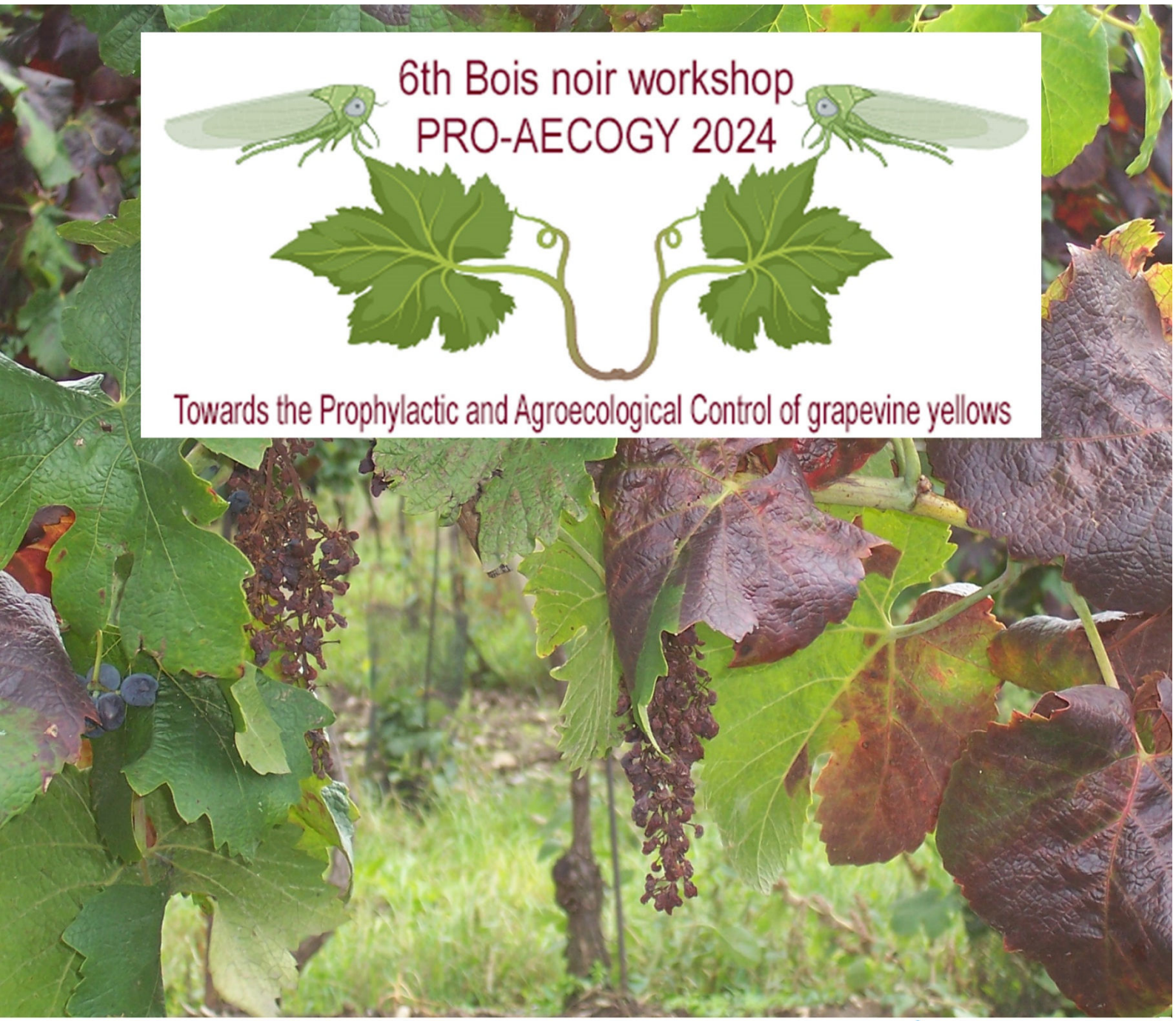
Proceedings of the
6th European Bois noir workshop
1st International Pro-AECOGY conference

Bordeaux 14-16 May 2024



INRAE université de BORDEAUX ISVV INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN BORDEAUX AQUITAINE PLAN NATIONAL DÉPÉRISSEMENT DU VIGNOBLE

Proceedings of the:

6th European Bois Noir Workshop

1st International Pro-AECOGY Conference

Towards the Prophylactic and Agroecological Control of grapevine yellows

14-16 May 2024

Bordeaux

France

Organized by

UMR-1332 Fruit Biology and Pathology,
University of Bordeaux and INRAE

Proceedings of the 6th European Bois Noir Workshop
and 1st International PRO-AECOGY conference
Edited by: Sandrine Eveillard and Xavier Foissac

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Invited Speakers

Elena Gonella is Associate Professor at the Department of Agricultural, Forestry and Food Sciences, University of Turin, where she teaches General and Applied Entomology, Grapevine Protection, Zoology and Parasitology, and Microbial interactions in Arthropods. She is involved in research activities concerning insect-microorganism interactions, including vector transmission of phytoplasmas and insect-symbiont interactions; pest management techniques based on symbiotic control; bio-ethology, epidemiology and management of emerging indigenous and exotic insects of agricultural interest; interactions natural enemies between invasive pests and indigenous and introduced natural enemies. She is the author and co-author of numerous articles.

Professor Saskia Hogenhout obtained her MSc at de Vrije University, Amsterdam in 1994 and her PhD at Wageningen University and Research Centre in 1999. She was appointed as Assistant Professor at The Ohio State University, USA in 1999 and obtained tenure as Associate Professor in 2005. She moved her research group to the John Innes Centre, UK, in June 2007, and became Honorary Professor at the University of East Anglia, Norwich, UK, in 2013. Research in the Hogenhout lab focuses on molecular plant-microbe-insect interactions. Her team investigates how virulence proteins of the insect-transmitted phytoplasmas modulate plant development and resistance to insect vectors. They have developed functional genomic resources for hemipteran sap-feeding insects, including aphids, leafhoppers and froghoppers, to study their interactions with plants, including how salivary virulence proteins modulate plant processes. General research theme: Molecular plant-microbe-insect interactions. Key words: Microbes / bacteria/ insects / plants / pathogens / virulence / effectors / proteolysis / development / immunity / cell biology / biochemistry / protein-protein interactions / genomics / evolution

Valerio Mazzoni leads the Unit of Plant Protection at Fondazione Edmund Mach. As an entomologist, he earned his PhD in 2005 at the University of Pisa with a focus on the taxonomy and ecology of Auchenorrhyncha. Currently, his research primarily focuses on sustainable crop protection methods, including biocontrol through antagonists and behavioral manipulation. Mazzoni is internationally recognized for his pioneering work in applied biotremology, which has significantly advanced the understanding and application of biotremology in agricultural practices. In 2017, the Tremos emitters for vibrational mating disruption against Scaphoideus titanus, developed in Mazzoni's Biotremology lab in collaboration with CBC-Europe, received the prestigious 'Bernard Blum Biocontrol Product of the Year' award for their innovative approach to agricultural pest control. Similarly, in 2022, the bimodal 'Shindo' traps targeting Halyomorpha halys earned the 'Bernard Blum Biocontrol Product of the Year' award for their role in assisting biocontrol uptake.

Nicola Mori is Associate Professor in General and Applied Entomology at the Biotechnology Department - University of Verona where he teaches Grapevine pest management and Protection and sustainability in food production systems. His main research areas are Epidemiology of phytoplasma diseases in grapevine and stone fruits, Integrated pest management in agricultural crops, Side effects of insecticides on beneficial organisms in agro-ecosystems. Currently his research focuses on the development of effective, innovative and practical strategies to protect grapevines from recently introduced pests and on the investigation of efficient pest monitoring methods in agriculture. As public engagement he coordinates working groups at local and national level for the advancement of ecofriendly and sustainable wine production.

Epidemiology of Bois noir

Chairs: Jordi Sabate and Michael Maixner

BOIS NOIR EPIDEMIOLOGY AND MANAGEMENT

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BOIS NOIR EPIDEMIOLOGY

Bois noir (BN) is the most important disease of the phytoplasma-associated grapevine yellows (GY) complex, causing important economic losses in all major wine-growing areas by reducing fruit quality and yields. BN has been associated with '*Candidatus* Phytoplasma solani' (CaPsol, subgroup 16SrXII-A or stolbur group) (Quaglino et al., 2013). The main insect vector of CaPsol is the planthopper *Hyalesthes obsoletus* Signoret (Maixner, 1994), erratically transmitting CaPsol to grapevine and living preferentially on stinging nettle, bindweed and other herbaceous plants and shrubs present in the vineyard and surrounding areas (Mori et al., 2020). Molecular approaches along with transmission trials unveiled that other polyphagous insect species can play a role in vectoring CaPsol to grapevine, including *Reptalus panzeri* Löw, *Aphrodes makarovi* Zachvatkin and *Dicranotropis remaniaca* Guglielmino (Quaglino et al., 2019). In addition, *Reptalus artemisiae* (Becker), *Macrosteles quadripunctulatus* (Kirschbaum), *Anaceratagallia ribauti* (Ossiannilsson), and *Pentastiridius leporinus* (L.) were also identified as putative CaPsol insect vectors, still lacking defined epidemiological role. Moreover, many other weedy hosts were found infected by CaPsol, showing the existence of several reservoir plants in the different agroecosystems (Chuche et al., 2018). The multifaceted ecology of BN highlighted the great genetic diversity and adaptability of CaPsol phytoplasma.

BOIS NOIR MANAGEMENT

The complex epidemiology of BN, involving several insect vector species transmitting CaPsol from multiple plant hosts to grapevine, makes very difficult the disease containment in vineyards. Sustainable Bois noir management is based on grapevines and insect vectors strategies.

<u>Bois noir management strategies focused on grapevine</u>

The selection of healthy propagating material and the choice of grapevine varieties showing low susceptibility to the pathogen, can play a key role in the prevention of BN. To eliminate phytoplasma from infected mother plants, hot water treatments, cryotherapy, and tissue cultures were used, underlying the possible and promising applications of such methodologies on a large scale (Pierro et al., 2024). Nevertheless, after the introduction of phytoplasma-free planting materials in vineyards, the presence of insect vectors able to transfer phytoplasma into healthy plants still represents a concrete risk, thus the BN management strategy needs to be implemented by multiple management approaches. About curative strategies, the utilization of agronomical practices (partial uprooting and pulling of symptomatic plants, grafting of materials from recovered vines to symptomatic plants) and the treatments of the canopy of BN-affected grapevines with resistance inducers or biostimulants showed promising results in inducing recovery and increasing berry production in some grapevine cultivars. The application of these strategies should be extended to wider geographic areas and grapevine varieties to have further confirmation of their effectiveness, optimizing the formulations of resistance inducers, and clarifying the involved mechanism of actions.

Bois noir management strategies focused on insect vectors

Since *H. obsoletus* (and many other BN insect vectors) is a polyphagous species, whose life cycle is not restricted in the vineyard, but also involves mainly surrounding areas, hedges and forests, the use of insecticides cannot significantly reduce neither the vector population, nor BN incidence (Mori et al., 2008). For the control of *H. obsoletus* on herbaceous plant hosts, chemical weeding, soil tilling, and frequent cuts were proposed (Mori et al., 2016); however, it should be considered that weed management may produce a relocation of insects, including those transmitting CaPsol, from wild species to grapevine obtaining a fast and significant raising of BN incidence in vineyards. Alternatively, in organic vineyards, programmed cuts until late summer are highly needed to promote the development of perennial grasses, competitive with stinging nettle (Maixner et al., 2010). Novel and sustainable vector control strategies have been recently developed such as the application of the biocontrol agents (entomopathogenic fungi and nematodes) against *H. obsoletus* (Moussa et al., 2021) and the microbial resource management (endosymbionts) in insect vectors (Gonella et al., 2011). In some area the use of chaste tree in the vineyard surroundings as a trap plant for *H. obsoletus* to prevent BN spreading in the vineyard was suggested (Sharon et al., 2005).

Considering that BN epidemiology involves multiple plant hosts and several insect vectors, the disease management should include an integrated approach based on the direct and indirect vectors control and the use of resistant grapevines.

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Different '*Candidatus* Phytoplasma solani' strains in grapevine cv. Chardonnay and their association with bois noir symptom severity and evolution

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INTRODUCTION

Bois noir (BN) is one of the most important and widespread grapevine yellows (GY) diseases in Europe. It is associated with '*Candidatus* Phytoplasma (*Ca*. P.) solani' (16SrXII-A), whose main vector is the Cixiidae *Hyalesthes obsoletus* Signoret (Quaglino et al., 2013). This study aims to investigate the possible influence of different '*Ca*. P. solani' strains on 1) BN symptom severity and 2) BN disease outcome.

MATERIALS AND METHODS

We performed our study in two Chardonnay vineyards in northeastern Italy (Cormons, Friuli Venezia Giulia), which have been monitored for the presence and incidence of BN since 2007 and 2012, respectively. In the two vineyards, both BN ecological pathosystems are present, the one that has bindweed (*Convolvulus arvensis* L.) as a natural host plant of '*Ca*. P. solani' and the other that instead has stinging-nettle (*Urtica dioica* L.) (Mori et al., 2020). Phytoplasma molecular detection and '*Ca*. P. solani' characterization were performed according to the schemes below (Fig. 1). A comprehensive molecular typing of representative '*Ca*. P. solani' strains was conducted in 2022 based on amplification and sequencing of *tuf*, *secY*, *vmp1* and *stamp* genes. Statistical analyses were performed by Fisher's Exact Test to determine whether the tuf-type of '*Ca*. P. solani' significantly influences the severity (3 classes of symptoms, 2022) and evolution of BN symptoms (symptom persistence, plant death, or recovery, 2015-2021).

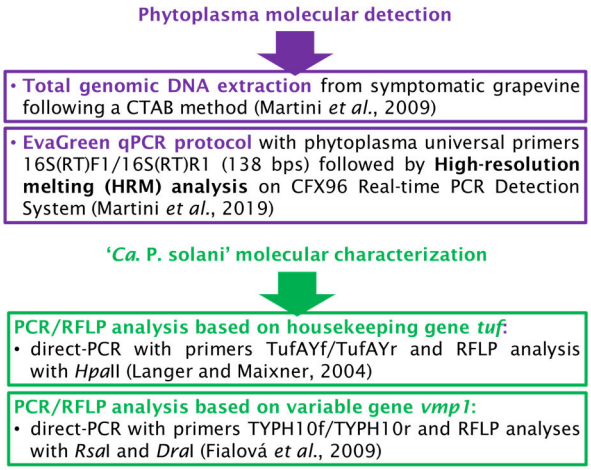

Figure 1. Schemes of the procedures used for phytoplasma molecular detection and '*Ca.* P. solani' characterization.

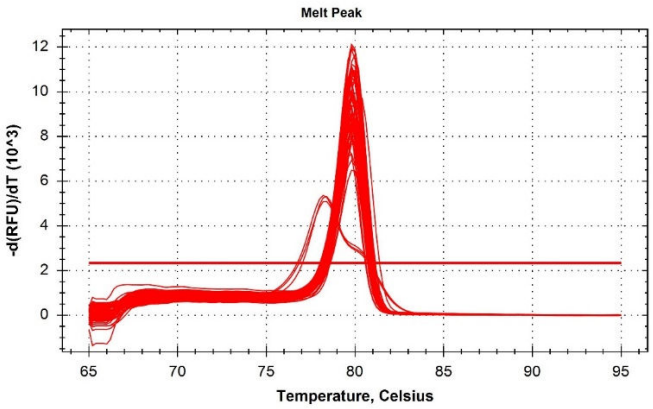

Figure 2. Melting peaks corresponding to 79.6-79.8 °C indicate '*Ca*. P. solani' infection in grapevine samples.

RESULTS AND DISCUSSION

In the two vineyards, the majority of samples collected in 2022 were positive for '*Ca*. P. solani' in qPCR analyses (Fig. 2 and Table 1). Molecular typing of 'Ca. P. solani' strains revealed that the most prevalent genotypes were tuf-a/secY26/Vm15/St9, tuf-b1/secY1/Vm76/St1 and tuf-

b1/secY1/Vm43/St10. In 2022, the percentage of grapevines belonging to the class of most severe symptoms (third class: absent production) was significantly higher for those infected with '*Ca*. P. solani' tuf-a strains versus those infected with tuf-b1 strains (52.0% vs 25.6%; p=0.0377) (Table 1). Furthermore, the mapping over the years of symptomatic plants in the two vineyards revealed that the percentage of dead grapevines was significantly higher in cases of infection with '*Ca*. P. solani' tuf-a strains (47.9% vs 20.4%; p=0.0012), whereas the percentage of recovered plants was significantly higher in cases of infection with '*Ca*. P. solani' tuf-b1 strains (38.8% vs 13.3%; p=0.0007). Therefore, all these results suggest a higher virulence of '*Ca*. P. solani' tuf-a strains in grapevine.

Table 1. Samples of GY symptomatic grapevines grouped by symptom severity (2022), by farm/vineyard and by '*Ca*. P. solani' tuf-type (tuf-a or tuf-b1).

Class of symptom severity	n° samples	n° samples/ vineyard	n° '*Ca*. P. solani' tuf-a/ tot (+ BN)	n° '*Ca*. P. solani' tuf-b1/ tot (+ BN)	n° negative (-), n° positive (+) other GY
1 (typical but mild symptoms, no productive losses)	**22**	12/viney. A	6	6	
		10/viney. B	1	6	3 (-)
		tot	**7/19 (+ BN)**	**12/19 (+ BN)**	3 (-)
2 (moderate symptoms and moderate productive losses)	**26**	14/viney. A	5	8	1 (+ FD)
		12/viney B	0	9	3 (-)
		tot	**5/22 (+ BN)**	**17/22 (+ BN)**	3 (-), 1 (+ FD)
3 (severe symptoms and absent production)	**24**	14/viney. A	9	4	1 (+ FD)
		10/viney. B	4	6	
		tot	**13/23 (+ BN)**	**10/23 (+ BN)**	1 (+ FD)

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Hyalesthes obsoletus and nettle-associated '*Ca*. Phytoplasma solani' epidemiological cycle in Serbia and the Balkans: Is it closed and specific?

