



Electrophysiological and Behavioral responses of *Philaenus spumarius* and *Neophilaenus campestris* females to host plant volatiles



BENAKI
PHYTOPATHOLOGICAL
INSTITUTE

**Eirini Anastasaki¹, Aikaterini Psoma¹, Georgios Partsinevelos¹,
Dimitrios Papachristos² and Panagiotis Milonas^{1*}**

¹ Laboratory of Biological Control, ² Laboratory of Agricultural Entomology,
Scientific Directorate of Entomology and Agricultural Zoology, Benaki
Phytopathological Institute

*corresponding author: p.milonas@bpi.gr

SCOPE & OBJECTIVES

- ❑ The spittlebugs, *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae): xylem-feeder insects → main vectors of *Xylella fastidiosa* in Europe.
- ❑ Investigation of the response of bacterial insect vectors to volatile organic compounds emitted by their host plants.
- ❑ Carification of the key parameters of the host selection.
- ❑ The plant material that was studied:
 - ✓ *Olea europaea* & *Polygala myrtifolia* → highly susceptible plant species to *X. fastidiosa*
 - ✓ *Pinus halepensis* → common plant where *N. campestris* is found during summer
 - ✓ *Cistus creticus*, *Medicago sativa*, *Cynodon dactylon*, *Festuca arundinacea*, *Apium graveolens*, *Petroselinum crispum* → cover crops or natural vegetation in olive orchards.



***Philaenus
spumarius***



Olea europaea



Pinus halepensis

*the photos of the plants and insect's species that were used in this e-poster were available online under the Creative Commons CC0 1.0 Universal Public Domain Dedication.



P. myrtifolia



C. creticus



M. sativa



C. dactylon



F. arundinacea



A. graveolens



P. crispum

EXPERIMENTAL : 4 STEPS

Dynamic
Headspace

GC-MS Analysis

GC-EAD

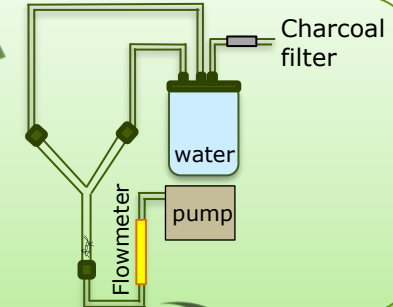
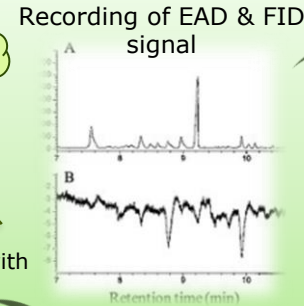
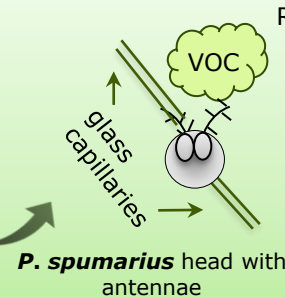
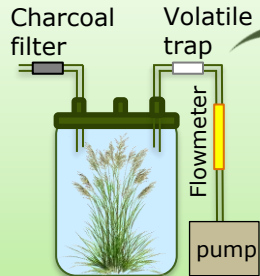
Dual Choice Bioassays

Collection of VOCs

Identification of VOCs

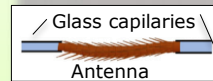
Identification of
electrophysiological active VOCs

