



Proceedings of the  
Barcelona 20-21 March 2013  
3rd European Bois Noir Workshop



Generalitat de Catalunya  
Departament d'Agricultura, Ramaderia,  
Pesca, Alimentació i Medi Natural



Proceedings of the:

# 3<sup>rd</sup> European Bois Noir Workshop

20-21 March 2013

Barcelona

Spain

**Organized by**

Institut de Recerca i Tecnologia Agroalimentàries (IRTA)

Laboratori de Sanitat Vegetal. DAAM. Generalitat de Catalunya.

Institució Catalana d'Estudis Agraris (ICEA)



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## Proceedings of the 3<sup>rd</sup> European Bois Noir Workshop

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## DETAILED PROGRAM

**Tuesday, 19th March 2013**

18:00-20:30	Registration of participants, reception, poster set up
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**Wednesday, 20th March 2013**

8:30-9:15	Registration and poster set up
9:15-9:30	Opening addresses
9:30- 10:30	<b>Invited review: Xavier Foissac</b> <i>'Candidatus Phytoplasma solani'</i> genome project and genetic diversity in the Euro-Mediterranean basin. Xavier Foissac, Patricia Carle, Anne Fabre, Pascal Salar, Jean-Luc Danet and the STOLBUR-EUROMED consortium.
10:30-11:00	Coffee Break
11:00-12:45	<b>Session 1. Epidemiology</b> The role of <i>Reptalus panzeri</i> in transmission of "bois noir" disease in Serbian vineyards. Tatjana Cvrković, Jelena Jović, Milana Mitrović, Oliver Krstić, Ivo Toševski Microsatellite and mtDNA evidence for genetic differentiation of <i>Hyalesthes obsoletus</i> populations associated with a new major host, stinking hawk's-beard ( <i>Crepis foetida</i> ), in Southeast Europe. Andrea Kosovac, Jes Johannessen, Oliver Krstić, Milana Mitrović, Tatjana Cvrković, Michael Maixner, Ivo Toševski, Jelena Jović "Bois noir" phytoplasma and Auchenorrhyncha species in Bosnia and Herzegovina vineyards. Duška Delić, Zorica Đurić, Jelena Jović, Biljana Lolić, Ivo Toševski, Ana Karačić Epidemiology of "bois noir" disease in Veneto region through phytoplasma molecular identification and spatial analyses data. Fabio Quaglino, Nicola Mori, Alberto Pozzebon, Paola Casati, Fabio Tessari, Giovanni Zanini, Anna Zorloni, Piero Attilio Bianco Analysis of the temporal and small-scale distribution of <i>Hyalesthes obsoletus</i> , vector of "bois noir", in a vineyard in Baden (Germany). B. Panassiti, J. Fahrentrapp, M. Breuer, R. Biedermann Mass occurrence of <i>Hyalesthes obsoletus</i> on <i>Urtica dioica</i> in Austria and sole presence of tuf-type b stolbur phytoplasma on stinging nettles grapevine and in the transmitting insects. Monika Riedle-Bauer, Judith Mörtel, Milica Pastar, Amal Aryan, Günter Brader Searching for vectors: molecular epidemiology of "bois noir" in southern Banat viticultural region of Serbia. Tatjana Cvrković, Jelena Jović, Milana Mitrović, Oliver Krstić, Ivo Toševski
12:45-13:15	<b>Poster session 1</b>
13:30	Lunch
15:00-23:00	Technical Tour and Social Dinner