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INTRODUCTION

The planthopper *Hyalesthes obsoletus* Signoret, 1865 (Hemiptera: Cixiidae) is a major vector and driver of '*Ca*. Phytoplasma solani' epidemiology (Maixner, 1994; Jović & Toševski, 2023) and associated diseases of cultivated plants, including the grapevine-Bois noir (BN) pathosystem. Because of its strong association with its host plants as a subterranean nymph and short life span as an adult (Cargnus et al., 2012), the epidemiological cycle is host plant-specific and is determined by the vector's host range and the pathogen's reservoir range (Imo et al., 2013; Maixner et al., 2014). Although *H. obsoletus* is commonly viewed as a polyphagous insect, there is plenty of proof of host plant adaptation in its populations, which are referred to as host races, biotypes, or cryptic species (Imo et al., 2013; Maixner et al., 2014; Kosovac et al., 2016; 2018; 2019). The first described and most prevalent epidemiological cycles linked with distinct strains of '*Ca.* P. solani' are driven by nettle and bindweed as pathogen reservoir plants and *H. obsoletus* host plants (Langer & Maixner, 2004). The genetic distinction between the two pathosystems was initially identified on the *tuf* gene and then verified by *secY*, *stamp*, and *vmp1* gene typing (Langer & Maixner, 2004; Johannesen et al., 2012; Aryan et al., 2014). In Serbia and the Balkans, nettle-associated '*Ca.* P. solani' genotypes are not commonly found in BN-affected grapevine (Atanasova et al., 2015; Kosovac et al., 2016); hence, research on this epidemiological pathway is neglected and available data are scarce. During more than a decade of research on '*Ca.* P. solani' epidemiology and *H. obsoletus* biology, ecology, and genetics in the Balkans, we have frequently found the presence of "wrong" '*Ca.* P. solani' genotypes in vector specimens obtained from nettles. Here, we assess these findings and call into question the geographic specificity of nettle-associated epidemiology in southeastern Europe.

MATERIALS AND METHODS

Adult *H. obsoletus* specimens were selectively collected from nettles across the Balkans and screened for the associated '*Ca*. P. solani' genotype. The collections were made in mid-July between 2011 and 2023. Some of the material or locations of the *H. obsoletus* collecting sites have previously been reported (Atanasova et al., 2015; Kosovac et al., 2016; 2018; 2019; Jović & Toševski, 2023). The material included specimens collected in Serbia, Hungary, Romania, Bulgaria, Montenegro, North Macedonia, and Greece on natural sites of nettle-growing habitats. In 2023, transmission experiments using nettle-associated *H. obsoletus* adults collected from selected locations in eastern Serbia (Braničevo district, Požarevac) with a high local abundance of the insect were performed on periwinkle plants. The nettle plants were sampled from the same sites and tested for the presence of '*Ca*. P. solani'. Total DNA was isolated from insects using a nondestructive SDS-based method (Kosovac et al., 2018). Initial identification of '*Ca*. P. solani' was done by nested PCR amplification and typing of the *stamp* gene, followed by *tuf*, *secY*, and *vmp1* typing (Johannesen et al., 2012) whenever possible, to correlate the pathogen's genotype with either nettle or the bindweed-derived epidemiology. The same methods were applied to periwinkle plants that had been exposed to naturally infected nettle-associated *H. obsoletus* individuals that were used in transmission trials. All '*Ca*. P. solani'-positive individuals genotyped on the *stamp* gene were subsequently tested for pathogen concentration with a real-time SYBR-Green qPCR method (Hren et al., 2007).

RESULTS AND DISCUSSION

When utilizing nested PCR *stamp* gene typing to identify '*Ca.* P. solani' in *H. obsoletus* individuals collected from nettles, "wrong" genotypes (bindweed-associated genotypes) were frequently found. This disparity was detected in more than 20% of all analyzed *ex* nettles *H. obsoletus* '*Ca.* P. solani'-carrying individuals, and in more than 80% in some locations. However, further typing of these isolates was not possible, as other marker genes of '*Ca.* P. solani' could not be amplified. In contrast, all isolates initially identified by *stamp* typing as "correct", i.e., nettle-associated, were successfully genotyped on all markers and verified as "typical nettle-associated" genotypes commonly occurring in central and western Europe, e.g., tuf-a/S7/ST19/V3 and tuf-b2/S6/ST6/V18. Individuals carrying '*Ca.* P. solani' bindweed genotypes had low phytoplasma titers (Cq > 33), likely due to adult erroneous feeding on non-host plants. However, individuals with nettle-associate genotypes carried '*Ca.* P. solani' in high concentrations (16 < Cq < 21). The genotypes identified in transmission trials, as well as those naturally infecting nettle plants, were all nettle-associated, which thus confirmed the specificity of the nettle-associated epidemiology and closed cycle of transmission in the Balkans.

ACKNOWLEDGEMENTS

We are grateful to X. Foissac for providing information on the SEE-ERA NET '*Ca*. Phytoplasma solani' classification. The research was supported by Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contract No. 451-03-66/2024-03/200010).

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Geographical and genetic diversity of '*Candidatus* Phytoplasma solani' isolates in Croatian grape growing regions

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INTRODUCTION

'Candidatus Phytoplasma solani' (CPs), a phytoplasma endemic to the Euro-Mediterranean basin is associated with several plant diseases, including the grapevine yellows disease „bois noir" (BN) (Quaglino *et al.* 2013). CPs spreads through highly complex disease cycle comprising several, possibly intermixed epidemiological networks which include diverse insect vectors and a broad range of host plant species. *Hyalesthes obsoletus* is considered to be the principal vector of CPs associated with BN. It feeds on various herbaceous plants but is most commonly associated with *Convulvulus arvensis* and *Urtica dioica* as hosts for its nymphs and adults (Langer and Maixner, 2004). A vector role in BN transmission has been proven also for *Reptalus panzeri* (Cvrković *et al.* 2014) and *Dictyophara europea* (Cvrković *et al.* 2022). The epidemiology of CPs is highly correlated with distribution of the host plants - tentative inoculum source in vineyards from which the insect vectors acquire phytoplasma. As different CPs strains have been shown to have different ecological reservoirs and pathways for spread, the genetic characterization of CPs strains is essential. The variability, prevalence, and distribution of the CPs genotypes from various hosts involved in the BN pathosystems was assessed by MLST approach to better document the BN situation in the Croatian vineyards.

MATERIALS AND METHODS

In the frame of a national survey programme, from 2009 to 2020, overall number of 3336 samples of grapevine, 89 weed and other plant samples, along with 267 of known and presumed CPs vectors from the suborder Auchenorrhyncha were collected from all Croatian grape-growing regions and tested for the presence of CPs. Total nucleic acids were extracted according to the previously described CTAB extraction protocol from grapevine or other plants (Plavec *et al.* 2019) or with the commercial kit OmniPrep™ (G-Biosciences, St. Louis, MO, USA) from individual insects. Samples were then analysed by triplex real-time PCR assay according to Pelletier et al. (2009). From most CPs foci in the country representative isolates were chosen and further analysed by MLST including *tuf, secY, vmp1* and *stamp* genes (Schneider *et al.* 1997; Fialova *et al.* 2009; Cimerman *et al.* 2009; Fabre *et al.* 2011). Amplicons were sequenced in both directions followed by editing and assembly with Geneious (http://www.geneious.com/) and alignment with ClustalX 2.0. Phylogenetic analyses were conducted by MEGA 7 and nucleotide sequences attributed to sequence type by their comparison with sequences deposited in GenBank following the previously designed nomenclature (Foissac *et al.* 2013).

RESULTS AND DISCUSSION

The presence of CPs was confirmed in grapevine, *Ailanthus altissima, Robinia pseudoacacia* and *Polygonum aviculare* as well as in insects *H. obsoletus*, *D. europaea* and *Cixius wagneri.* The MLST analyses performed on 78 plant samples and 20 insect samples revealed two new genotypes for *stamp* and *vmp1* genes, designated as ST59 and V28, respectively. *Stamp,* with its presumed important role in the interactions with insect vector was found to be a highly discriminative marker with 13 genotypes detected. Overall, analysis identified 28 different CPs MLST genotypes. The prevalent MLST genotype in grapevine CPsSqt21 (S6/ST6/V18/tuf-b2) found across central and north-western Croatia

was affiliated to *H. obsoletus* and *U. dioica*. The other two most frequent genotypes were the *U. dioica*-associated CPsSqt28 (S39/ST46/V3/tuf-a) and the *C. arvensis*-associated CPsSqt2 (S1/ST9/V4/tuf-b1). Especially interesting was CPsSqt11 (S4/ST4/V28/tuf-b1) shared by two hosts, *A. altissima* and *C. wagneri*, which encompasses a new vmp1 genotype (V28). Moreover, three positive *C. wagneri* specimens found in different regions shared the same vmp1 genotype. Nevertheless, our results with prevalent MLST genotypes are in accordance with regional distribution of CPs strains in neighbouring countries (Mehle *et al.* 2022). Although the *H. obsoletus* is a principal vector of CPs in Croatia, our survey revealed that its population did not correlate with the distribution and high number of CPs positive plants. Even though this finding could be attributed to the limited insect sample number or locally determined interactions of hosts and vectors that favour adaptive mutations, the substantial number of MLST genotypes found indicates the co-existence of several independent epidemiological cycles. This is certainly a consequence of a unique geographical position of Croatia, bridging the different eco-climatic areas of central and south-eastern Europe.

ACKNOWLEDGEMENTS

This study was partially supported by Croatian Science Foundation grant no. UIP-2014-09-9744 and IP-2019-04-2469 and by the Ministry of Agriculture (National Survey Programme).

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Grapevine cultivation and Bois noir in China – lessons from other phytoplasma diseases

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Grapevine cultivation and wine production in China

Grapevine cultivation in China has a long history and is widely distributed. According to the Chinese National Bureau of Statistics (CNBS, 2023), in the year 2022, the total grapevine cultivation area in China is 705,110 ha, and the total production is 15,377,900 ton. Of all the grapevine production, about 80% is table grape, 15% is wine grape, and 5% is raisin grape. According to China National Light Industry Council (CNLIC, 2023), the total wine production in China is 143,000 kL in 2022. Red wine share 86.3% of the total production in China (OIV, 2023).

Table grape cultivation is distributed all over China, the main production provinces including Xinjiang, Liaoning, Shannxi, Jiangsu, Guangxi, Yunnan and Shandong. The most common cultivars include Summer Black, Kyoho, Shine-Muscat, and Centennial Seedless. In the central and southern provinces, where heavy rain occurs during the grapevine ripening, rainproof cultivation is usually applied to avoid cracking (Liu, 2017).

Wine grape cultivation is mainly distributed in north-west provinces, Xinjiang, Gansu and Ningxia, as well as north provinces, Hebei and Shandong. The most planted cultivars are Cabernet Sauvignon, Cabernet Franc, Cabernet Gernischt, Merlot, Syrah, and Riesling (Liu, 2017)

Raisin grape is mainly distributed in Xinjiang province, and the cultivar is Centennial Seedless (Liu, 2017). This province is located in northwest of China, with a land area of 1 664 897 km^2, almost four times of California. Semiarid or desert climate prevails in Xinjiang. In places with plenty irrigation water, it is very suitable for fruit and wine production.

Bois noir disease in China

During a short visit to China in the year 2008, Bertaccini *et al*. found phytoplasma symptoms on grapevine (*Vitis vinifera*), apple (*Malus* spp.), apricot (*Prunus armeniaca*) and Chinese scholar tree (*Sophora japonica*) in Shanxi province, north China (Duduk *et al*., 2010). Polymerase chain reaction/ restriction fragment length polymorphism (PCR/RFLP) on the phytoplasma 16S ribosomal gene followed by sequencing showed that the phytoplasmas are closely related to "stolbur" ('*Candidatus* Phytoplasma solani'). This is the first and only "bois noir" case reported in China where now it is a quarantine disease (Jiang *et al*., 2011).

Phytoplasma diseases in China

Record of phytoplasmas could be tracked back to about 1000 years ago. Chinese famous poet Ou Yangxiu, in Song dynasty (1031), mentioned about the "Yaohuang" ("Yao-yellow") tree peony, a green flower peony cultivar, which was late confirmed associated with the presence of mycoplasma-like organisms by electronic microscope observation (Wang & Maramorosch, 1988). According to a bibliometric study, about 300 plant species from more than 80 families were reported associated with phytoplasmas in mainland China and Hainan Island. The phytoplasmas are from eleven 16Sr groups, namely group -I, -II, -III, -V, -VI, -XI, -XII, -XIV, -XIX, -XXX, and -XXXII (J. Li, unpublished data). Economically important phytoplasma diseases in China include mulberry dwarf (16SrI-B), wheat blue dwarf (16SrI-C), paulownia witches' broom (16SrI-D), jujube witches' broom (16SrV-B), rice yellow

dwarf (16SrXI-A), and sugarcane white leaf (16SrXI-B). These phytoplasmas are associated with severe damage in crops and trees. New phytoplasma associated disease are being reported continuously (Wang *et al*., 2022).

One systematically studied phytoplasma in China is jujube witches' broom (JWB), associated with JWB phytoplasma ('*Ca.* P. ziziphi', 16SrV-B). Jujube (*Ziziphus jujuba*) is a fruit tree species originate and dominated in the middle and lower reaches of the yellow river in northern China. As one of the oldest cultivated fruit trees in the world, jujube has a long cultivation history of more than 7,000 years. The traditional jujube cultivation areas distribute in Henan, Hebei, Shanxi, Shannxi and Shandong provinces. In the new century, the main production area has transferred to Xinjiang, which contribute 55.98% of the total dry fruit production of China in year 2020. In Xinjiang, the climate is dry, the extreme low temperature in winter exceeding -20°C. Thus, the bacteria and insect vectors can barely survive, and JWB disease rarely occur. In the traditional jujube cultivation areas, JWB disease is well controlled by keeping the tree vigor by irrigation, fertilizer, and keeping the orchard phytoplasma-free by sanitation methods and pesticides application. However, since farmers put less labor in management for economic reasons in these years, the JWB disease is getting more and more serious (Guo *et al*., 2023).

Lessons learned and perspectives

The global warming makes the environment more suitable for phytoplasma associated disease and their insect vectors in the warm temperate zones. An appropriate management may keep the occurrence of phytoplasma diseases at an economical threshold level. More knowledge about the phytoplasma diseases, especially for their early detection and efficient containment methods should be disseminate to the public.

ACKNOWLEDGEMENTS

The authors would thank China Scholarship Council for funding Li Jidong's visiting research at University of Bologna

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Genomic, Diversity of grapevine phytoplasmas

Chairs: Martina Seruga-Music and Fabio Quaglino

Molecular genotyping of '*Candidatus* Phytoplasma solani' strains identified in different crops in Jordan

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INTRODUCTION

Recent surveys on phytoplasma-associated diseases in Jordan highlighted that '*Candidatus* Phytoplasma solani' (CaPsol) is the prevalent phytoplasma throughout the Country. It was largely reported in several crops exhibiting different symptoms, in wild plants (CaPsol plant hosts), and putative insect vectors (Abu Alloush et al., 2023a,b, 2024). To improve the knowledge of CaPsol ecology, this study investigated the genetic diversity within CaPsol strain population identified in multiple crops in Jordan.

MATERIALS AND METHODS

DNA extracts from 51 CaPsol-infected plants of 8 crops, selected from previous studies (Abu Alloush et al., 2023a,b, 2024) (Table 1), were used as templates in direct and nested PCRs for the amplification of *tufB*, *stamp,* and *vmp1* genes, carried out as previously described (Aryan et al., 2014; Fabre et al., 2011). To genotype CaPsol strains identified in Jordan, nucleotide sequences of obtained PCR products were compared with those of representative CaPsol strains previously described (Pierro et al., 2018; Jamshidi et al., 2022).

Table 1. CaPsol strains infecting different crops in Jordan selected for molecular typing

Host	Symptoms	No. CaPsol strains
Almond	witches'-broom, yellowing, dieback	10
Cherry	yellowing	7
Grapevine	leaf reddening/yellowing and rolling	17
Peach	witches'-broom, yellowing	4
Pear	leaf reddening	1
Persimmon	leaf scorch and rolling	2
Plum	witches'-broom, yellowing	6
Pomegranate	yellowing, witches'-broom, little leaf	4

RESULTS AND DISCUSSION

Expected amplicons of *tufB*, *stamp,* and *vmp1* genes were obtained from all the 51 CaPsol-infected plants analyzed. Surprisingly, considering the high genetic diversity generally present within CaPsol strain populations in a specific geographic area (Quaglino et al., 2021; Jamshidi et al., 2022), nucleotide sequence analyses revealed that all 51 CaPsol strains share identical *tufB*, *stamp,* and *vmp1* gene sequences, highlighting there is no genetic variability in CaPsol strain populations in Jordan. Comparison with previously described CaPsol genotypes revealed that Jordanian CaPsol strains share sequences identical to genotypes tuf b-1 (strain CrHo12_601, Acc. No. KJ469708), St15 (strain P7, Acc. No. FN813258), and Vm53 (strain P7, Acc. No. AM992100), previously identified in Lebanon and Georgia (Caucasus region) and associated with bindweed-related pathosystem (Quaglino et al., 2016; Pierro et al., 2018). Further studies will investigate the diffusion of CaPsol genotype tuf b-

1/St15/Vm53 in additional plant hosts and putative insect vectors to study the epidemiological patterns of CaPsol-associated diseases in Jordan.

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Genetic diversity in putative effector genes among FDp and FDp-related strains from northern Italy

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INTRODUCTION

Flavescence dorée (FD) is the most important leafhopper-transmitted grapevine disease associated with a quarantine pathogen (FD phytoplasma, FDp). Recent studies demonstrated the presence of many FDp genotypes that differ in their epidemiological cycle, suggesting genotype-specific biological behaviour (Malembic-Maher *et al*., 2020; Rigamonti *et al*., 2023; Rizzoli *et al*., 2021). The recent availability of full FDp genome (Deboneville *et al*., 2022) can allow a deeper understanding of the dynamics that regulate this devastating disease, considering that, to date, phytoplasmas cannot be isolated in a pure culture *in vitro*, so they can be studied only at molecular level. The knowledge of genetic variability of putative effector genes in different FDp strains and how this variability can influence the disease development and severity will be useful to develop more targeted management strategies in agroecosystems. This study reports first results obtained by molecular typing of different FDp strains, identified in North Italy, based on sequence analyses of putative effector genes.

MATERIALS AND METHODS

Twenty-six 16SrV phytoplasma strains, previously identified in grapevine and other plant hosts in northern Italy, have been selected: 23 FDp strains (14 M54, 5 M51, 4 M50), and three FDp-related strains (2 M43, 1 M39) (Table 1). According to the FDp genome annotation by Deboneville *et al*. (2022), two putative effector genes (*hp1*, locus tag M6G77_00200; *hp4*, locus tag M6G77_02130), and a gene encoding the Bax inhibitor 1 (*baxI*, locus tags M6G77_00035 and M6G77_00935) have been selected for molecular typing. Primer pairs for each gene have been designed for direct and nested-PCR amplification carried out as follows: 94°C for 5 minutes followed by 35 cycles at 94°C for 1 min, 50°C (52°C in nested-PCR) for 1 min, 72°C for 2 min, and final elongation at 72°C for 7 minutes. PCR products have been sequenced in both directions. Nucleotide sequences and *in-silico* translated proteins have been compared to identify mutations within the analyzed FDp strains.

