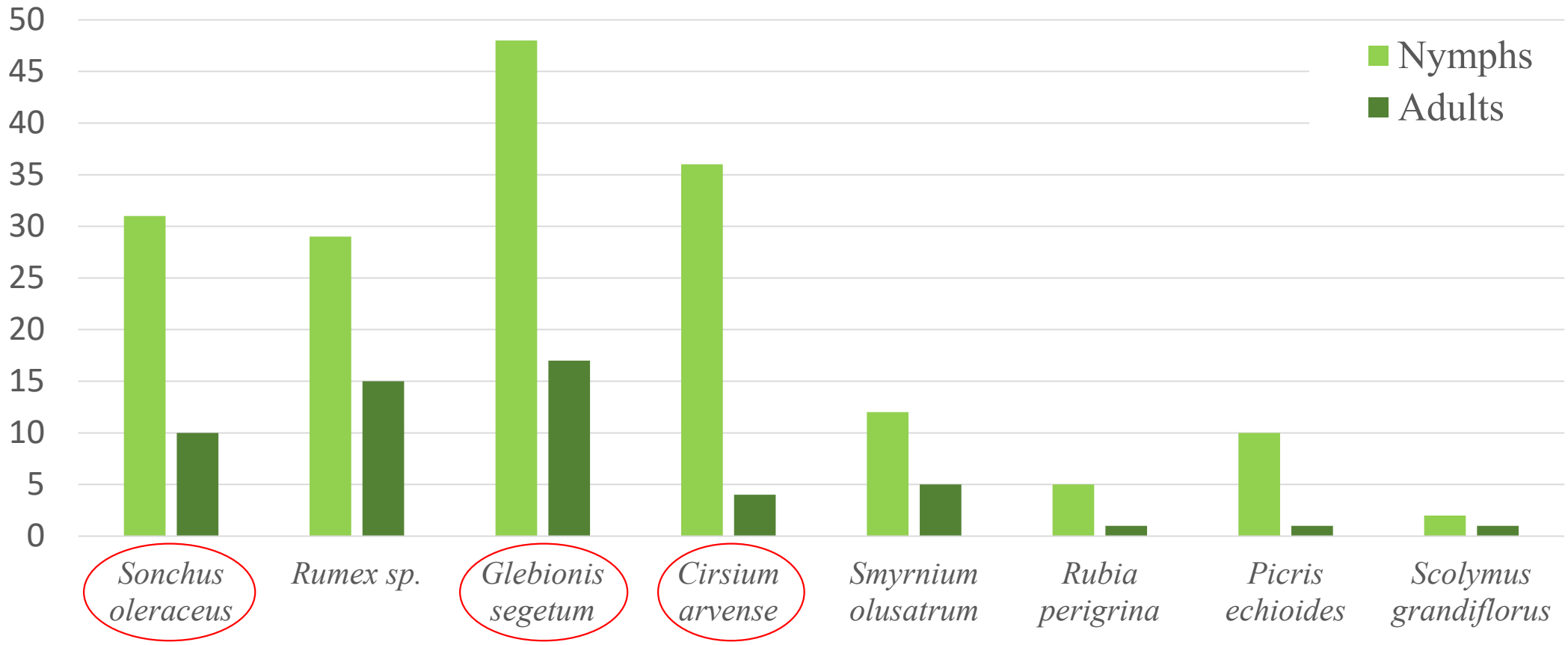
Rubia perigrina

Daucus carota

Cirsium arvense

Smyrnium olusatrum

Spittlebug nymphs and adults of *Philaenus tesselatus* on diverse weeds, Dar Chichou April 2021