**Thursday, 21 th March 2013**

09:00-10:00	<b>Session 1. Epidemiology</b>
	<p>Multiple infections of “bois noir” phytoplasma and grapevine leafroll viruses in Turkey. Filiz Ertunc, Didem Canik-Orel, Yagmur Turkmen</p> <p>A case study of “bois noir” phytoplasma pathosystem: multilocus sequence typing approach. M. Šeruga Musić, J. Plavec, I. Križanac, Ž. Budinšćak, D. Škorić</p> <p>Interaction between host plants and region on the population structure of <i>Hyalesthes obsoletus</i> (Cixiidae) in Switzerland: implications for “bois noir” management. Jes Johannessen, Benjamin Maniyar, Michael Maixner, Patrik Kehrlí</p> <p>The spatiotemporal distribution of <i>Hyalesthes obsoletus</i>, nettle host plants and stolbur phytoplasma in a fallow vineyard. Michael Maixner, Jes Johannessen</p>
10:00-11:00	<b>Session 2. Detection and Characterization</b>
	<p>Triplex qPCR assay for the detection of “flavescence dorée” and “bois noir” with internal control. Giuseppe Durante, Elena Zacchi, Camilo Gianinazzi, Paola Casati and Piero Attilio Bianco</p> <p>Stolbur and “bois noir” phytoplasma strains represent a distinct novel species, ‘<i>Candidatus Phytoplasma solani</i>'. Fabio Quaglino, Yan Zhao, Paola Casati, Daniela Bulgari, Piero Attilio Bianco, Wei Wei, Robert Edward Davis</p> <p>Survey of ‘<i>Candidatus Phytoplasma solani</i>' and its potential vectors in Northern regions of Azerbaijan. Gulnara Balakishiyeva, Jean-Luc Danet, Alamdar Mammadov, Irada Huseynova and Xavier Foissac</p> <p>Are the “bois noir” - associated phytoplasmas molecularly differentiable from stolbur phytoplasmas infecting other species? Nicoletta Contaldo, Samanta Paltrinieri, Nicola Mori, Alessandro Canel, Loidy Zamora, Bojan Duduk, Assunta Bertaccini</p>
11:00-11:30	Coffee break
11:30-12:15	<b>Poster session 2</b>
12:15-13:15	<b>Session 3. Interaction host-pathogen and Bois noir Control</b>
	<p>Classification trees based on the gene expression information from selected genes for the prediction of the “bois noir” disease status of grapevine. Ana Rotter, Petra Nikolić, Maja Ravnikar, Kristina Gruden, Marina Dermastia</p> <p>Expression of scFv antibodies in plants using Grapevine virus A (GVA) based vectors in an attempt for controlling phytoplasma. Munir Mawassi, Ludmila Maslenin, Liliya Kotliarevski</p> <p>Effects of the treatment with resistance inducers on disease symptoms, plant physiology and production of “bois noir” affected grapevines. Gianfranco Romanazzi, Sergio Murolo, Erica Feliziani</p> <p>How effective is a herbicide treatment for the control of <i>Hyalesthes obsoletus</i>? Angelika Schartl</p>
13:15-13:45	Conclusions
13:45	Lunch

### Poster session

#### Epidemiology

Molecular detection of stolbur phytoplasma from natural foci associated with grapevine yellows in Romania. Constantina Chireceanu, P. G. Ploaie, Anamaria Petrescu, Silvia Cazacu

Occurrence and epidemiology of “bois noir” disease in Czech vineyards. Dana Šafářová, Pavla Válová, Pavel Lauterer, Petr Ackermann, Martin Starý, Milan Navrátil

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#### Characterization

Phylogenetic investigation on “stolbur” isolates from grapevine, insect vector and alternative host plants. Andrea Gentili, Luca Ferretti, Federico Punelli, Elisa Costantini, Graziella Pasquini

*GroEL* gene characterization of “bois noir” phytoplasma from Serbia, Bulgaria and Italy. Jelena Mitrović, Nicoletta Contaldo, Zhelyu Avramov, Marija Smiljković, Assunta Bertaccini and Bojan Duduk

Genetic diversity of “bois noir” phytoplasma in two vineyards of the Bekaa valley Lebanon. Christina Mortada, Fouad Jreijiri, Elia Choueiri and Xavier Foissac

#### Control

Efficacy of entomopathogenic fungi and nematodes against *Hyalesthes obsoletus* nymphs in field conditions. Nicola Mori, Gabriella Frigimelica, Francesca Marcato, Nazareno Reggiani, Francesco Pavan

Biochemical alterations in primary and secondary metabolism of grapevine variety ‘Chardonnay’ (*Vitisvinifera* L.) infected by “bois noir”. Denis Rusjan, Heidi Halbwirth, Karl Stich, Andreja Škvarč, Maja Mikulič-Petkovšek