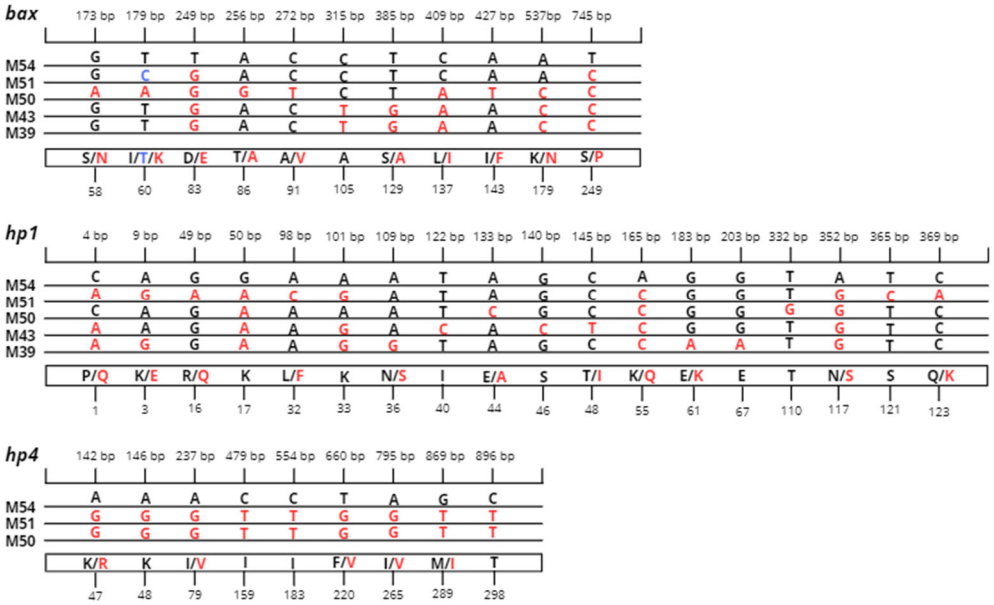
RESULTS AND DISCUSSION

Most of the 16SrV phytoplasma strains were positive to *baxI* gene amplification (25 out of 26), while 20 strains out of 22 and 18 out of 22 were positive to *hp1* and *hp4* amplification, respectively (Table 1). FDp and FDp-related strains belonging to the same *map*-genotype share identical *baxI*, *hp1*, and *hp4* nucleotide gene sequences, while several SNPs (11 in *baxI*, 18 in *hp1*, 9 in *hp4*) were found distinguishing strains of distinct *map*-genotypes. Most of such SNPs (10 in *baxI*, 11 in *hp1*, 5 in *hp4*) were non-synonymous mutations, determining amino acidic variation in the *in-silico* translated proteins (Figure 1). None of the SNPs produced stop-codon interfering with the protein functionality. Differences observed in the putative effector genes and *in-silico* translated proteins, distinguishing FDp *map*-genotypes, could influence their interaction with hosts and determine their specific ecological niches. Further studies will be conducted to evaluate the genetic diversity of FDp and FDp-related *map*-genotypes in other putative effector genes, and to investigate and clarify the functional role of these putative effectors.

Table 1. Results of PCR-based amplification of *baxI*, *hp1*, and *hp4* genes from 16SrV phytoplasma strains

N. of 16SrV phytoplasma strains	Plant species	*map*-genotype	*baxI*	*hp1*	*hp4*
5	*Vitis vinifera*	M54	+	+	+
1	*Hedera helix*	M54	+	n.d.	n.d.
1	*Robinia pseudoacacia*	M54	+	n.d.	n.d.
1	*Convolvulus arvensis*	M54	+	n.d.	n.d.
3	*Euonymus spp.*	M54	+	-	+
2	*Acer spp.*	M54	+	+	+
1	*Ailanthus altissima*	M54	-	+	-
3	*Ailanthus altissima*	M51	+	+	+
1	*Euonymus spp.*	M51	+	+	+
1	*Quercus spp.*	M51	+	+	+
1	*Alnus glutinosa*	M50	+	-	-
3	*Ailanthus altissima*	M50	+	+	+
1	*Ailanthus altissima*	M43	+	+	-
1	*Alnus glutinosa*	M43	+	+	-
1	*Alnus glutinosa*	M39	+	+	-

Figure 1. Single nucleotide polymorphisms and amino acidic variations in *baxI*, *hp1*, and *hp4* genes within analyzed FDp and FDp-related *map*-genotypes



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Genes encoding collagen-like repeats are promising Variable Numbers Tandem Repeats (VNTR) markers for the differenciation of Bois noir-associated '*Candidatus* Phytoplasma solani' strains

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INTRODUCTION

In population genetics, repeated DNA sequences are widely targetted to describe genetic diversity. In bacteriology, Variable Numbers of Tandem Repeats (VNTR) are widely used as such repeats do not vary at the same pace as do neutral genes (Hood et al., 1996; Frotingham and Meeker-O'Connell, 1998) and usually evolve in a *rec*A-independent manner (Puopolo *et al.,* 2001). When used in combination (Multiple Loci VNTR Analysis, MLVA) (Johanss0n et al., 2004), they produce fingerprints of bacterial populations, especially at the site of emergence which translate as a bottle-neck in terms of genetic variability. In the frame of a '*Candidatus* Phytoplasma solani' (CaPsol) genome survey of the PO strain, a gene, named *coll-like*, was found to encode GXY amino acid repeats reminiscent of collagen structure (Cimerman et al., 2006). Search for other GXY repeats in CaPsol strain PO draft genome revealed the presence GXY repeats in a gene encoding a surface protein. The encoded protein is also possessing large repeated domains upstream of the collagen-like repeats, a signal peptide and a C-terminal hydrophobic alpha-helix, a structure reminiscent of Vmp1 (Cimerman et al., 2009). This gene will be referred as *vmp3-coll.* The objective of this study was to assess the variability of these two potential VNTRs in grapevine Bois noir-associated CaPsol isolates.

MATERIALS AND METHODS

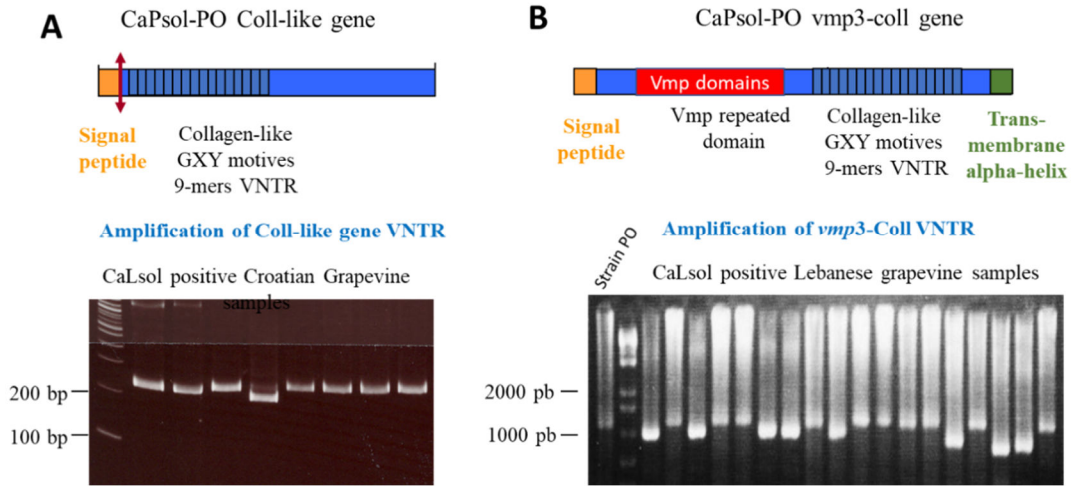
Total nucleic acids were extracted from 1 g of grapevine petioles according to CTAB standard procedure from samples collected in Bekaa valley (Lebanon) and from various location in Croatia. These samples had been shown to be positive for CaPsol infection (Mortada et al., 2013; Plavec et al., 2015). They were submitted to nested-PCR amplification with the primers described below. For *coll-like*, the first primer pair was collF1 (5'-CCTTTTATCATAACCTGTTT-3') / collR1 (5'-CAATAGGATATAGATAG-3'), followed by the primer pair collF2 (5'-GCTATTATTTATGCTGGCTC-3') / collR2 (5'-CGCTGTCCGTCTTTTGCTAA-3'), and PCR conditions were 95°C 2 min, 35 cycles of 95°C 30 sec, 55°C 30 sec, 72 °C 15 sec. For *vmp3-coll*, the first primer pair was vmp3-F5 (5'-GCTTCAAATAAGAATAGCATCAG-3') / vmp3-R4 (5'-GTTGCTGTATCTGGTGAAGT-3'), followed by vmp3-F4 (5'-CAACTAATT-TTGGACCTAACGG-3') / vmp3-R3 (5'-GTTTGTAGCTGGTTGATCTGG-3'), and PCR conditions were 95°C 2 min, 35 cycles of 95°C 30 sec, 55°C 30 sec, 72 °C 1 min. PCR products were analysed on 1.5 % and 0.7 % agarose gel electrophoresis for *coll-like* and *vmp3-Coll*, respectively.

RESULTS AND DISCUSSION

All selected CaPsol-associated Bois noir isolates from Croatia showed nested-PCR amplifications with *coll-like* primer pairs, with three different electrophoretic mobility profiles observed on gel electrophoresis among the eight Croatian samples tested (Figure 1-A). All selected CaPsol-associated Bois noir isolates from Lebanon showed nested-PCR amplifications with *vmp3-coll* primer pairs, with at least five different electrophoretic mobility profiles observed on gel electrophoresis among the

eighteen Lebanese samples tested (Figure1-B). For both genes, the sequencing of the obtained amplicons will indicate the actual number of VNTR repeats.

Figure 1: Structure of Coll-like and Vmp3-coll proteins and nested-PCR amplification of corresponding VNTR in Croatian (A below) and Lebanese grapevine samples (B below).



The amplification and sequencing of *vmp3-coll* VNTR among a set of sixteen CaPsol isolates from eleven Euro-mediterranean countries of the SEE-ERANET/ STOLBUR-EUROMED network (Foissac et al., 2013), indicated that the number of *vmp3-coll* VNTR was highly variable among CaPsol isolates and ranging from 28 to 64. Only three CaPsol isolates gave no *vmp3-coll* VNTR amplifications indicating either the absence of *coll-like* and *vmp3-coll* genes in some of the CaPsol strains tested or sequence variability at the site of primers.

ACKNOWLEDGEMENTS

This study was supported by the Plan National Dépérissement du Vignoble grant RENOV and the French-Lebanese bilateral project CEDRE 13E-L2.

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Diversity of effectors and potential mobile unites in '*Candidatus* Phytoplasma solani' genomes

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INTRODUCTION

The recent ascent of metagenomic era and new genomic technologies delivered new tools and possibilities in finding potential effectors by which phytoplasmas successfully manipulate their hosts. However, challenges in cultivation of phytoplasmas in a pure *in vitro* culture and enrichment of phytoplasma DNA together with possible high percentage of repetitive sequences still hamper attempts to obtain complete assembly for some of the phytoplasma genomes. Nonetheless, almost 20 completely assembled phytoplasma genomes have been deposited in GenBank so far, together with substantial number of phytoplasma genome drafts (Wei and Zhao 2022, https://www.ncbi.nlm.nih.gov/genome/browse#!/overview/phytoplasma) including only 3 draft genomes for *'Candidatus* Phytoplasma solani' (Mitrović et al 2014, Seruga Music et al. 2019). '*Ca.* P. solani' is a cosmopolitan pathogen with a broad host range transmitted by many insect species and one of the most important phytoplasmas in Croatia, associated with widespread *bois noir* (BN) disease (Quaglino et al. 2013, Plavec et al. 2015). In our previous work, we sequenced a genome of '*Ca*. P. solani' SA-1 strain from micropropagated periwinkle (*Catharanthus roseus* (L.) G. Don.) tissue infected with phytoplasma originating from grapevine. The assembly, annotation and prediction of secreted proteins revealed the presence of 38 putative effectors with 20 of them being located within potential mobile units (PMUs) or PMU-like elements (Seruga Music et al. 2019). However, the within-species diversity of effectors remains unclear. The aim of this study was to obtain more genomic data by sequencing two '*Ca*. P. solani' strains originating from different plant hosts in order to get better insight into the *'Ca.* P. solani' effector repertoire and diversity.

MATERIALS AND METHODS

Isolation of total genomic DNA was performed from periwinkle plants infected by single '*Ca.* P. solani' strains (ST19 and STOL) originating from different plant hosts and maintained by grafting, followed by generation of libraries and sequencing by using Illumina MiSeq (GENEWIZ Azenta Life Sciences) and ONT MinION platforms (Academia Sinica, Taipei, Taiwan). Raw reads were mapped to the reference genome and reads originating from periwinkle were filtered out. *De novo* assembly was performed by using Unicycler v0.4.9b (hybrid assembly), followed by annotation of genome drafts (RNAmmer, tRNAscan-SE, Prodigal), and prediction of putative effector genes (SignalP v5.0). Whole genome alignment and comparative analyses were also performed. For selected putative effectors, including homologues of SAP11 and SAP54, specific primers were designed by using Geneious Prime software (https://www.geneious.com/, Biomatters Ltd., Auckland, New Zealand). Thirty isolates from our lab collection of '*Ca*. P. solani' originating from different hosts were used in assessment of variability of selected effectors. Complete sequences of *SAP11-like* and *SAP54-like* genes were amplified and sequenced (*GENEWIZ* Azenta Life Sciences), followed by editing by using Geneious Prime software and alignment of sequences by using ClustalX (http://www.clustal.org/clustal2/). Subsequent phylogenetic analyses were performed by using MEGA 11 software (https://www.megasoftware.net/).

RESULTS AND DISCUSSION

De novo assemblies generated 2 draft genomes with total size of 707,036 bp (ST19) and 656,141 bp (STOL) in 28 and 19 contigs, respectively. Prediction of putative effector genes in the draft genomes and comparative analyses revealed the presence of 22 and 20 putative effector genes, respectively, including some of the homologues of AYWB secreted proteins (Bai et al. 2006) such as *SAP11-like*, *SAP54-like*, *SAP55-like*, *SAP25-like*, *SAP44-like*, *SAP19-like* and *SAP61-like*. Also, some of the predicted effector genes were identified as species- and strain-specific ones. Moreover, our study has shown that different '*Ca*. P. solani' isolates possess different array of effectors with some effectors being present in all isolates and conserved (*SAP11-like*), some seem to be species specific, while some are present in more than one variant among the strains (*SAP54-like*). Further detailed genome analyses revealed the presence of PMU-like regions and elements (14 in ST19 and 6 in STOL strain) of a different composition and size, up to 12 kbp. In STOL strain some of the PMUs were completer and resembled those already described in '*Ca.* P. solani' and '*Ca.* P. asteris', while in ST19 more diversification in PMU-like elements was revealed. In both, effector and putative secreted protein genes were frequently found within PMU-like regions. Molecular phylogeny of selected PMU genes demonstrated different origin and horizontal gene transfer, suggesting their role in host adaptation and pathogenicity. All the results of this studies are in corcondance with '*Ca.* P. solani' great adaptability potential and pathogenicity affecting numerous plant hosts. This study sets a base for functional studies of putative *'Ca.* P. solani' effectors and their interactions with host targets, which can facilitate deciphering the pathogenicity strategies of this successful and versatile pathogen.

ACKNOWLEDGEMENTS
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Biology of grapevine phytoplasma insect vectors

Chairs: Tatjana Cvrkovic and Gudrun Strauss

Manupulating microbial symbioses in phytoplasma vectors as a tool to interrupt grapevine yellows spread

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INTRODUCTION

Symbiotic interactions with microbes are a central trait for a wide range of insects, including the vectors of phytoplasmal agents of grapevine yellows (GY), which all belong to the Auchenorrhyncha suborder within Hemiptera (Cao et al., 2022). In Auchenorrhyncha, an ancient co-diversification has been reported with primary symbionts, which provide hosts with essential nutrients and show intracellular localization into specialized insect organs and strict vertical (transovarial) transmission. Additionally, facultative symbionts, often with unknown function, have been described in many phytoplasma vectors with multiple localizations in the host body and mixed vertical-horizontal transmission modes (Brentassi & de la Fuente, 2024). The broad distribution and abundance of microbial symbioses in Auchenorrhyncha suggested they may be exploited to reduce the chance of phytoplasma transmission by vectors, providing an additional tool for disease management (Gonella et al., 2019a). This approach may help going beyond the practical limitations of insecticide applications, especially for polyphagous vectors that are mostly found in the wild compartment of vineyard agroecosystems.

PEST MANAGEMENT STRATEGIES BASED ON SYMBIONT MANIPULATION

Several methods have been described to reduce the pest status of insects (i.e., reducing their fitness or altering their vector competence for pathogens) through the alteration of microbial symbioses, under the strategy named symbiotic control. The main approaches to reach this objective are funded either on establishing new (heterologous) associations, producing genetically modified symbionts, or disrupting obligate symbioses (Arora & Douglas, 2017). The first technique is mostly implemented through the exploitation of the reproductive manipulator *Wolbachia*, both using strains that induce cytoplasmic incompatibility as well as those showing pathogen blocking phenotypes. *Wolbachia*-based strategies may aim suppressing insect progeny, through the incompatible insect technique, or disturbing vectored pathogens in live insects (Gong et al., 2023). The production of genetically modified symbionts (paratransgenesis) has been proposed against several pest insects, including vectors of agricultural relevance, however its application is widely debated, and it is still not authorized in Europe. The disruption of obligate symbioses has been recently introduced as a pest management measure against some stink bug pests (Hemiptera, Pentatomidae), where the interruption of vertical transmission can be conducted as this process undergoes an environmental phase (Gonella & Alma, 2023).