Behavioural test of the
electrophysiological active VOCs



In laboratory or in situ depending
by the host plant tested

carrier gas and
other gas supplies

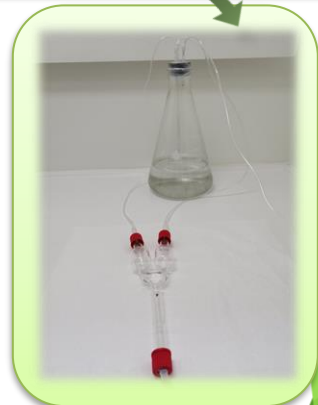


EAD

Injector

Volatiles' glass
transfer tube

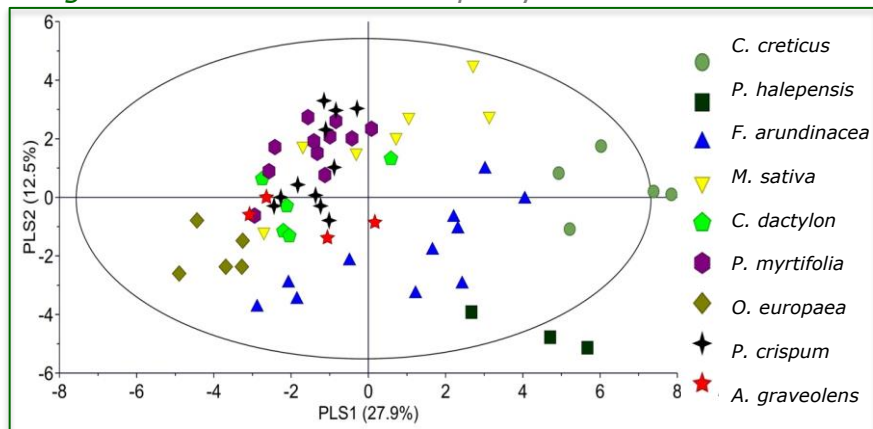
GC/FID



RESULTS & DISCUSSION

Chemical profile

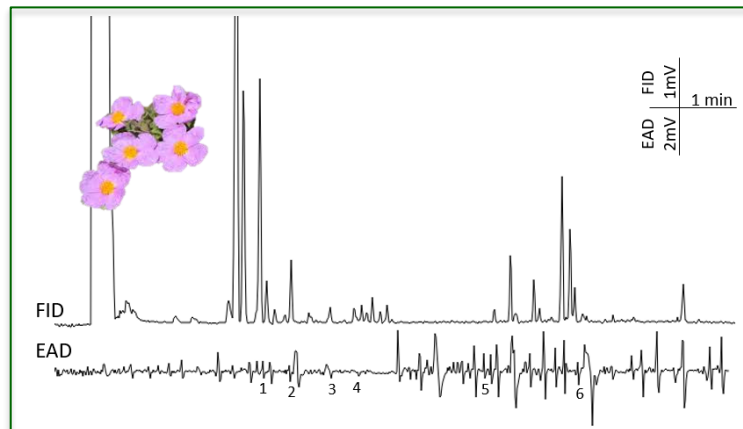
- *C. creticus*: the richest profile. Main compounds α -pinene, 4,5-di-*epi* aristolochene.
- *P. halepensis*: dominated by β -myrcene, *E*- β -ocimene, α -pinene, linalool.
- *P. myrtifolia*: majorly alkanes, α -pinene, camphene, camphor.
- *O. europaea*: *E*- β -ocimene, α -pinene, α -copaene.
- *C. dactylon*: camphene and α -pinene.
- *F. arundinacea*: α -pinene, 3*Z*-hexenyl acetate, limonene and 2,4-dimethyl heptane.
- *M. sativa*: 2,4-dimethyl heptane, camphor, limonene, 2-ethyl-1-hexanol and nonanal.
- *P. crispum*: 1,3,8-*p*-menthatriene and β -phellandrene.
- *A. graveolens*: limonene and β -myrcene.



PLS-DA of volatile compounds identified in the different plant species.

Electrophysiological active compounds

- In total 65 compounds elicited consistent EAD responses for *P. spumarius* and 10 compounds for *N. campestris*.
- α -pinene was found in all tested plants but elicited consistent EAD responses of *P. spumarius* in five plants.
- Antennae of *P. spumarius* females responded to camphor, limonene, 4-methyl octane and sabinene. These compounds were found in at least 5 out of 8 examined plant species.

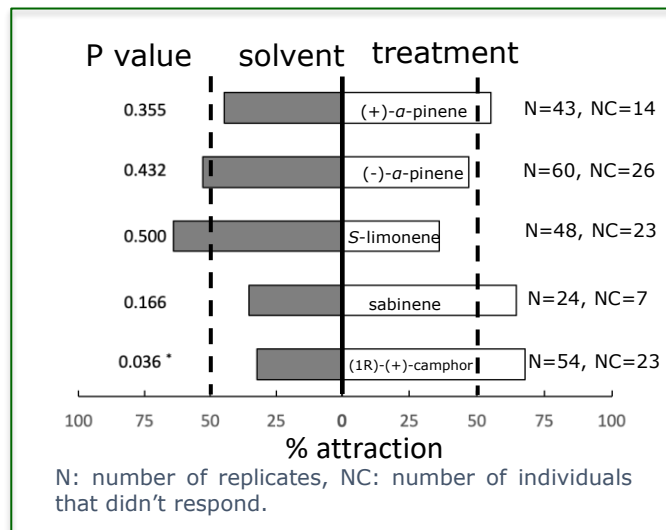


Representative GC-EAD traces of female *P. spumarius*, to VOCs of *C. creticus*, n= 15. Electrophysiologically active compounds are numbered: 1) β -pinene, 2) limonene, 3) *cis*-sabinene hydrate, 4) isoborneol, 5) δ -elemene, 6) β -selinene.

RESULTS & DISCUSSION

Dual Choice Bioassays

- ✓ The VOCs that triggered the most frequent EAD responses in *P. spumarius* females' antennae were selected for olfactometer behavioral bioassays depending on availability of synthetic compounds.
- ✓ Significant preference of *P. spumarius* females was observed for (1R)-(+)-camphor. *P. spumarius* females did not discriminate any of the other compounds tested from control.
- ✓ Overall, 60% of the tested females make a choice towards all treatments.



CONCLUSIONS

- ❑ The VOCs blend, from the headspace of various plant species that have a major role in the epidemiology of *X. fastidiosa* has been determined.
- ❑ Several compounds elicited consistent EAD responses for both *P. spumarius* and *N. campestris*.
- ❑ The electrophysiological responses recorded for *P. spumarius* and *N. campestris* towards the VOCs blend confirmed the wide range of VOCs that the aforementioned species respond to.
- ❑ Olfactometer bioassays showed significant attraction to (1R)-(+)-camphor for *P. spumarius* females.