Nymphs of ***P. maghresignus*** on *Asphodelus* plant collected from Feija







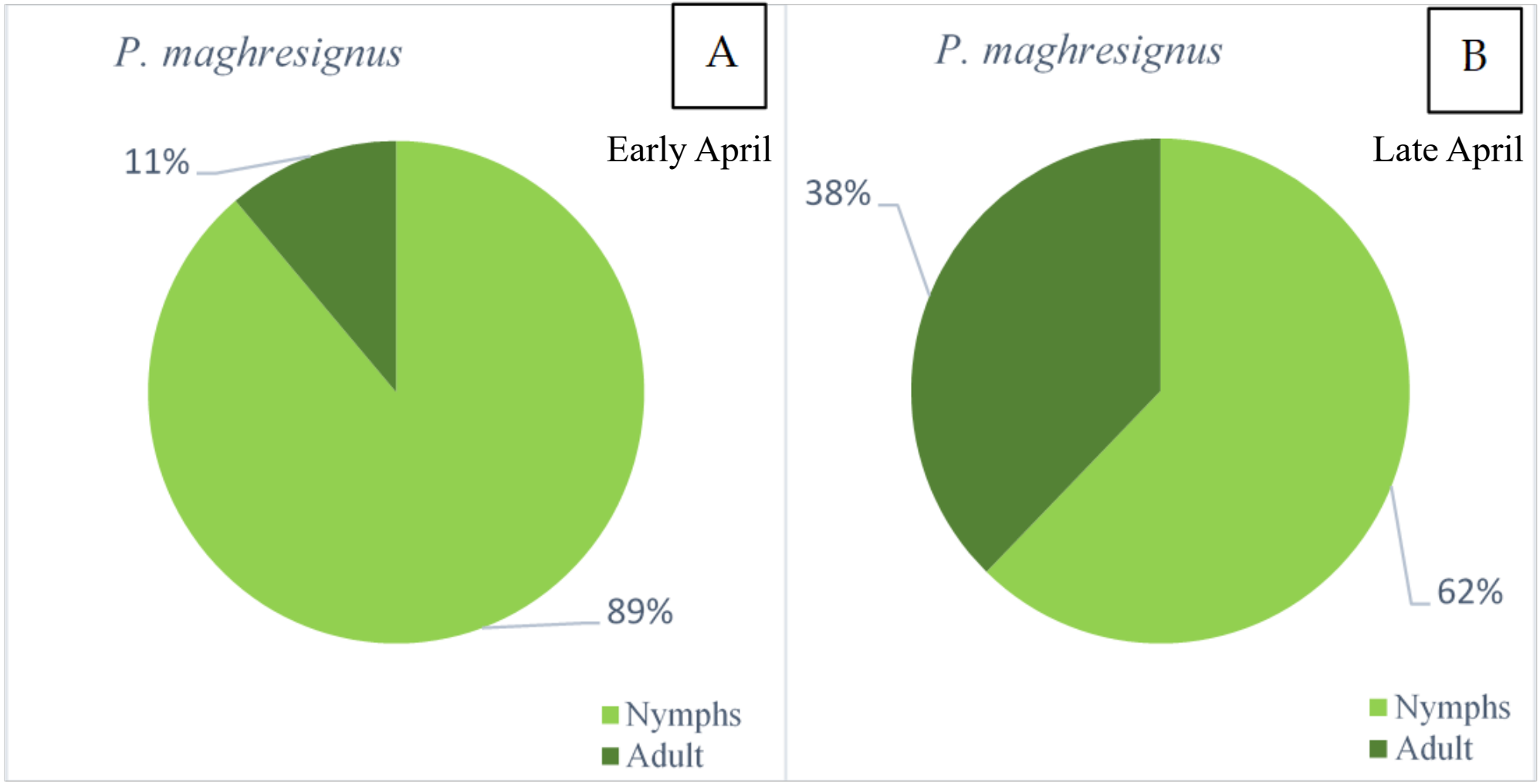




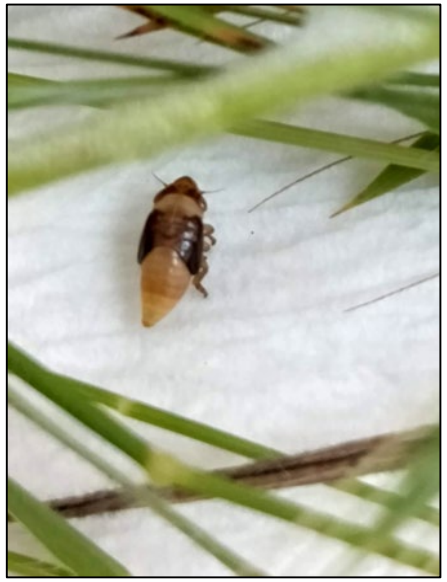



Monophagous

P. maghresignus nymphs and adults on *Asphodelus microcarpus*, Feija 2021



Nymph and Adult of *N. campestris* on Poaceae (Dar Chichou)



Detection of *X. fastidiosa* in insects

Extraction of ADN from the upper part of Philaenus and qPCR

Morphological identification by dissection of male genitalia (lower insect part)

Analyze insects, potential vectors to identify whether they are contaminated by the bacteria

No Xylella identified in insect until now

- Remember when *Xylella fastidiosa* is detected in the (pre)cibarium of the insect, that does not mean it will be an effective vector for a given plant
- Vector transmissions are necessary.

Enemies ?

Diptera

Hymenoptera

Not identified yet

Evaluation of the Risk

- Occurrence of a confirmed vector in Tunisia : *Neophilaenus campestris*
- Occurrence of an important potential vector : *Philaenus tesselatus*, very close in morphology and behavior to *Philaenus spumarius*
- Occurrence of *P. maghresignus* similar to *P. italosignus* a vector too
- Plant trade and exchanges are currently made

What is the necessary measures ?


Thank you for
your interest