APPLICATIONS IN GY VECTORS

In consideration of the main characteristics of the microbial symbioses in Auchenorrhyncha, the implementation of symbiotic control strategies against GY phytoplasma transmission is not trivial. While the application of methods based on paratransgenesis is currently limited, due to regulatory and ethical issues, the disruption of obligate symbioses may be hampered by the protective strategies that are the result of the long-term co-evolution between primary symbionts and Auchenorrhyncha. These symbionts are indeed intracellular in the host body, and they are transmitted in a transovarial way; hence the only way to suppress them would be by using systemically delivered antibiotics to feed vectors, which would produce several harmful side effects on the plant and the whole food web. Therefore, the most acknowledged technique in the case of GY vectors may be the promotion of new

symbiotic interactions, which could gather the specific requirements for a successful control. So far, different bacteria have been shown related with a reduction of phytoplasma infection in vectors. The acetic acid bacterium *Asaia* sp. was reported to limit phytoplasma acquisition in the experimental vector of flavescence dorée (FD) phytoplasma *Euscelidius variegatus* under laboratory conditions, by means of a combination of physical exclusion, due to high production of an air-liquid interface biofilm capable of sequestrating phytoplasma cells, and the activation of immune genes in host to counteract infection (Gonella et al., 2018, 2019b). In the field, a negative correlation was found between the presence of *Wolbachia* in the FD vector *Dictyophara europaea* and phytoplasma infection (Krstic et al., 2018), suggesting that the symbiont may display pathogen blocking phenotypes against the phytoplasma, and/or drive the host reproduction to maintain this phenotype over generations. Since *Wolbachia* is widespread in other GY vectors ─ e.g. the bois noir vector *Hyalesthes obsoletus* (Gonella et al., 2011) ─techniques based on promoting infections by this symbiont may be extended to the control of multiple diseases. On the other hand, in the main FD vector *Scaphoideus titanus* the most abundant symbiont capable of reproductive manipulation is *Cardinium* (Marzorati et al., 2006), which occurs only in the populations introduced into Europe. Therefore, the design of control strategies based on replacing *Cardinium* with *Wolbachia* may be proposed as a control tool against FD.
Taken together, the most recent research advances regarding microbial symbioses in the vectors of GY are certainly providing a framework for the implementation of new disease control strategies that should complement insecticide treatments, and possibly lead to the reduction of pesticide sprays. However, the potential that has been uncovered is far from being applied in the field, and much work is still required to exploit the promising opportunity represented by insect symbioses in the context of phytoplasma management.

ACKNOWLEDGMENTS

This research is partially based on study carried out within the Agritech National Research Center that received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

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Euscelis incisus in Bois noir-affected vineyards of Istria (Croatia): vector competence and syntopic occurrence with *E. lineolatus*

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INTRODUCTION

Bois noir (BN) disease of grapevine ('*Ca.* Phytoplasma solani', CaPsol,16S rRNA XII-A) was first molecularly confirmed to affect vineyards in Croatia more than two decades ago (Škorić et al., 1998). Presently, BN is widespread, occurring in most of the wine regions of the country (Plavec et al., 2018). The diversity of CaPsol genotypes in Croatian vineyards indicates complex epidemiology, which was recently confirmed in Istria, the westernmost grape-growing county of Croatia in the northeastern Adriatic Sea (Delač et al., 2023). *Euscelis incisus* (Kirschbaum, 1858) is a nitrophilous, polyphagous leafhopper, widely distributed throughout the Western Palaearctic region, inhabiting diverse habitats (Nickel, 2003). Due to its wide distribution and ability to inhabit a variety of ecologically distinct environments, this leafhopper can encounter diverse phytoplasmas that invade a wide range of plant species. It has been established as a vector of the 16Sr I-B/C, III-B, and XII-A phytoplasma subgroups and a carrier of the I-F/R, II-E, IX-C/E, and XI-G subgroups (Jakovljević et al., 2020). Its congener, *E. lineolatus* (Brullé, 1832) was found to harbor I-B/C and XII-A subgroups, while its ability to act as a phytoplasma vector has not been confirmed so far (Landi et al., 2013). The aim of this study was to investigate the epidemiological and vector roles of *E. incisus* and co-occurring *E. lineolatus* in transmitting CaPsol in BN-affected vineyards of Istria.

MATERIALS AND METHODS

Specimens of *E. incisus* (Ei) and *E. lineolatus* (Elin) were collected in two vineyards in the Vižinada grape-growing area (Istria, Croatia) in July and September of 2019 and 2020. Insects collected in the first experimental year were identified by morphological examination and DNA barcoding and screened for CaPsol presence. In the next year, to test their vectoring ability, specimens collected on the same locations were allowed to feed on experimental *Catharantus roseus* plants (Table 1). Total DNA was extracted from individual insects and experimental plants following a previously described protocol (Jakovljević et al., 2020). The CaPsol identification was performed by SYBR Green-based real-time PCR (qPCR) (Hren et al., 2007). Positive plants were characterized by MLST of *tuf*, *secY*, *vmp1* and *stamp* genes. To differentiate Ei and Elin, sequence comparison of the *mtCOI* barcode region was performed using between groups mean distance calculation in MEGA7.

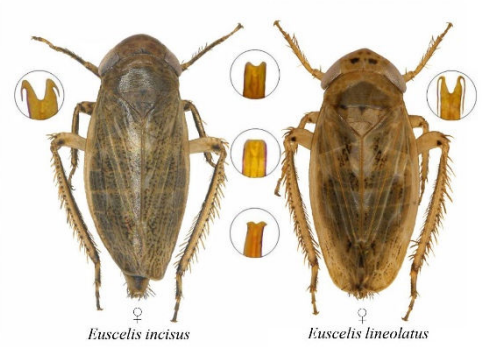
RESULTS AND DISCUSSION

A total of sixteen Ei/Elin specimens were gathered from vineyard 1 in the first year. Twenty-one specimens were collected in vineyard 1 during the second year, while fifteen Ei/Elin were taken in vineyard 2 (Table 1). In both vineyards, Ei and Elin specimens lived syntropically. The results of morphology and *mtCOI* barcode identification showed that there was no clear species separation based on outer morphology or copulatory structures of males (Figure 1), while average genetic divergence between haplotypes of the two species was 15%.

Table 1. Surveyed vineyards with *Ei* and *Elin* populations, with information of collected and infected specimens

Location	Taxon	Date of collection	No of analyzed specimens	
			Collected	CaPsol+
Vineyard 1	*E. incisus*	July-2019	5	1
		Sep-2019	4	-
		July-2020	13	7
	E. lineolatus	July-2019	4	1
		Sep-2019	3	-
		July-2020	8	1
Vineyard 2	*E. incisus*	July-2020	9	3
	E. lineolatus	July-2020	6	-

Figure 1. *Ei* and *Elin* specimens with aedeagus types



Among 16 Ei/Elin specimens collected in 2019, single Ei and Elin were positive for CaPsol presence (Cq 34). Three months after inoculation in 2020, both *C. roseus* plants were phytoplasma positive. Characterization revealed the presence of two different MLST genotypes: SB5g (tuf-a/S7/ST19/V3) and GGYg (tuf-b1/S4/ST4/V4). Among the analyzed specimens from the SB5g-positive plant, 7/13 Ei and 1/8 Elin tested positive, whereas 3/9 Ei from the GGYg plant were positive ($29 < Cq < 34$). The ability of Ei to transmit STOLg (tuf-b1/S1/ST13/V2-TA) has been experimentally confirmed (Jakovljević et al. 2020). However, current research suggests that this leafhopper may play a part in the epidemiological cycles of diverse CaPsol-induced diseases and their natural plant reservoirs, thus contributing to their natural spread across the ecosystem. Up to now, there is data about Elin's capability of carrying the XII-A phytoplasma subgroup (Landi et al. 2013), but those are the first findings about its potential to act as a vector. Given its syntopic presence with Ei, it is of epidemiological importance to evaluate its role as a phytoplasma vector, and to apply the molecular-genetic tools of separation of Ei and Elin in the Mediterranean basin.

ACKNOWLEDGEMENTS

We are grateful to X. Foissac for providing information on the SEE-ERA NET CaPsol classification. The research was supported by Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contract No. 451-03-66/2024-03/200010) and by bilateral project between Serbia and Croatia (No. 337-00-205/2019-09/38).

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High-throughput monitoring of Auchenorrhyncha as an approach for a simultaneous detection of vectors and quarantine pests in viticulture

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INTRODUCTION

Global trade of plant products, in addition to climate change, represents a major driving force for the spread of invasive pests in agricultural crops (Chapman et al., 2017; Schneider et al., 2022). Within the European territory, the bacterium *Xylella fastidiosa* (Xf) and the grapevine flavescence dorée phytoplasma (GFDp) are currently of great concern in viticulture. Classified as Union quarantine pests (QPs), they cause considerable economic damage whereas high levels of infestation result in a decline in viticulture and thus a negative impact on the traditional landscape. Apart from this, '*Candidatus* Phytoplasma solani', a Union regulated non-quarantine pest (RNQP) and causal agent of Bois noir (BN), impairs the phytosanitary quality of grapevine's planting material.
While in case of Xf, the vectors of the European outbreaks, the spittlebugs *Philaenus spumarius* and *Neophilaenus campestris*, are commonly distributed, and the introduction of the Xf is feared (Cornara et al., 2019), the vector of GFDp, the leafhopper *Scaphoideus titanus*, has not yet been confirmed in all areas (Chuche & Thiery, 2014). Therefore, it is crucial to detect not only infestations but also invasive vectors at an early stage of introduction in order to prevent further spread of the diseases. Thus, monitoring measures are essential, but rely currently on time-consuming visual inspections for disease symptoms and/or the monitoring of vectors. As Xf, GFDp and BN are transmitted by plant sap-feeding Auchenorrhyncha, the goal was to develop an efficient monitoring strategy using a high-throughput sequencing (HTS) methodology based on insect mass catches, which simultaneously detects vectors and associated QPs or RNQPs in an arthropod pool.

MATERIALS AND METHODS

Insect sampling and barcoding

An efficient active mass trapping technique should be identified for vineyards that i) reflects the highest possible spectrum of existing candidate and identified vectors of the QPs and RNQP and ii) can be used for molecular analyses without restrictions. Insect mass catches were generated between July and August 2021 in 12 vineyards by two common sampling methods, sweep netting and collection by leaf vacuum device. Samples were taken from the ground cover between the rows of vine as well as the vine canopy. As, compared to other arthropod groups, only few molecular reference sequences are available for the identification of Auchenorrhyncha, species in these mass catches were morphologically identified using the key of Biedermann & Niedringhaus (2004). Subsequently, DNA barcode sequences were elaborated for individual insects, while DNA was extracted with CTAB method (Maixner et al., 1995). The Folmer region of the COI was amplified by PCR using the primers LCO1490/HCO2198, adapted from EPPO (2021). PCR products were sequenced externally (MicroSynth Seqlab, Göttingen, Germany) while analyzed in CLC workbench.

High throughput-sequencing

To establish a reliable HTS methodology for the identification of Auchenorrhyncha species within an arthropod mass catch, identified Auchenorrhyncha were remixed with the remaining arthropods and used for COI amplicon sequencing. Mixed samples analyzed consisted of either a) specified Auchenorrhyncha, b) specified Auchenorrhyncha and small bycatch (arthropods < 1 cm) or c) specified Auchenorrhyncha and total bycatch. Illumina MiSeq PE250 reads were generated after amplification of a section of the COI gene (AllGenetics & Biology SL). Filtered and merged reads were used to generate ZOTUs (Zero-radius Operative Taxonomic Units), which were blasted against different databases, generated from published or own barcode sequences. The results were then compared to the species list from taxonomic analysis.

RESULTS AND DISCUSSION

For Auchenorrhyncha, sampling with the sweep net method proved to be less efficient than with the leaf vacuum device, as more species and individuals were recorded with the latter. Overall, 10,057 Auchenorryncha were collected in vineyards, while 53 species could be clearly identified by morphological analysis. These belonged to the Cicadellidae (subfamilies: Deltocephalinae, Cicadellinae, Agallinae and Megophtalminae), Aphrophoridae, Cercopidae, Dictyopharidae and Cixiidae families. Specimens of Typhlocybinae and Delphacidae were not analyzed, as representatives of these families have not yet been described as vectors of phytopathogens on grapevines. Of these species found, 5 are relevant for GFDp, 12 for BN and 5 for Xf. The respective DNA barcodes could be generated and implemented in the HTS workflow.

Preliminary results of the HTS analyses show that the “bycatch” plays a role as dilution factor in the arthropod mass catch, since the number of identified species in samples of category “b” and “c” are lower than in samples of category “a”. A high number of species was identified in category “a”, consistent with the list from taxonomic analysis. Therefore, the results are a promising approach for an early, efficient and labor saving detection of pests in the field and the maintenance of plant health.

ACKNOWLEDGEMENTS

This research was enabled by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support Program Number 2818711X19.

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A plea for using the correct taxon names of phytoplasma vectors: a case of *Reptalus artemisiae*, a vector of '*Candidatus* Phytoplasma solani'

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INTRODUCTION

The planthopper *Reptalus artemisiae* (Becker, 1865) (Hemiptera: Cixiidae) is an emerging vector of '*Ca*. Phytoplasma solani', and a well studied species for nearly two decades for its tentative, and later proven, vector role in phytoplasma transmission (Trivellone et al., 2005; Chuche et al., 2016). However, until recently, there was a misidentification of this taxon, leading to the incorrect nomenclature of *Reptalus quinquecostatus* (Dufour, 1833) (Webb et al., 2013), which is actually the name of another cixiidae species, previously known as *Reptalus melanochaetus* (Fieber, 1872) and now considered as its junior synonym. The case of misidentification was presented and documented in detail by Webb and coauthors (2013), while the question of naming *Reptalus quinquecostatus sensu* Fieber was resolved by Emeljanov (2020), who made the necessary nomenclatural changes. The changes were made following the International Code of Zoological Nomenclature, Article 70.3. We present here an overview of the (in)correct use of the taxon names for the two species in the last three years since the changes were made and make a plea for using the correct taxon names.

MATERIALS AND METHODS

To find how many times the taxon names *R. artemisiae* and *R. quinquecostatus* were used in scientific literature since the nomenclatorial changes were made (Emeljanov, 2020) we performed a Google Scholar search on 20 March 2024 for the terms which included species names with full and abbreviated genus. We imposed date limits on the search to include results from 2021 to 2024. Then we filtered the results, i.e., we checked each reference from the list if the use of the taxon names was done in correct manner or not, or if the use of name was a consequence of citation in the literature or phytoplasma strain designation. In the latter two cases, the use of the name was treated as correct.

Taxon		
Correct name	*Reptalus artemisiae* (Becker, 1865)	*Reptalus quinquecostatus* (Dufour, 1833)
	Flata artemisiae Becker, 1865	*Cixius quinquecostatus* Dufour, 1833
Synonyms	= *Reptalus quinquecostatus* (Fieber, 1872) = *Oliarus quinquecostatus* Fieber, 1872	= *Reptalus melanochaetus* (Fieber, 1872) = *Oliarus melanochaetus* Fieber, 1872
Non-synonym	*Reptalus quinquecostatus* (Dufour, 1833)	

RESULTS AND DISCUSSION

The search returned a total of five separate results for the use of the name '*Reptalus artemisiae*' and more than 70 for '*Reptalus quinquecostatus*', which, after manual filtration, resulted in 36 distinct results, i.e., publications. For the purpose of avoiding "negative citations", we are referring in the reference list only to publications that used the taxon names correctly. In total, correct use of the taxon name *R. artemisiae* was in all five publications (Quaglino et al., 2021; Pierro et al., 2022; 2024; Moussa et al., 2023; Jović & Toševski, 2023), while *R. quinquecostatus* as the correct taxon name was used in only two publications (Bucher et al., 2023; Jović & Toševski, 2023). In addition, 13 more publications were referring to the name *R. quinquecostatus* correctly, but this was not used as a taxon name but as a citation or phytoplasma strain designation. However, it is worrying that in only three years after the nomenclatorial changes in the genus *Reptalus* were made (Emeljanov, 2020), a total of 21 publications used the taxon name *R. quinquecostatus* incorrectly. The vast majority of these publications (70%) covered studies on the epidemiology of '*Ca.* Phytoplasma solani' or the vector role of the cixiid planthopper. This is the main reason why we make a plea to the scientific community of researchers working on phytoplasma epidemiology and on the diversity and vector role of cixiids to provide correct taxon names in their studies. The reason for the high number of cases of incorrect use of the taxon name *R. quinquecostatus* is because the taxon *R. artemisiae* is an emerging pest with an important role as a vector of '*Ca*. Phytoplasma solani', which makes the situation even more worrying. We hope that this plea, alongside the two publications explaining the misidentification of the two taxa in question (Webb et al., 2013; Emeljanov, 2020), will raise awareness of the importance of providing correct taxon names to avoid any future confusion, which could have negative implications, especially in the case of economically important species.

ACKNOWLEDGEMENTS

The research was supported by Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contract No. 451-03-66/2024-03/200010).

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EMERGREEN: Greenhouses Platform for Studying Emerging Plant Diseases

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PRESENTATION

The EMERGREEN platform (EMERging plants pathogens in GREENhouses and labs) was established in 2023 from the desire of INRAE's scientific departments to create a high-tech tool dedicated to regulated phytopathogenic agents and their vectors. This platform is located on the INRAE Nouvelle-Aquitaine Bordeaux campus in Villenave d'Ornon, France.
The experiments conducted at EMERGREEN aim to:

- Anticipate the emergence of diseases caused by quarantine phytopathogens:
  - o Exploration of the biology of QOs (Quarantine Organism): life cycle, pathogenicity, life history traits, vectors, and hosts
  - o Study of QOs' tolerance to environmental conditions such as temperature, humidity, and ultraviolet radiation
  - o Analysis of QOs' adaptive potential, implementation of experimental evolution approaches
- Improve detection and surveillance methods:
  - o Study of QOs' genetic diversity
  - o Development of early detection methods for emerging disease outbreaks
- Propose actionable levers:
  - o Development of risk management strategies considering various environmental, biological, and socio-economic factors
  - o Study of host resistance to QOs
  - o Study of the effectiveness of antagonists and potential auxiliaries for biological regulation of QOs and control the spread of diseases

PLATFORM OFFER

EMERGREEN is dedicated to studies on emerging plant diseases characterized by complex interactions among a host plant, a quarantine or regulated pathogen, its potential vectors, and the environment and meets the needs of the national and international academic scientific community and industrial stakeholders. Manipulating these phytopathogenic agents (listed on the QO list) requires authorization from government services, and EMERGREEN's experience in applying for accreditation and monitoring compliance with the handling of quarantine organisms (record keeping, audits, etc.) enables us to meet the needs of the scientific community for quarantine organism manipulation and preparation of accreditation dossiers. EMERGREEN also offers support services for project management, provision of scientific equipment in confined facilities, and support for experimental activities such as phenotyping of pathogen-plant host-vector interactions, contributing to ongoing research projects.