#### Posters related to other phytoplasma diseases

Detection of aster yellows phytoplasma in date palm in Egypt. Maha Alkhazindar

Aster yellows and X-disease phytoplasmas in wild plants in Southern Finland. Johanna M. Boberg and Jari P.T. Valkonen

Phytoplasma diseases in potatoes in Russia in 2012. Natalia Girsova, Tatyana Kastalyeva, Karina Mozhaeva, Damir Bogoutdinov, Ing Ming Lee

## Presence of stolbur phytoplasma in grapevine and other natural hosts in the republic of Macedonia

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### Introduction

Bois noir (BN) is the main phytoplasma disease of grapevine in the Republic of Macedonia. Presence of stolbur phytoplasma start investigated since 2001 (Seruga *et al.* 2003). Since then, Bois noir is under scrutiny in all grapevines growing region in Macedonia. Typical BN symptoms appear on the grapevine leaves, shoots and clusters, with different extents seen according to cultivar (Fig. 1).

**Figure 1.** Typical phytoplasma symptoms on white variety (Chardonnay) and red variety (Vranec)



### Material and Methods

In this study the epidemiology of the grapevine yellow disease is determined using grapevine samples and wild spontaneous vegetation samples, including the period from 2010 till 2012.

The main aims of this study were: i) to check the presence of grapevines phytoplasmas on grapevines and wild vegetation in 15 localities investigated vineyards in Macedonia, ii) to molecularly characterize and compare the isolates from grapevine with those from other natural host plants iii) to evaluate spreading of BN in all vineyard localities with a particular attention to the recovery phenomenon.

**Figure 2.** Collected material from wild spontaneous vegetation (*Cuscuta* spp. and *Convolvulus arvensis*)



## Results and discussion

A total of 301 grapevine samples and 34 samples from spontaneous vegetation under grapevine symptomatic samples (*Convolvulus arvensis* L., *Cuscuta* sp. L., *Chenopodium album* L., *Solanum nigrum* L., *Amaranthus retroflexus* L.) were tested by PCR/RFLP analyses.

Using nested-PCR with ribosomal primers P1/P7 followed by R16(l)F1R1, and by RFLP analysis (*TaqI*, *Tru9I*, *HpaII*) stolbur phytoplasma on grapevine and some other natural hosts from 15 localities, was tested.

In 185 grapevine samples (61.4%), STOL phytoplasma (16SrXII – A – stolbur phytoplasma group) was identified (Table 1). From spontaneous vegetation, in cuscuta (20%) and convolvulus plants (35.7%), we identify also positive samples with PCR tests. On the contrary, solanum plants were always negative to PCR tests (Table 2).

In two of fifteen localities (Stip and Veles), in the second year of investigation, recovery phenomenon were recorded on "Chardonnay" cultivar – from 38 symptomatic in 2010, 25 recovery plants (did not present symptoms until 2011/12).

**Table 1.** Percentage of symptomatic grapevine samples in different localities

Localities	N°positive/total	%
Negotino	11/19	57.9%
Kavadarci	15/33	45.5%
Gevgelija	5/10	50.0%
Valandovo	12/21	57.1%
Skopje	21/32	65.6%
Kumanovo	14/18	77.7%
Veles	13/26	50.0%
Stip	7/12	58.3%
Argulica	19/24	79.1%
Sarcevo	28/33	84.8%
Amzibegovo	17/26	65.3%
Erdjelija	5/9	55.5%
Kocani	6/12	50.0%
Radovis	3/10	30.0%
Strumica	9/16	56.2%

**Table 2.** Percentage of positive spontaneous vegetation samples in different localities

Samples	N°positive/total	%
<i>Cuscuta</i> spp	2/10	20%
<i>Convolvulus arvensis</i>	5/14	35.7%
<i>Solanum nigrum</i>	0/5	0%
<i>Chenopodium album</i>	0/3	0%
<i>Amaranthus retroflexus</i>	0/2	0%

**Key words:** *Bois noir*, PCR/RFLP, recovery, *Chardonnay*.

## References

- Seruga M., Skoric D., Kozina B., Mitrev S., Krajacic M., Curkovic P., 2003. Molecular identification of a phytoplasma infecting grapevine in the Republic of Macedonia. *Vitis* **42**, 181-185.