CONFINED FACILITIES

The EMERGREEN platform comprises three biosafety level 2 and 3 modules, all organized to meet biosafety requirements. Currently, EMERGREEN hosts:

1) a 180 m² biosafety level 2 "Vection" module, including a leafhopper (*Euscelidius variegatus*) rearing unit, a hatching unit for the natural vector of grapevine flavescence dorée phytoplasma (*Scaphoideus titanus*), and a phytoplasma collection maintained on periwinkle (*Catharanthus roseus*) for acquisition/transmission studies by insect vectors.

2) a 265 m² "High Containment" module, including 148 m² of biosafety level 3 greenhouse, with activities such as a virus collection on *Prunus*, the study of plant virus etiology, and the transmission/acquisition of grapevine yellows phytoplasma with the natural vector.

3) a 420 m² "Insect-Free" module of biosafety level 2, including laboratories, technical rooms and 132 m² of greenhouse, for the study of pine diseases (agreement pending).

Funding from the Nouvelle Aquitaine Region and INRAE has been obtained for the construction of biosafety level 2 and 3 greenhouses and laboratories (each approximately 400m²) to support EMERGREEN's future ambitions. This project aims to increase experimentation capacity, offer operational flexibility, and achieve high energy performance.

EMERGREEN, through its agents' involvement, is represented in numerous scientific and technological networks, which serve as strong means of access to technological innovations within the EMERGREEN platform's scope of expertise. The skills and know-how of the platform's agents, as well as the privileged link with users, are sources of continuous improvement of research equipment. Additionally, the platform is positioned within strategic frameworks such as INRAE 2030, contributing to scientific advancements and addressing key challenges in the field.

At the academic level, the EMERGREEN platform benefits from the scientific environment of INRAE, Bordeaux University, Bordeaux Sciences Agro, and the CNRS.

Thus, meeting the needs of the scientific community to progress in the identification and characterization of quarantine organisms (prioritized or non-prioritized), to elucidate their life cycle, and to find management strategies in changing agricultural and forest landscapes, the platform is involved in 14 regional, national, and international projects in these first two years, where its expertise is recognized and valued.

Interaction of grapevine phytoplasmas with insects and plants

Chairs: Martina Seruga-Music and Jordi Sabate

Multi-level exploration of interactions between '*Ca.* P. solani' and grapevine

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INTRODUCTION

To this day, cultivation of phytoplasma in a pure axenic cultures reliably remains an elusive task. Therefore, studying these organisms within hosts while considering host-pathogen interactions remains the most viable approach. In order to survive and to promote infection, phytoplasmas must be capable of modifying host defense responses and metabolism. This can be achieved with the use of effector proteins. Among them, few are thoroughly researched and have been investigated in multiple disparate phytoplasma. However, many of the predicted effector proteins remain unexplored. In our research, we have focused on the interaction between the phytoplasma '*Ca*. P. solani' and grapevine cv. Zweigelt, which is considered a susceptible genotype.

MATERIALS AND METHODS

Grapevine leaf vein material from infected and healthy plants was collected from a commercial vineyard in Vienna on two separate years, in July and September. RNA was extracted, and mRNA-Seq and sRNA-Seq analyses were performed. In addition to the standard differential expression and target prediction analysis, analysis at the level of communities of genes and mRNA-microRNA interaction networks was performed to detect communities that formed and disintegrated with respect to time and sanitary status (Škrlj et al., 2021). This was complemented with enzyme activity assays. By analyzing the '*Ca*. P. solani' genome, six potential effector proteins were selected for further investigation. They were tagged with YFP and transiently expressed in *Nicotiana benthamiana* by *Agrobacterium*-mediated transformation. Sub-cellular localization analysis was performed via confocal microscopy. The same expression system was also used to obtain *N. benthamiana* leaves expressing the potential effector proteins for enzyme activity assay. The material was collected 3- and 14-days post-inoculation. Protein pulldown was performed on the transiently transformed *N. benthamiana* material to find potential interactors of the PoStoSP28 putative effector protein.

RESULTS AND DISCUSSION

Extensive changes in the transcriptome of infected grapevines were observed in the early growing season, even before the emergence of any symptoms. They persisted at a similar level until the late growing season, when the symptoms become apparent. Major changes in genes related to jasmonic and salicylic acid synthesis and signaling appeared already in the early growing season and endured throughout the growing year. In the early growing season, gene expression was characterized by a

pattern of general upregulation, particularly in biotic stress response and signaling, amino acid production, secondary metabolism, and DNA and protein synthesis. By the late growing season, the pattern has reversed considerably, with a profile characterized mostly by downregulation, particularly of various transporters, transcription factors, and protein synthesis. sRNA analysis indicated that most of the differentially expressed miRNAs and phasiRNAs belonged to groups associated with the regulation of genes involved in proteolysis, LRR receptors and cellular signaling, with most being predominantly downregulated in both growing seasons.

To investigate the phytoplasma side of the interaction, we identified six potential effector proteins (PoStoSP04, PoStoSP06, PoStoSP13, PoStoSP14, PoStoSP18, and PoStoSP28) that were present in the strain SA-1. Upon functional domain analysis, two have been found to have known functions. Namely, PoStoSP18, a maltose binding protein within ant ABC-type sugar transporter, and PoStoSP28, an antigenic membrane protein StAMP. *N. benthamiana* leaves transiently transformed with the putative effectors were collected for enzyme activity assays. Since the disruption of sugar metabolism is a common symptom of phytoplasma infection, 12 key enzymes of carbohydrate metabolism were analyzed. The activity of seven enzymes was modulated by the presence of various potential effector proteins. Among them, five exhibited similar changes in '*Ca*. P. solani' infected grapevine cv. Zweigelt, both on the level of transcripts, as well as enzyme activity analysis. Furthermore, a pulldown assay revealed that the PoStoSP28 interacts directly with phosphoglucomutase in *N. benthamiana.* In addition to carbohydrate metabolism, enzymes involved with oxidative stress were also investigated. Similarly to the carbohydrate analysis, all putative effector proteins seemed to affect at least one of the investigated enzymes in *N. benthamiana*, and the overall results were like ones obtained in enzyme activity assays in '*Ca*. P. solani' infected grapevine, with a major effect on the ascorbate-glutathione cycle. Subcellular localization of the YFP-tagged putative effector proteins showed that they were mostly diffused throughout the nucleus and cytoplasm. However, the presence of PoStoSP28 appeared to induce a greater number of autophagosomes, and moreover, colocalized with them.

The combined results from the investigations of infected grapevine and putative effector expressing *N. benthamiana* indicate that the putative effectors play a role in the carbohydrate metabolism and reactive oxygen species response during phytoplasma infection, with possibly redundant functions (Dermastia et al., 2021, 2023). This is consistent with the emerging evidence that effector proteins target multiple processes in the host plants as well as converging to similar targets.

ACKNOWLEDGEMENTS

This work was supported by the Slovenian Research Agency (ARRS) grants J4-2544 and J1-7151, young researcher fellowship to Špela Tomaž, fellowship of The Jožef Stefan International Postgraduate School to RS, Austrian Science Fund (FWF) grants I-2763- B29and I-5042B, and the National Sustainability Programme I (NPU I) of the Ministry of Education, Youth and Sports of Czech Republic, grant LO1415.

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Infection with Flavescence dorée phytoplasma results in a general repression of the lignin synthesis pathway in *Vitis vinifera* cv. Pinot noir

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INTRODUCTION

In grapevine (*Vitis* sp.), various phytoplasmas are associated with Grapevine Yellows (GY). These diseases are present in all wine-growing areas, inducing significant economic losses. Flavescence dorée (FD) is one of the most harmful and damaging ones (Tramontini et al., 2020) and is classified as a quarantine disease in the EU and Switzerland. It is associated with the Flavescence dorée phytoplasma (FDp) and an epidemic propagation occurs only in the presence of the Nearctic leafhopper *Scaphoideus titanus* Ball (Chuche et Thiéry, 2014). Typical symptoms of the disease are normally observed in late summer and consist of (i) downward rolling of the leaves with premature color alterations (red / yellow for the red / white cultivars, respectively); (ii) inflorescence abortion or berry withering, and (iii) lack of lignification in new shoots. The lignification defect is characterized by shoots remaining green at the end of the season, which fail to rigidify, are flexible, and unable to withstand winter frost. The first two symptoms described above are often associated with other plant pathogens, while developmental defects such as lack of lignification are characteristic of phytoplasmas. Nevertheless, the mechanisms by which they cause these symptoms are not well known. Lignin is one of the most abundant polymers found in vascular plants. Its deposition serves crucial physiological and developmental functions including responses against biotic and abiotic stresses (Lourenço et al., 2016). The regulation of lignin biosynthesis, especially in grapevine, is not yet fully understood. In this study, a combined approach using metabolomics, untargeted transcriptomics, and gene expression analysis was used to better understand how monolignol biosynthesis is affected in FDp-infected shoots.

MATERIALS AND METHODS

Three replicates of healthy and FDp-infected shoots from cv. Pinot noir were collected in Roche, Switzerland. Healthy vines in close proximity to infected ones were selected. Samples were collected at three different time points in 2022: on June 23rd (when the first FD symptoms on leaves were barely visible), on September 1st, and on October 19th. Sections of about 20 cm of shoots were cut, immediately placed in liquid nitrogen and transported to the laboratory. The procedure developed by Jaini et al. (2017) to quantify phenylpropanoid pathway intermediates in *A. thaliana* served as a basis to develop the analytical method used in this work. Each sample was extracted three times to obtain technical replicates. Fifty mg of dried weight (DW) were used for the analysis. For RNA extraction, 1-2 g of plant material was used. To perform RT-qPCR analysis, specific primer pairs and TaqMan probes were designed for each selected enzyme from the lignin biosynthesis pathway. Additionally, RNAs from samples collected in September 2022 were sent to an external platform for library preparation (TruSeq stranded mRNA kit) and sequencing (Novaseq 6000 platform; 150 bp paired-end reads). Differential gene expression analysis was performed with DESeq2.

RESULTS AND DISCUSSION

Twelve intermediates of the phenylpropanoid/monolignol pathway were selected for targeted investigations by quantitative UHPLC-MS analysis. These analyses showed that infection by Flavescence dorée phytoplasma affects downstream metabolites such as ferulic acid, sinapic acid,

coniferaldehyde, sinapaldehyde, p-coumaroyl alcohol, and sinapyl alcohol very early in the season, while upstream metabolites such as shikimate and phenylalanine accumulate later in the season. Expression analysis of enzymes from the monolignol pathway by RT-qPCR confirmed the early downregulation of certain enzymes involved in lignin biosynthesis. The expression of *Vitis vinifera* C4H-1, C4H-2 / C4H-3 (cinnamate 4-hydroxylase), C3H (p-coumarate 3-hydroxylase), and F5H-3 (ferulic acid 5-hydroxylase) was significantly reduced in FD-positive shoots compared to healthy ones. A closer examination of the pathway within the RNAseq data also corroborated its early overall downregulation.

Previous studies revealed that genes involved in phenylpropanoid biosynthesis are also affected in Bois noir phytoplasma-infected *Vitis* sp leaves or canes (Hren et al, 2009; Albertazzi et al, 2009; Negro et al, 2020) favouring the synthesis of some flavonols, stilbenoids, and phenolic compounds (Rusjan et al, 2014). In these works, several transcription factors involved in lignin biosynthesis were repressed in infected plants. Transcription factors are known to be common targets for phytoplasma effector proteins (Oshima et al, 2023). Indeed, numerous pathogens including fungi and bacteria produce effectors influencing the expression of genes involved in the lignification process (Bauters et al, 2021). Together with our results, these data suggest that phytoplasmas may directly target lignin synthesis. Given that effectors are the tools used by phytoplasmas to manipulate the host plant, it makes them priority candidates for further investigation of the lignification disorder associated with grapevine yellows.

ACKNOWLEDGEMENTS

We thank Michel Jeanrenaud, Olivier Viret, and Canton of Vaud for giving us access to field material and partial funding of the research.

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New insights on the '*Ca.* Phytoplasma solani' transmission by *Neoaliturus fenestratus* (Cicadellidae: Deltocephalinae)

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INTRODUCTION

Neoaliturus fenestratus (Herrich-Schäffer 1834) is a polyphagous leafhopper widespread throughout Europe, particularly in ruderal habitats, i.e., abandoned fields, vineyards, and sparsely vegetated meadows, commonly associated with different plants of the family Asteraceae (Nickel 2003). *Neoaliturus fenestratus* is confirmed to transmit the safflower phyllody associated with phytoplasmas from the 16SrI-B subgroup (Raccah & Klein 1982), *Picris hieracioides* bushy stunt caused by phytoplasma from the 16SrII-E subgroup (Mitrović et al. 2012), lettuce phyllody and wild lettuce phyllody associated with phytoplasmas belonging to the 16SrIX group (Salehi et al. 2007), and chicory phyllody phytoplasma of the 16SrIX-C subgroup (Ermacora et al. 2013). Furthermore, Mitrović et al. (2019) experimentally confirmed its vector role in transmitting '*Ca.* P. solani' of the 16SrXII-A subgroup to lettuce and carrot. In this study, *N. fenestratus* was investigated for its capacity to transmit the '*Ca*. P. solani' to grapevine (*Vitis vinifera*) and native plants commonly found in and/or around vineyards in Serbia (*Convolvulus arvensis*, *Calendula officinalis*, *Chenopodium album*, and *P. hieracioides*).

MATERIALS AND METHODS

Transmission trials were conducted with *N. fenestratus* specimens collected from the abandoned vineyard in the locality Jasenovik, South Serbia with previously confirmed high incidence of '*Ca.* P. solani' in the plants of wild compartment. Separate trials were set up with seedlings of *V. vinifera, C. arvensis, C. officinalis, C. album* and *P. hieracioides.* All tested plants were grown from seeds in controlled conditions. A total of 70 *N. fenestratus* were separated into groups of 10 to 20 specimens and offered with seedlings of the test plants. Fresh plants were changed after 24h in each replication until there were no surviving adults. Plants were monitored for symptoms development for the following three months prior to molecular analysis. Molecular identification was based on a nested PCR analysis with '*Ca*. P. solani'- specific stol11F2/R1 and F3/R2 primers and then characterized by amplifying and sequencing the *stamp* gene encoding the antigenic membrane protein by nested PCR with the primers pair stampF/R0 followed by stampF1/R1 (Fabre et al. 2011).

RESULTS AND DISCUSSION

In the transmission trial with *P. hieracioides,* eight plants were exposed to ten field collected *N. fenestratus*. Four plants tested '*Ca.* P. solani' positive (50%), expressing the symptoms of leaf yellowing. All *N. fenestratus* specimens were confirmed to carry the '*Ca.* P. solani'. The STOL genotype of the *stamp* gene was amplified from both, *P. hieracioides* and leafhopper specimens. In total five fresh seedlings of *C. arvensis* were offered to ten *N. fenestratus* specimens. Analysis showed that three bindweed plants (60%) and six exposed leafhopper specimens were tested positive for the '*Ca*. P. solani' presence. Infected *C. arvensis* plants expressed no symptoms. Characterization of the *stamp* gene showed the presence of the M5 genotype in the test plants and leafhopper specimens. Four *C. album* seedlings were exposed to ten *N. fenestratus* with two plants (50%) tested positive for

phytoplasma, both with symptoms of discoloration and desiccation. Six out of ten leafhopper specimens (60%) were tested positive. The same *stamp* genotype Rqg50 was detected in insects and exposed *C. album* plants. In the test trial with *V. vinifera,* three seedlings were exposed to 20 specimens of *N. fenestratus*. One asymptomatic grapevine (30%) and 15 leafhopper specimens were tested positive; however, the *stamp* gene failed to be sequenced successfully. In total, ten leafhoppers were offered with three seedlings of *C. officinalis.* Eight *N. fenestratus* (80%) were tested positive and determined to carry Rpm35 and STOL genotypes. Two *C. officinalis* plants developed symptoms of yellowing, carrying the same *stamp* genotypes as the leafhopper specimens. *Neoaliturus fenestratus* has a wide distribution in Serbia in abandoned vineyards and grasslands, associated with many native plants known as hosts of '*Ca.* P. solani'. This is in coherence with significant rate of infected *N. fenestratus* specimens (64.3%) and diversity of the *stamp* genotypes detected within (STOL, Rqg50, M5, Rpm35). These results are complementary to the previous studies conducted by Mitrović et al. (2019), in which *N. fenestratus* specimens collected from the same vineyard in Jasenovik successfully transferred the genotypes Rqg31, STOL and BG4560 to carrots and lettuces. Diversity of the *stamp* genotypes detected in the field sampled leafhoppers match the isolates previously reported from the grapevine, potato, maize and other crops in Serbia, including common weeds and several other polyphagous vector species. This indicates a wide range of possible scenarios how *N. fenestratus* could have acquired the '*Ca.* P. solani' phytoplasma in the field. The association with common weeds known to host the phytoplasma, the wide distribution of leafhopper populations across Serbia, the diversity of harbored '*Ca.* P. solani' phytoplasma genotypes, and the experimentally confirmed vector status qualify *N. fenestratus* as an important link in the chain of events that comprise the complex epidemiology of '*Ca*. P. solani' in Serbian vineyards. This could also be the case in other regions, given that *N. fenestratus* has previously been reported to harbor the '*Ca*. P. solani' in vineyards across Europe, and recent data in Italy indicate its importance as a phytoplasma carrier and potential epidemiological constituent of "bois noir" (Pierro et al. 2022).

ACKNOWLEDGEMENTS

The research was supported by Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contract No. 451-03-66/2024-03/200010).

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Visualization of '*Candidatus* Phytoplasma solani' SAP11-like and SAP54-like effectors interactions with plant transcription factors by using BIFC assay

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INTRODUCTION

Phytoplasmas are obligate intracellular pathogenic bacteria whose mechanism of virulence and pathogenicity have not been entirely unfolded, yet. Still, characteristic symptoms of disease, such as yellowing, witches' broom, phyllody or changes in leaf morphology might indicate the presence of phytoplasma infection in plants and effector proteins' activity (Pecher et al., 2019). By secreting effector proteins, phytoplasmas strongly impact the developmental and behavioural setting of their hosts (Sugio et al., 2011). Research on effectors and their host-cell targets molecular mechanisms has been conducted for only a few '*Candidatus* Phytoplasma' species thus far. Most studies concern Aster Yellows phytoplasma strain Witches' Broom (AY-WB) secreted proteins (SAPs) (MacLean et al., 2011). As already shown previously, SAP11 mainly targets the plant nucleus and alters host immune response by destabilizing TCP transcription factors (TFs), inducing stem proliferation. On the other hand, SAP54 affects cellular processes crucial for plant development by degrading the Type II MADS-domain transcription factors (MTFs) resulting in formation of flowers into leaf-like structures (Bai et al., 2023; MacLean et al., 2011, 2014). Presumably, wider range of phytoplasma hosts, would be associated with more complex effector protein properties and interactions.
One of such phytoplasmas with the wide host range is '*Ca.* P. solani' – the causal agent of 'bois noir' of grapevine and stolbur disease in wild and cultivated herbaceous and woody plants, and several other diseases of, for example, maize, tomato, pepper and strawberry in Europe and Mediterranean basin.
Our recent sequencing and comparative genome analyses of SA-1 '*Ca.* P. solani' (Acc. No. MPBG01000000) strain and 2 additional strains revealed the presence of SAP11-like and SAP54-like effectors and redirected the research focus to their potential interaction features (Seruga Music et al., 2019; unpublished).

MATERIALS AND METHODS

Genomic DNA of '*Ca.* P. solani' has been isolated from *Catharanthus roseus* L. G. Don plants infected with two different strains of '*Ca.* P. solani' as well as from healthy *Arabidopsis thaliana* plants. Specific primers were designed on the basis of previously published SAP11-like_SA-1_PSSA1_v1c1150 (Acc. no. MPBG01000000) and unpublished SAP54-like_STOL_ph74_0110 sequence for PCR amplification of effector genes of interest. Also, TFs and MTFs as potential interactor genes from *A. thaliana* were amplified by PCR from previously designed plasmid contructs. Appropriate plasmid constructs were prepared by using InFusion cloning technology (Clontech, Takara). For the bimolecular fluorescence complementation (BIFC) assay, genes were cloned into BamHI site of plasmid vectors pSPYNE and pSPYCE in order to investigate potential interactions of '*Ca.* P. solani' SAP11-like effector with TFs TCP2 and TCP4 and SAP54-like with MTFs AP1 and SEP3. *In planta* BIFC assay was conducted in agroinfiltrated *Nicotiana benthamiana* leaves. Approximately 60 hours after agroinfiltration of *N. benthamiana*, protein interactions were detected by confocal microscopy of lower leaf epidermis samples, using Leica TCS SP8 X laser scanning confocal microscope/HC PL APO CS2 40x/OIL objective.

RESULTS AND DISCUSSION

BIFC assay is widely used to detect interactions in subcellular compartment in any aerobically-growing organism or cell that can be genetically modified to express the fusion proteins (Kerppola T., 2019). Our results have shown that fluorescence signal of SAP11-like effector and TCP4 interaction was detected in cell cytoplasm, while the signal of SAP11-like and TCP2 interaction was localized both in nuclei and cytoplasm of agroinfiltrated leaf epidermal cells. SAP54-like effector interacted with AP1 and SEP3 fluorescing predominantly in nuclei of epidermal cells.
To the best of our knowledge, this is the first research on any '*Ca.* P. solani' protein effector potential interactions. In order to corroborate these results, further methods such as yeast-2-hybrid (Y2H) assay and MS analyses will be used.

ACKNOWLEDGEMENTS

This work was supported by Croatian Science Foundation grant. IP-2019-04-2469.

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Preliminary results of BIFC assay: '*Candidatus* Phytoplasma solani' protein Stamp interacts with insect-vector actin

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INTRODUCTION

Phytoplasmas, (*'Candidatus* Phytoplasma) are uncultivable plant pathogenic bacteria. Regarding the absence of cell wall, phytoplasmas are classified as members of class Mollicutes and mandatory inhabitant of the phloem sieve cells of plants and insect gastrointestinal system. They are associated with numerous plant diseases causing devastating yield losses in diverse crops worldwide (Hogenhout & Loria, 2008; Namba, 2019). The spread of phytoplasmas by insect-vectors mostly rely on their specific surface proteins. For instance, flavescence dorée (FD) phytoplasma has a range of variable membrane proteins (Vmps) present on its surface and detected in the midgut and salivary glands of *Euscelidius variegatus* infected with FD phytoplasma (Arricau-Bouvery et al., 2018). Antigenic membrane protein (AMP) of '*Ca.* Phytoplasma asteris' has been observed to interact with the insect microfilament protein – actin (Oshima et al., 2004; Suzuki et al., 2006). Another transmembrane protein, known as Stamp, is 16 kDa antigen membrane protein found only in '*Ca.* P. solani'. It shares 26–40% sequence ID with protein AMP of '*Ca.* P. asteris' strains and 40% ID with AMP of '*Ca.* P. japonicum'. Based on this resemblance, the aim of this study was to investigate the potential novel protein-protein interaction of phytoplasma protein Stamp and insect-vector actin (Fabre et al., 2011).

MATERIALS AND METHODS

'*Ca.* P. solani' genomic DNA has been isolated from *Vitis vinifera* L. sample, while the actin sequence was obtained after RNA isolation from insect vector *Hyalesthes obsoletes* Sign followed by cDNA synthesis, TA-cloning and Sanger sequencing. Our approach involved use of *bimolecular fluorescence complementation* (BIFC) and *yeast – 2 – hybrid* (Y2H) assays as *in-vivo* methods. Appropriate plasmid constructs for both BIFC and Y2H were prepared by using In-Fusion cloning technology (Clontech, Takara). For BIFC assay, two genotypes of *stamp* – ST6 and ST9 were used to design pSPYNE plasmid constructs in order to obtain the expression of both genotypes with the intention of probing the interaction with an expressed actin by *E. coli* (HST08 strain) transformed with pSPYCE plasmid construct. BIFC assay was conducted in agroinfiltrated *Nicotiana benthamiana* leaves. Around 40 hours following the agroinfiltration process in *N. benthamiana*, protein interactions were observed by confocal microscopy of samples taken from the lower leaf epidermis. This was carried out using a Leica TCS SP8 X laser scanning confocal microscope with a HC PL APO CS2 40x/OIL objective.
For Y2H assay, we cloned ST6 and ST9 genes into EcoRI and SalI sites of pGBT9 plasmids, to construct a bait vector (BD). To construct a prey vector (AD), actin gene was cloned into the same restriction sites of pGAD424 plasmid. Cloning was followed by *Saccharomyces cerevisiae* co-transformations and yeast growth on LB -Leu-Trp, SD-Leu-Trp-His, SD-Leu-Trp-His-Ade medium with and without 20 mM 3AT added.

RESULTS AND DISCUSSION

The results of the first attempt of BIFC assay suggested the presence of the presumed interaction of Stamp and actin as a weak fluorescence signal in cell cytoplasm of agroinfiltrated *N. bentahamiana* leaves was observed. For the Y2H assay, in our preliminary experiments no interactions of Stamp6 or Stamp9 with actin were detected. In our future experiments we will aim to further optimize Y2H assay for detection of an interaction of '*Ca.* P. solani' highly variable Stamp and highly conserved insect-vector's actin or consider other methods for protein-protein interaction detection.
To our current understanding, these preliminary results of BIFC assay are the first report of Stamp interaction with insect-vector actin, which opens a new insight into '*Ca.* P. solani' epidemiology. Studies upon these protein – protein interaction are still ongoing.

ACKNOWLEDGEMENTS

This work was supported by Croatian Science Foundation grant. IP-2019-04-2469.

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Other plant diseases associated to '*Candidatus* Phytoplasma solani'

Chairs: Jelena Jović and Xavier Foissac

Vectors, hosts and isolates of '*Candidatus* Phytoplasma solani' in Spain

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INTRODUCTION

'Candidatus Phytoplasma solani' has been identified in a wide range of wild and cultivated plants in the Iberian Peninsula in the last decades, causing severe and mild symptoms. Between the plants affected were vine, strawberry, potato, nettle and bindweed. BN is present in the main wine production areas of northern Spain with low incidences in general and reemerging focus in Aragon and La Rioja regions. In contrast in the last years important stolbur outbreaks have been reported on strawberry nurseries, where a holistic phytoplasma control strategy has been implemented. The main pathosystem for stolbur on Iberian Peninsula is *Convolvulus arvensis/Hyalesthes obsoletus/ tuf-b*/crop, except in La Rioja and Navarre where *Tuf-a/Urtica dioica* is present in plots near the rivers.

MATERIALS AND METHODS

Between 2014 and 2023 incidence of *'Ca. P.* solani' and *H. obsoletus* population have been followed on strawberry nurseries and its edges, with the objective to know the epidemiological cycle of the phytoplasma and propose control strategies for the vector and the disease. Strawberry and other wild and cultivated plants were analyzed for stolbur and other phytoplasmas. The insects were captured weekly from June to September with a D-vac aspirator, over strawberry and weeds within and on the edges of the plots. The detection of the phytoplasma in plants and insects was realized by means of qPCR with universal and specific primers and probes for '*Ca.* P. asteris' and '*Ca.* P. solani' (Angelini et al., 2007). The characterization of stolbur-associated phytoplasma isolates in these nurseries, vines and weeds of other parts of Spain was realized with the primers *tuf* and *vmp*1 genes (Maixner & Langer, 2004; Cimerman et al., 2009; Fialovà et al., 2009.). The amplification products were sequenced and compared with the sequences in the Genbank.

RESULTS AND DISCUSSION

The prevalent isolate on plants and insects was tuf-b/V1, but for nettle and *the H. obsoletus* captured on it, the main isolate was tuf-a/V3. Infected nettles are present in a restricted area in the regions of Navarre and La Rioja, matching all with tufa strain.
The main host for *H. obsoletus* is the bindweed, and secondarily nettle in Navarre and La Rioja, but captures on other plants are common. The *H. obsoletus* adults flight was one month later on nettle than bindweed all the years of the study. This difference was constant even with the great fluctuations between the years by temperatures.
With the objective to reduce the incidence in strawberry nurseries, strategies of control focused on the vector have been implemented, controlling the population eliminating the wild plant hosts and with insecticide treatments against nymphs and adults. The results showed a high incidence of stolbur in strawberry in 2014 that was gradually reduced till 2018 and static till 2021 when a little reemergence was observed. '*Ca*. P. solani' was also detected in *C. arvensis, U. dioica, Lycopus europaeus, Asparagus* sp., *Calystegia sepium* and cultivated *Chicorium intybus*. *H. obsoletus* adults were captured on strawberry, *C. arvensis* and cultivated *C. intybus*, with high populations the first years. There were no captures of *H. obsoletus* on *U. dioica, C. sepium, L. europaeus*, and wild *Cichorium intybus*. Nymphs of *H. obsoletus* were observed in May in the roots of *C. arvensis* and *C. intybus*. Cicadellidae

as *Anaracetagallia laevis, Euscellidius variegatus, Austroagallia sinuata, Hardya tenuis, Psammotettix* spp.*, Empoasca* spp. and *Zyginidia scutellaris* were captured in the plots with a very low percentage of positives for '*Ca.* P. solani'. '*Ca.* P. asteris' and '*Ca.* P. solani' were detected in higher percentages on *Neoaliturus fenestratus* and *Macrosteles* sp. The dates of first *H. obsoletus* adults emergence ranged from 5th of June (2015) to the 4th of July (2018), The *H. obsoletus* flight lasts approximately 45 days, the peak being two weeks after the first adults appearance. The first symptoms on strawberry and sentinel *Catharanthus roseus* periwinkle appeared one month after the flight peak. The amplified products of *tuf* and *vmp*-1 genes in the nurseries showed Tuf-b profile in all the samples from strawberry, endive, *C. arvensis* and *H. obsoletus*, and for the *vmp*1 the V1 profile, the main isolate in Spain in vineyards in Catalonia, Aragon and Basque Country where V4 is also present. In Navarre and La Rioja regions, *C. arvensis* hosts tuf-b/V1 strains, and tuf-a/V3 is the main strain in vineyards near creeks where *U. dioica* is present with *H. obsoletus* populations.

The incidence of Bois Noir in vineyards of Spain is low, but there is great dispersion of '*Ca.* P. solani' in a wide range of plants, including crops, in the north of the Iberian Peninsula. The main epidemiological cycle is characterized by *C. arvensis* hosting tuf-b/V1 strain. The tuf-a/V3 strain continues to be restricted to the high Ebro valley, where *U. dioica* hosts *H. obsoletus*. In other regions as Catalonia or Castille, *H. obsoletus* has not been captured on *U. dioica*, and the few positive plants host tuf-b/V1 strain.

The implemented measures in the strawberry nurseries allowed a great reduction on the populations of *H. obsoletus* and consequently on the incidence of '*Ca.* P. solani' on strawberry. *H. obsoletus* populations were controlled first of all by eliminating the plant hosts (*C. arvensis and C. intybus*) as much as possible, tilling and removing the roots in the coldest days in winter and retiring the surviving plants in spring. In the edges, the carving in winter was complemented with the cutting of the weeds to favor the establishment of non-host plant communities such as grasses or alfalfa. Where endives endured mechanical measures, an application of systemic insecticide was done in May to control the nymphs before the beginning of the flight. Taking the advantage of the insecticide treatments for other insect pests, during the adult flight mainly in June, strawberry plants were treated weekly with a combination of contact and systemic insecticides to control the *H. obsoletus* individuals that could arrive to the crop.

ACKNOWLEDGEMENTS

This work was financed by grant RTA05-156 of the programa sectorial de I+D, M.A.P.A., España. We would like to thank Dr Maixner for sharing the predictive model for *H. obsoletus* adult flight.

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Diversity of '*Candidatus* Phytoplasma solani' in potato and grapevine in Germany

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INTRODUCTION

'*Candidatus* Phytoplasma solani' (CaPsol) has long been known as a causal agent of Bois Noir in grapevine associated with *Hyalesthes obsoletus* as the principal vector. Since 2022 Stolbur-like symptoms were observed in potato fields associated with *Pentastiridius leporinus*, which had previously been reported in sugar beet (*Beta vulgaris* ssp. *vulgaris*) in Germany transmitting '*Ca*. Arsenophonus phytopathogenicus' (CaAp) (Behrmann et al., 2023). Both Cixiids were identified as CaPsol-vectors (Gatineau et al., 2001). The rapid spread and adaption of *P. leporinus* to these economically important crops poses problems for agriculture and food production. Initially, CaPsol-strains were attributed to the 16 SrXII-A subgroup, but more subgroups have been identified since (Duduk et al., 2023; Quaglino et al., 2017) that affect various wild plants and agricultural crops such as potato (*Solanum tuberosum* ssp. *tuberosum*) and grapevine (*Vitis vinifera*) in Europe and the Mediterranean area. In order to understand the means of spread and to assess potential risks for crop production, CaPsol-strains from potato, grapevine and associated wild plant hosts as well as leaf- and planthoppers as potential vectors, were genetically characterized by multilocus sequence typing (MLST) and phytoplasma populations compared across various locations within Rhineland-Palatine.

MATERIALS AND METHODS

Insect and plant sampling

Adult planthoppers were collected from 15 potato fields and 25 vineyards with sweeping nets and mouth aspirators at different time points. Insects from potato fields, unmanaged habitats and adjacent fields were used to archive isolates of CaPsol and CaAp from potato in periwinkle (*Catharanthus roseus*). Dead insects were removed and identified morphologically according to the key of Biedermann & Niedringhaus (2004). Female Cixiidae were analyzed by COI barcoding according to the EPPO standard protocol (EPPO, 2021). Plant tissues were taken from symptomatic potato plants and grapevine and kept frozen at -20 °C until DNA extraction.

Transmission

Up to 12 insects of the same species were transferred onto a single periwinkle in a cylindrical acrylic insect proof cage on the day of collection. The trials were carried out under controlled conditions in a climatic insect chamber with an inoculation access period of 10 days. Cages were checked daily for dead insects, which were removed and stored at -20 °C for further analysis.

DNA extraction and PCR

Both, insects and plant tissues, were tested for the presence of CaPsol, while samples from potato fields were additionally tested for CaAp. DNA was extracted according to Maixner et al. (1995). Pathogen-infection was assessed by PCR, using group specific primers as *tuf*, *f/r Stol*, *Stol11* and *Fra4/5* (Clair et al., 2003; Maixner et al., 1995; Schneider et al., 1997; Zreik et al., 1998). PCR-positive samples were further characterized by MLST based on *tuf* and *vmp1* RFLP as well as *stamp*, and *secY* sequence analysis (Aryan et al., 2014; Cvrković et al., 2014).

RESULTS AND DISCUSSION

MLST results were compared between crops. In addition to crop-specific "stolbur" isolates, some common isolates were found in both cropping systems. In total 18 different genotypes were identified in plant and insect samples, of which 9 genotypes originated from potato fields and 9 from vineyards. In addition, 3 identical genotypes were identified in both cropping systems. Beside *P. leporinus* and *H. obsoletus*, other Auchenorrhyncha species such as *Cixius wagneri*, *Reptalus panzeri* and *Reptalus quinquecostatus* or *Anaceratagallia spp.* were caught in potato fields, and *Agallia consobrina*, *Neoaliturus fenestratus*, *Psammotettix* ssp. and *Dictyophara europaea* and others in vineyards, respectively. *D. europaea* were also caught in unmanaged habitats. The infection status of these species is under investigation. Phytoplasmas of the subgroup 16 SrXII-P described by Duduk et al. (2023) from sugar beet were detected in *P. leporinus* collected from potato fields. Twenty-seven periwinkle plants were successfully inoculated by *H. obsoletus*, *P. leporinus* and *D. europaea*, respectively, containing 7 comprehensive genotypes. Our preliminary data show that some genotypes affect both grapevine and potato, while others are so far considered unique to potato. There are indications that traditional *H. obsoletus*-supported pathosystems may branch on both crops, but may develop distinct cycles in potato with *P. leporinus*.

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Genomics of the SBR-associated '*Candidatus* Phytoplasma solani' driving the Outbreak in Germany

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INTRODUCTION

Over the last decade, a disease associated with sugar beet (*Beta vulgaris* subsp. *vulgaris*), known as Syndrome Basses Richesses (SBR), has spread eastwards from Burgundy (France), reaching southern Germany in 2008. It now affects Europe's two largest sugar beet producing countries. In Germany, the epidemic is driven by rapidly growing populations of the cixiid *Pentastiridius leporinus* L. which was identified in the 1990s as a vector of '*Candidatus* Arsenophonus phytopathogenicus' (*Morganellaceae*) and '*Candidatus* Phytoplasma solani' (*Acholeplasmataceae*). SBR causes absolute sugar losses of 2-4%, but also reduces biomass (Gatineau et al., 2002; Pfitzer et al., 2022). The disease was primarily associated with a '*Ca*. A. phytopathogenicus' infection. Although initially rare, mixed infection with the phytoplasma lead to more severe symptoms. In 2018, '*Ca*. A. phytopathogenicus' was found to be widespread in symptomatic sugar beet when the two pathogens were monitored by real-time PCR (Christensen et al., 2004; Zübert & Kube, 2021). In most areas, the stolbur pathogen was detected in less than 15% of the samples. With the huge vector populations and rapid spread of *P. leporinus* in Germany, a drastic increase in mixed infections by a new dominant '*Ca*. Phytoplasma solani' 16SrXII-P subgroup was observed (Duduk et al., 2023), taxonomically distinct from the taproot stolbur reported in southeastern Europe (Ćurčić et al., 2021). These mixed infections often led to complete sugar beet losses due to primary pathogens, but also due to various secondary infections. The high losses threaten the future of sugar beet cultivation in Germany. In addition, the polyphagous vector transmits the phloem-restricted pathogens to other important crops, including potato (*Solanum tuberosum*), resulting in bacterial wilt (Therhaag et al., 2024). Little is known about this new stolbur pathogen. We have investigated the genome sequence of this strain to gain insight into the pathogen-host interaction and to provide data for the development of diagnostic assays.

MATERIALS AND METHODS

Experimentally infected *P. leporinus* were used to generate high molecular weight DNA using a solid phase DNA purification approach. Shotgun sequencing of the metagenomic samples was performed using SMRT (Pacbio), followed by taxonomic binning of reads and assembly of selected long reads (Duckeck et al., 2023). The genomic data were used for a comprehensive functional reconstruction (Böhm et al., 2023).

RESULTS AND DISCUSSION

Preliminary analysis of the genomic data revealed typical phytoplasma characteristics and a number of particularities within the stolbur group. In addition, the genomic data allowed a critical review of common phytoplasma PCR assays and the development of improved detection approaches needed in vector management projects. The dataset also supports an ongoing screening of the pathogen's gene expression in susceptible and tolerant sugar beet varieties.
The ongoing epidemic should be seen as a major challenge to a wide range of crops in Europe, with phytoplasma as a key driver.

ACKNOWLEDGEMENTS

We would like to thank all our cooperation partners and in particular the funding provided by the Industrial Collective Research (IGF) project no. N22943 of the "Gemeinschaft zur Förderung von Pflanzeninnovation e.V." (GFPi) financed by the German Federal Ministry for Economic Affairs and Climate Action via the German Federation of Industrial Research Associations (AiF).

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Genetic diversity of '*Candidatus* Phytoplasma solani' infecting Cucurbit in Turkey revealed using multilocus sequence typing analysis

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INTRODUCTION

Many species of the Cucurbitaceae family constitute important crop groups in nutrition and are widely cultivated in many countries of the world. However, there are many phytopathogens that are responsible for cucurbit diseases and reduce product quality and yield. One of these is '*Candidatus* Phytoplasma solani' (CaPsol), which has a wide host range and is known to be widespread in the Euro-Mediterranean basin.

The highly genetically variable stolbur antigenic membrane protein (*stamp*) and variable membrane protein-1 (*vmp1*) genes have been used as epidemiological markers to provide information on the complex epidemiological cycles of CaPsol spreading naturally and in agroecosystems (Pacifico et al. 2009). Moreover, the *tuf* gene may also play an important role in adapting to these complex and variable environmental conditions (Murolo et al. 2010). The mentioned gene regions can be used in multilocus sequence (MLS) typing analyses, providing insights into molecular ecology and improving our understanding of the epidemiology of phytoplasma diseases (Murolo and Romanazzi, 2015). Thus, within the scope of this study, molecular characterisation of multiple gene region sequences of CaPsol strains obtained in cucurbit production areas of Marmara region, which has the largest agricultural production volume in Turkey, was carried out. In addition, by using haplotype network analysis method of *stamp* and *vmp1* gene region sequences of these strains, their relationships with other genotypes were revealed.

MATERIALS AND METHODS

Phytoplasma sources and molecular assays

Field surveys were carried out in the watermelon and cucumber growing regions in Tekirdağ and Çanakkale provinces, in the Marmara region of Turkey, during the 2019-2020 plant development periods. To extract total nucleic acids (TNA) from cucurbit samples, 250 mg of leaves were utilized along with a slightly modified CTAB procedure. Molecular characterization of the 16S rDNA gene and detection of phytoplasmas in the samples were accomplished using nested PCR investigations using universal primer (R16mF2/R16mR1 and R16F2n/R16R2) pairs. As stated by Schneider et al. (1997), the *tuf*gene was amplified using nested PCR using the primers fTuf1/rTuf1 and fTufAY/rTufAY. Using the primers StampF/StampR0 and StampF1 /StampR1, the *stamp* gene was amplified as described by Fabre et al. (2011). The *vmp1* gene was amplified using the primers H10F1/H10R1, followed by nested PCR with the TYPH10F/TYPH10R pairs (Cimerman et al., 2009; Fialova et al., 2009).

Sequencing and molecular evolutionary analyzes

Using the Sanger technique, the nested PCR fragments were sequenced bidirectionally. BlastN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to perform nucleotide sequence similarity studies after the raw sequence data was edited and combined using the CLC Main Workbench V.20.3 software. Phylogenetic trees were generated based on *stamp* and *vmp1* gene regions using the neighbor-joining (NJ) method in MEGA X software. For the *vmp1* and *stamp* gene regions, the

Tamura-Nei and the Tamura-3 parameter models were used to construct the unrooted trees in MEGA X software.

RESULTS AND DISCUSSION

Symptoms such as yellowing, small and crisp leaves, and leaf/flower deformities were observed in Marmara region cucurbit growing areas, and BLAST analyses showed that phytoplasmas detected in infected plants were associated with the 16SrXII-A subgroup. In a Russian Federation study, mosaic symptoms were observed on melon leaves that were naturally infected with CaPsol (Girsova et al., 2021). On the other hand, a different study carried out in Türkiye's Eastern Mediterranean region revealed that cucurbit plant symptoms as phyllody, virescence, and yellowing leaves are related to 16Sr-VI group infection (Çarpar & Sertkaya 2022). Despite having distinct or comparable symptoms, multiple phytoplasma groups in cucurbits might result in mixed viral infections. Therefore, in this study, cucurbit plantations had a higher prevalence of virus-like symptoms than phytoplasma symptoms.

Amplification studies yielded fragments of 1250 bp from seven cucurbit samples and BLAST analyses showed that these were 16Sr XII-A. Furthermore, from these seven novel cucurbit strains, amplicons of 800 bp, 470 bp, and 1290 bp in size were obtained for the *tuf, stamp,* and *vmp1* gene regions, respectively. BLAST analyzes showed that the cucurbit strains had more than 99% nucleotide identity with the V4 and V14 genotypes for the *vmp1* gene region and clustered with these genotypes in the phylogenetic trees. Similarly, for the *stamp* gene, cucurbit strains had over 99% nt homology with five different genotypes obtained from eastern European countries (STOL, Rqg50, Rpm35, BG4560, and Rqg31) and clustered with these genotypes. Furthermore, the strains had nt identity ranging from 99.88 to 100% with the sequences of *tuf-b1* type strains. On the other hand, there is very limited information on MLS analyzes of stolbur in Turkey, and these non-ribosomal gene regions of tomato strains obtained in another study showed close similarities with Eastern European strains (Randa-Zelyüt, 2023). The findings clearly suggested that cucurbit strains are closely related to some genotypes from Eastern Europe according to multiple gene regions. This indicated that there may be a geographical relationship between genotypes.

ACKNOWLEDGEMENTS

This research was supported by a grant from Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, Project number FBA-2023-4471.

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Pro-AECOGY - Epidemiology of Flavescence dorée

Chairs: Nataša Mehle and Sylvie Malembic-Maher

From landscape to cultivated compartments: a proactive approach for the early detection of Flavescence dorée

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INTRODUCTION

"Flavescence dorée" (FD) is a detrimental grapevine disease associated with FD phytoplasmas (FDp) that are quarantine organisms in the European Union and in Switzerland. The epidemic transmission is mediated by the Nearctic leafhopper *Scaphoideus titanus*, which completes its biological cycle on the genus *Vitis*. FD control is based on three main components, namely, the application of insecticides targeting *S. titanus*; the removal of FDp-infected grapevines; and the use of certified propagation material. Despite major and systematic control efforts in FD-infested regions, the disease is still spreading and causing important economic losses (Tramontini et al., 2020). Possible reasons of the lack of success in controlling FD may be linked to the role of gone-wild grapevines and abandoned vineyards as latent and disregarded FDp inoculum reservoirs and main vector habitats as well as the existence of additional epidemiological cycles consisting of alternative vectors and host plant species. Among these, the association of the East Palearctic leafhopper *Orientus ishidae* and alder trees (*Alnus* spp.) seems to play a not negligible role in the local FD epidemiology of several winegrowing regions (Malembic-Maher et al., 2020; Rizzoli et al., 2021). In the specific case of Southern Switzerland, several FDp *map* genotypes were found in both *A. glutinosa* and *O. ishidae* specimens collected from the same trees, including the *S. titanus*- and grapevine-compatible M50 strain (Rizzoli et al., 2021). As shown by Jarausch et al. (2021; 2023), FDp may be acquired from alder trees and later transmitted to grapevine by alternative vectors even in the absence of *S. titanus*. In 2023, a pilot study was conducted in several winegrowing regions of Switzerland to assess the potential existence of alternative FDp epidemiological cycles in several vineyard pathosystems beyond the Southern Alps (Rizzoli et al., 2021). The goals were to (i) test phytoplasma infection in alder trees, (ii) test if known FDp alternative vectors were present on alder trees and were FDp-infected, and finally, (iii) verify the compatibility between the *map* genotypes found in tree and insect samples.

MATERIALS AND METHODS

Thirty-eight experimental sites situated in several Swiss winegrowing regions hosting alder trees were selected (Figure 1). In the summer of 2023, insects were collected from alder tree branches with a beating tray and transported to the laboratory for determination. During the winter 2023-2024, three-year-old wood from crown branches was collected from the previously sampled trees. Known FDp vectors and alder's plant material were subjected to molecular analysis. After attesting the FDp infection status, FDp-infected samples were further analyzed to identify the *map* genotypes.

RESULTS AND DISCUSSION

FDp-infected *O. ishidae* and alder trees were found in all the monitored winegrowing regions, including the northwestern part of Switzerland, where neither FD nor *S. titanus* are currently present in vineyards (Figure 1). Most importantly, several *map* genotypes, which were previously detected in grapevine from FD outbreaks and *S. titanus*, were identified. Thus, a potential establishment of FD in

S. titanus-free areas of Northern Switzerland is possible, as previously described in Germany by Jarausch et al. (2021). Considering natural and human-mediated spread, as well as climate change, it is foreseeable that *S. titanus* may soon colonize currently FD-free areas of Northern Switzerland. Then, *S. titanus* may trigger FD outbreaks by acquiring FDp compatible genotypes originally transferred from the surrounding landscape by alternative FDp vectors, such as *O. ishidae*. An in-depth understanding of the local FD pathosystem complexity in Western and Northern Switzerland is therefore urgently needed. Integrating or even starting from the landscape might consequently be a key component for FD risk assessment in FD-free areas, especially in winegrowing regions where other grapevine yellow diseases such as "Bois Noir" are already well established.

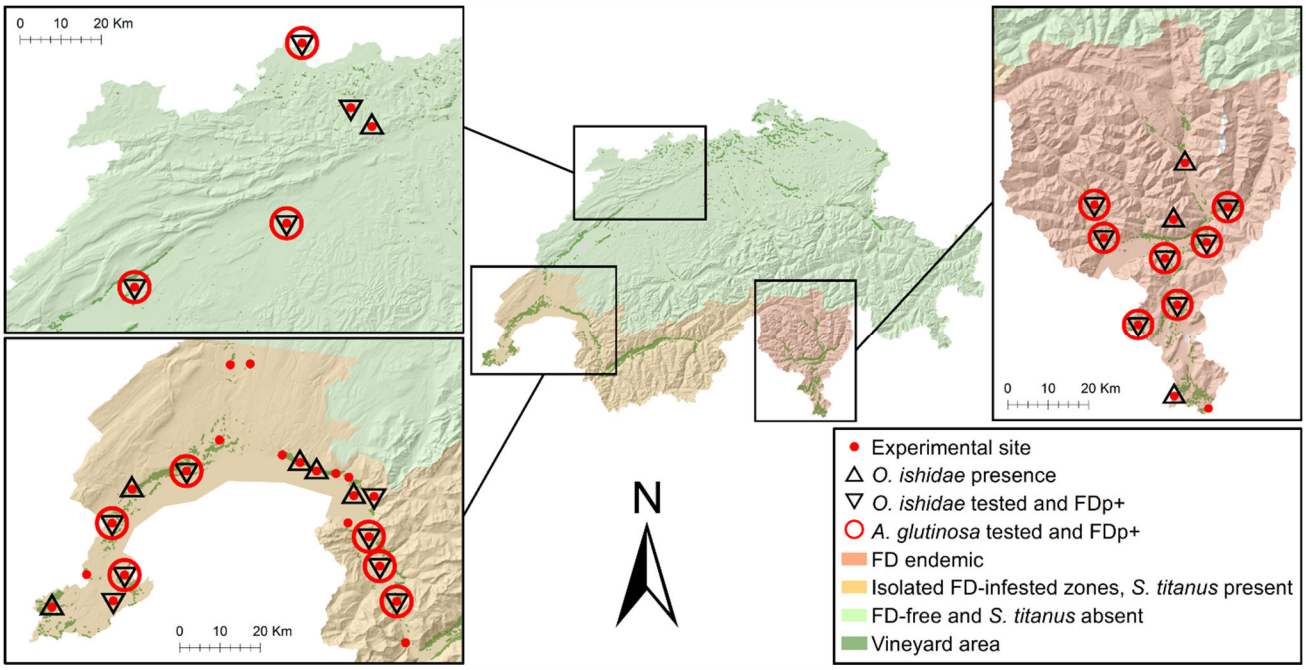

Figure 1. Distribution of sampling locations within Switzerland.

ACKNOWLEDGEMENTS

This research was partially funded by the Federal Office for Agriculture (grant nr. 627001075).

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Preliminary data on the natural reservoir plants and insect carriers of Flavescence dorée and related phytoplasmas in Croatia

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INTRODUCTION

The occurrence of Flavescence dorée (FD) disease of grapevine, its associated phytoplasma, FDp (a member of the 16SrV taxonomic group of elm yellows, subgroups V-C and -D), and the main insect vector, the deltocephalinae leafhopper of North American origin, *Scaphoideus titanus*, was first evidenced in vineyards in Croatia in 2009 (Šeruga Musić et al., 2011; Plavec et al., 2015). Contemporary identification of FDp in the climbing shrub *Clematis vitalba* as one of the main natural reservoir plants in North Italy and the Balkans was also confirmed for Croatia (Filippin et al., 2009). Subsequent studies aimed at determining the genetic diversity and structure of FDp populations and tracing transmission pathways in Croatia revealed the invasive tree *Ailanthus altissima* and the natural riparian tree *Alnus glutinosa* as natural FDp reservoirs (Plavec et al., 2019). However, the roles and epidemiological importance of each of the identified reservoir plants remained unclear due to the limited number of elaborated samples. Regarding the natural insect vectors of FDp from reservoir plants to grapevine and their association with natural reservoir plants on the wider territory of Croatia, particularly beyond vineyards in their natural habitats, not much is known. To understand the epidemiological importance of FDp natural plant hosts and insect vectors, we performed surveys targeting clematis, alders, and other tentative natural woody hosts of FDp in vineyard surroundings and natural habitats in wine-producing regions of Croatia.

MATERIALS AND METHODS

The tentative natural reservoir plants and insect vectors were surveyed between 2019 and 2022. The collections were made in the following wine-producing regions of Croatia: Adriatic northwest (Istria), Continental central (Koprivnica-Križevci County), and Continental north (Varaždin County). All plant samples collected for FDp analyses were non-symptomatic, and these included samples of clematis, alnus, ailanthus, and *Cornus sanguinea*. Putative insect vectors of FDp were collected using sweep nets and mouth aspirators, selectively sweeping the tentative FDp reservoir plants. Total DNA was extracted from individual plant and insect samples as previously described (Plavec et al., 2019; Krstić et al., 2022) and tested for the presence of 16SrV-group phytoplasmas using real-time PCR on the *map* gene (Pelletier et al., 2009). The phytoplasma-positive samples were genotyped on *map* and *vmpA* genes following previously published protocols (Malembic-Maher et al., 2020; Krstić et al., 2022). The *map* and the *vmpA* gene sequences were compared to FDp and related phytoplasma genotypes identified during our surveys in Serbia and in natural areas of the Balkans in search for the natural constituents of the FDp transmission cycle (Krstić et al., 2018; 2022; unpublished).

RESULTS AND DISCUSSION

The clematis plants were found as frequent (acc. 50% infected) natural FDp reservoirs of the Map-FD3 (Fig. 1) and VmpA-III genotype cluster (Vectotype III), thus confirming the previous findings for the Balkans (Filippin et al., 2009; Malembic Maher et al., 2020; Krstić et al., 2018). Of the tentative insect vectors obtained from clematis, the planthopper *Dictyophara europaea* collected in Istria was

found carrying FD3-M51 (>10%), while the leafhopper *Phlogotettix cyclops* collected in Koprivnica-Križevci was carrying M51 variants, identical as in clematis on the same location. All the insect-associated isolates were of Vectotype III (Malembic Maher et al., 2020). This confirmed previous findings on the importance of these insects as tentative natural vectors of FDp (Krstić et al., 2018; Plavec et al., 2019). The '*Candidatus* Phytoplasma ulmi' was for the first time identified in a single individual of *D. europaea*, while *C. sanguinea* was found as an additional reservoir plant of the M51 genotype in locations with frequently infected clematis and *D. europaea*. In the Varaždin, at Lake Ormož we identified syntopic occurrences of the genotypes Map-AldY in alders and Map-FD3 in clematis. Finally, we noted the widespread occurrence of mosaic leafhopper *Orientus ishidae* on natural plants in riparian areas of Croatia that carried AldY or FD2 genotypes when collected from alders.

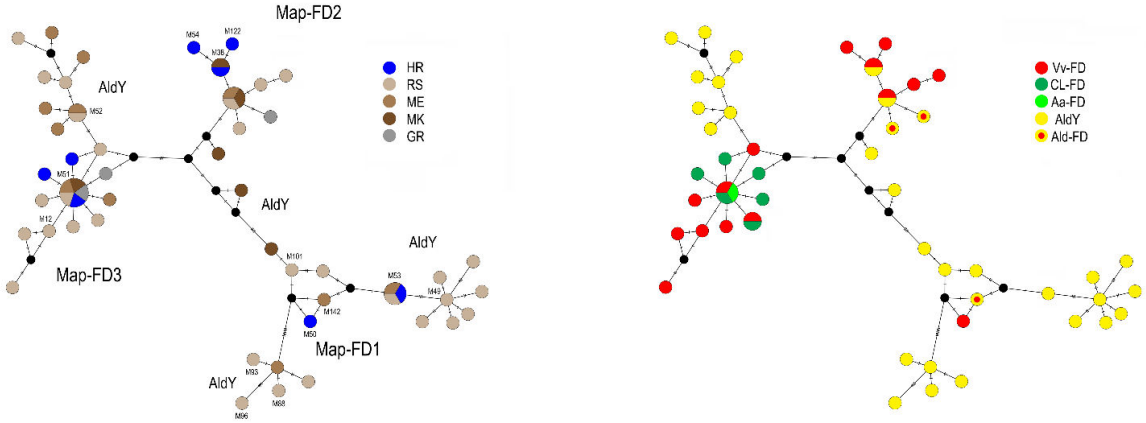

Figure 1. Median-joining networks inferred from a total of 48 *map* genotypes of FDp and related strains from Croatia and the Balkans (left), identified in grapevine and/or natural reservoir plants (right). Legends present countries of isolates carrying designates genotypes (2 letter code), and host association of genotypes (Vv-FD: *Vitis* FDp, CL-FD: *Clematis* FDp, Aa-FD: *Ailanthus* FDp, AldY: *Alnus* alder yellows phytoplasma, Ald-FD: *Alnus* FDp not yet found in Vv or St).

ACKNOWLEDGEMENTS

The research was supported by Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contract No. 451-03-66/2024-03/200010) and by bilateral project between Serbia and Croatia (No. 337-00-205/2019-09/38).

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New insights on Flavescence dorée epidemiology in Serbia

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INTRODUCTION

Flavescence dorée (FD) is a severe grapevine disease caused by FD phytoplasmas (FDp), impacting major viticultural regions in Europe. The epidemic spread of FD within vineyards is facilitated by the Nearctic leafhopper *Scaphoideus titanus*, completing its life cycle on *Vitis* sp. plants in Europe (Chuche & Thiéry, 2014). Through genotyping of the informative house-keeping gene *map*, FDp has been categorized into three genetic clusters, each associated with specific vector and reservoir plant epidemiology or geographic distribution (Arnaud et al., 2007; Malembic-Maher et al., 2020). Genotypes of the Map-FD2 cluster are most abundant in vineyards in France, Switzerland, Italy, Croatia, and Slovenia, while FD3 prevails in the Balkans (reviewed in Krstić et al., 2022). Epidemic outbreaks of FD in Serbian vineyards began in the early 2000s, and despite extensive preventive and curative measures, today FD is affecting vineyard areas in all administrative districts in Serbia(Krstić et al., 2022).Until recently, the M51 genotype belonging to the Map-FD3 cluster, which is common in all districts and wine-growing regions, was the only genotype associated with the FDp outbreaks in Serbian vineyards.Subsequent surveys in the late 2010s on the occurrence and diversityof FDp in Serbian vineyards revealed for the first timetheoccurrence of other genotypes, including members of the Map-FD2 cluster: M89, M148, and M155, indicating that FDp in Serbian vineyards is not monotypic and has a complex epidemiology(Krstić et al., 2022). This discovery prompted a more in-depth investigation into the specific ecological factors contributing to disease outbreaks to assess the significance of these new findings. Initial results from this research are presented herein.

MATERIALS AND METHODS

During 2022 and 2023, we collected grapevines with characteristic symptoms of FDp infection insix locations of the Srem district, including the traditional grape-growing region, Fruška Gora. On threeof these sites, adults of *S. titanus* were selectively collected from half of July to the end of August inside and around affected vineyards. Total DNA from grapevine plants was extracted from one gram of leaf midribs and petioles from each sample using a previously described CTAB protocol (Angelini et al., 2001). Insect DNA was extracted from individual specimens following a previously described non-destructive protocol (Jakovljević et al., 2020). Molecular typing of 16SrV-group phytoplasmas in plants and insects was performed by nested PCR on the housekeeping gene *map* and subsequent sequencing of the PCR products (Arnaud et al., 2007).

RESULTS AND DISCUSSION

The survey was conducted at six locationsof Vojvodina (Srem district), between rivers Sava and Danube. Genotyping of FDp-infected grapevine isolates resulted in identification of genotypes belonging to two different *map*clusters. As expected, according to all of our up to date analysis of FDp-infected grapevines as well as the published data, the majority (70%) of analyzed grapevine isolates carried M51 genotype from Map-FD3 cluster. This genotype was found in all inspected localities. The epidemiological pattern of the FDp genotype M51 in Serbian epidemics is inferred from prior studies, linking it to *Clematis vitalba* as the main host plant and potentially associating it with *Ailanthus altissima*, possibly connected to *C. vitalba* via polyphagous vectors like *Dictyophara europaea*. Besides M51, genotype M155 of the Map-FD2 cluster was found in 30% of grapevine

isolates. Up to now, this genotype was detected only in single grapevine infections inthe South Banat district (Krstić et al., 2022), situated adjacentto the Srem district, with the river Danube being the sole geographical barrier between them. *Scaphoideus titanus* was collected from three of five locations where the M155 genotype was present. At one location, equal numbers of insects were found infected with genotypesM51 and M155. However, at two other locations, only the M155 genotypes were detected in infected *S. titanus* specimens. European alders (*Alnus glutinosa* and *A. incana*) are the main reservoir plants for Map-FD2 genotypes, connected to grapevine through native leafhoppers *Oncopsis alni* and *Allygus modestus/mixtus*, as well as the alien leafhopper *Orientus ishidae* (Malembic-Maher et al., 2020). Despite alders not being in close surroundings of vineyards in grape-growing habitats in Serbia, their presence along riverbanks and riparian habitats, coupled with feral vitis, clematis and other FDp reservoir plants is characteristic of the plant communities in riparian zone throughout the country. Moreover, we have recently identified frequent infection of native leafhoppers *Allygus modestus* with FDp of the Map-FD2 cluster, commonly found in association with alder trees at numerous sites in Serbia, as well as in *O. ishidae* populations established at one location of a riparian habitat (Jović et al., 2024). These findings, along with discovery of the M155 genotype in both, grapevine and *S. titanus*, indicate the occurrence of new FDp epidemic outbreaks in Serbia and highlight the importance of determining the role of each leafhopper species in FDp epidemiology.

ACKNOWLEDGEMENTS

The research was supported by Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contract No. 451-03-66/2024-03/ 200010) and by the Science Fund of the Republic of Serbia, #GRANT No 6808, Endemics and epidemics of grapevine Flavescence dorée (FD) phytoplasma –tracing and tracking transmission routes - FDemic.

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Grapevine Flavescence dorée phytoplasma *map* genotypes associated with outbreaks in Slovenian vineyards

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INTRODUCTION

Grapevine Flavescence dorée phytoplasma (FD phytoplasma; phytoplasma of the 16SrV group) was first found in Slovenia in 2005 in the Primorska wine-growing region during the official survey. Despite control measures, new outbreaks continued to occur and this phytoplasma is currently causing damage and yield losses in all wine-growing regions in Slovenia.

To effectively prevent the spread of the phytoplasma that causes FD disease, a thorough understanding of the epidemiology of this disease is essential. To this end, we investigated the genetic diversity of phytoplasma isolates that caused FD outbreaks in Slovenian vineyards in 2023. We compared the *map* genotypes of FD phytoplasmas in grapevines with those found in grapevines in previous years, in alternative hosts and insect vectors. Based on these comparisons, possible pathways of spread of this phytoplasma in Slovenia are identified and the importance of alternative hosts and vectors is assessed.

MATERIALS AND METHODS

The grapevine samples were collected as part of the official survey of grapevine Flavescence dorée phytoplasma under the coordination of Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection. The study includes grapevine samples collected in 2023 in all wine-growing regions of Slovenia. Each sample consisted of leaves from at least three different parts of one or up to five plants from the same vineyard. DNA was extracted as described in Mehle *et al*. (2013). Samples which tested positive with 16SrV phytoplasma-specific real-time PCR (Hren *et al*., 2017) were subjected to further amplification of the *secY-map* locus (*map* gene) by nested PCR with FD9f5/MAPr1 and FD9f6/MAPr2 primer pairs (Arnaud *et al*., 2007). Nested PCR and sequence analysis of the nested PCR products were performed as described in Kogej Zwitter *et al.* (2023).

RESULTS AND DISCUSSION

In 2023, a nested PCR was performed on 302 grapevine samples in which we detected an infection with phytoplasmas of the 16SrV group. In 21 samples, the phytoplasma concentration was too low for amplification by nested PCR. In 281 grapevine samples that yielded a nested PCR product, 5 different *map* genotypes were detected: M54 was detected in 257 samples (91.4%), M38 in 18 samples (6.4%), M158 in 4 samples (1.4%), M122 in one sample (0.4%) and M50 in one sample (0.4%) (Table 1). All these *map* genotypes were also detected in Slovenian grapevine samples from previous years (Kogej-Zwitter *et al*., 2023). As in 2023, the M54 genotype was also dominant in 2019 and 2021. In 2023, we were unable to detect the M51 genotype in the grapevine samples, the presence of which was confirmed in a few samples from 2019 and 2021.

With the exception of M158, all *map* genotypes detected in Slovenian grapevines were also found in grapevine-*Scaphoideus titanus* pathosystem in some other European countries. M38 was also confirmed in *Alnus glutinosa* and in *Oncopsis alni*, M50 in *A. glutinosa*, *O. alni* and *Clematis vitalba*, and M51 in *C. vitalba* and *Ailanthus altissima* (Malembic-Maher *et al*., 2020; Plavec *et al*., 2019). Of the *map* genotypes detected in Slovenian grapevines, genotypes M38, M122 and M50 were also found

in cultivated symptomatic *Corylus avellana* shrubs and in *Orientus ishidae* in Slovenia (Kogej Zwitter *et al*., 2023). However, M54 has never been detected in *A. glutinosa*, *C. vitalba* and *O. alni* (Malembic-Maher *et al*., 2020). M54 was also not detected in any of the samples of *C. avellana* from Slovenia examined so far, nor in samples of *O. ishidae* caught in Slovenian hazelnut orchards (Kogej Zwitter *et al*., 2023). M54 is the only genotype detected so far in samples of *S. titanus* caught in Slovenia, although it should be noted that only 53 cumulative *S. titanus* samples (up to 10 specimens per sample) were examined in the period from 2021 to 2023 and that the presence of 16SrV phytoplasmas was detected in 19 samples, of which in 14 samples the concentration of phytoplasma was sufficient for the determination of the *map* genotype (data not shown). Future research will focus on investigations of the wild environment outside the vineyards which are mainly infected with M54 genotypes (e.g. analysis of non-cultivated *C. avellana* plants), as well as on the evaluation of other possible vectors of the most devastating phytoplasma genotype (M54) in Slovenian vineyards.

Table 1: Summary of discovered *map* genotypes from 2019 to 2023 in Slovenian grapevines

Map-FD cluster	Genotype	2019 (80 samples tested)*	2021 (90 samples tested)*	2023 (281 samples tested)
FD1	M50	2.5 %	1.1 %	0.4 %
	M158	2.5 %	0 %	1.4 %
FD2	M38	10.0 %	3.4 %	6.4 %
	M54	81.2 %	91.1 %	91.4 %
	M122	0 %	2.2 %	0.4 %
FD3	M51	3.8 %	2.2 %	0 %

*Data from Kogej Zwitter *et al*. (2023)

ACKNOWLEDGEMENTS

This study was supported by the Slovenian Ministry of Agriculture, Forestry, and Food through the Administration of the Republic of Slovenia for Food Safety, Veterinary, and Plant Protection. We thank Slovenian phytosanitary inspectors and experts from authorized institutions for providing the field samples.

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Pro-AECOGY – Innovative and Sustainable management of grapevine yellows

Chairs: Esmeraldina Souza and Gianfranco Ramanazzi

Vibrational mating disruption and other techniques of behavioral manipulation against grapevine yellows'vectors

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BIOTREMOLOGY

Biotremology is the scientific discipline that studies behavioral and physiological interactions among organisms mediated by vibrational signals (Mazzoni et al., submitted). In insects, vibrational communication is widespread and involves most of the orders. The largest manifestation of it is found in Hemiptera, and in particular in Auchenorrhyncha, which include many pest species including grapevine Yellows' vectors (Virant-Doberlet et al., 2023). In fact, both *Scaphoideus titanus*, vector of Flavescence Dorée, and *Hyalesthes obsoletus*, vector of Bois Noir, primarily use vibrational signals for intraspecific communication, especially for mating (Mazzoni et al., 2014).

THE MATING BEHAVIOR OF *SCAPHOIDEUS TITANUS* AND *HYALESTHES OBSOLETUS*

The American grapevine leafhopper, *S. titanus*, relies on vibrational signals from the early stage of pair formation when males adopt the so-called 'call-fly' strategy to search for available females on the vine canopy. During the twilight hours of July and August, they emit Calling Songs, which consists of series of pulses emitted at regular intervals (ca. 0.3s) for at total duration of 15-20 s. After emitting one or two songs, they fly away (from leaf to leaf) and repeat the call. This routine is interrupted as soon as a female replies to the call, thus establishing vibrational duets with the male. After the first 'identification duet', devoted to reciprocal assessment (of partner identity and quality), males start searching for the females thanks to the 'location duet'. By emitting pulses from a stationary position, males invite females to respond, thus revealing their position on the plant. After a female's pulse, the male moves a few centimeters in the direction of the female. Finally, when the male reaches the female's leaf, they perform the 'courtship duet', during which the male emits the 'buzz', a harmonic sound at high frequency (900-1200 Hz), and that precedes the mating (Polajnar et al., 2014).
The cixiid planthopper, *H. obsoletus*, also communicate through vibrational signals, albeit with distinct mating behavior and different roles and usage of vibrations compared to *S. titanus*. In *H. obsoletus*, females initiate mating communication by emitting a long series of monotone pulses from a static position without exhibiting the call-fly behavior. Males can respond with single pulses (male syllable 1), establishing a first 'duet of identification'. Following this, there is a clear role reversal: males emit long series of pulses (male syllable 2) to which females reply with single pulses, prompting males to approach ('location duet'). When males are in close proximity of females (less than 1 cm) they emit a 'courtship song' preceding copulation (Mazzoni et al., 2010).

SIMILARITIES AND DIFFERENCES BETWEEN THE TWO SPECIES

Pair formation in both species involves an escalation of behaviors associated to phase-specific signals and songs from males and females, engaging in a duet where the integrity and functionality depend on the reciprocal adherence to temporal rules. The scheme follows a fixed sequence: 1) calling; 2) identification; 3) location; 4) courtship; 5) copula. In both species, males actively search for stationary females, who are stimulated to emit signals by male songs (Mazzoni et al., 2014). Despite numerous similarities, some important differences exist, relevant for the development of behavioral manipulation strategies. The most important difference lies in the role of the caller: males in *S. titanus* and females in *H. obsoletus*. In the leafhopper, females never call, necessitating male-driven duets; females need to be continuously stimulated or communication ceases, impeding copulation. In contrast, planthopper

females signal their readiness to mate through calls, prompting males to emit series of pulses after identification, revealing their own position. However, a crucial point lies here, males could potentially locate females solely by following initial calls if they lasted long enough to determine their location.

IMPLICATIONS FOR BEHAVIORAL MANIPULATION

Different mating strategies lead to radically different approaches of behavioral manipulation, a pest control technique aimed at inducing target species to exhibit behaviors detrimental to their survival. By mimicking species-specific signals, it is possible to 'cheat' an insect into behaviors that, otherwise, would be advantageous in natural conditions. For example, sexual pheromones massively released in the field can compromise mating success in pest species like moths, where males cannot track natural pheromone trails released by females (Cardé, 1990; Foster and Harris, 1996). Similarly, in Auchenorrhyncha, vibrational signals can be used either to disrupt individuals from mating or attract them into traps. The former strategy has been developed over the last two decades to combat the infestations of *S. titanus* in vineyards (Mazzoni et al., 2019). In practice, by transmitting a disturbance noise (DN) mimicking the male's rivalry signal to vines, the *S. titanus* mating signals can be masked effectively reducing population density. This method, the 'Vibrational Mating Disruption (VMD)', after about 10 years of laboratory and semi-field trials, was tested in the field. Electromagnetic shakers were installed into the poles of a vineyard trellis at 50m intervals to transmit the DN to plants infested by wild *S. titanus* population. The field test was carried out for six consecutive years (2017-2022) in a commercial vineyard in the Province of Trento (Northern Italy) and promising results were observed. Monitoring of the pest population level was performed by visual inspections of immature stages and with yellow sticky traps for adults in two adjacent and similar areas: one vibrated by the shakers and one not-vibrated, the control. A significant reduction in *S. titanus* population density during the first three years of VMD application was observed. During this three-year period the shakers were capable to maintain the DN over the safety threshold (i.e., the least DN amplitude required to prevent male-female communication) in most of the plants. However, due to the senescence of the shakers, their efficacy diminished over time leading to *S. titanus* population rebounds (Nieri et al., 2023; Thiery et al., 2023).

Conversely, *H.obsoletus*, a species that occurs on vines only occasionally, renders VMD impractical. Transmitting disruptive signals to vines, which are not a host plants for the planthopper, would not cause any reduction in the species mating, while treating nettles and bindweed, the actual host plants, with vibrations does not appear feasible. On the other hand, laboratory tests confirmed males tracking the females' calls, suggesting the potential development of 'vibrotraps' to monitor their presence in the field and optimize protection strategies. A similar approach has been successfully developed for another pest, the brown marmorated stinkbug, *Halyomorpha halys*, with a bimodal trap releasing aggregation pheromone and sexual vibrational signals (Zapponi et al., 2022). In the case of *H. obsoletus*, an unimodal trap would suffice, considering the scarce or even null role of volatiles in pair formation (Mazzoni et al., 2010).

CONCLUSIONS AND PERSPECTIVES

In conclusion, the study of intraspecific communication and mating behaviors and their implications for behavioral manipulation in pest control strategies, particularly in the cases of Auchenorrhyncha, sheds light on innovative approaches to managing agricultural pests. These strategies underscore the importance of tailored approaches in pest management, which must account for the diverse mating behaviors of target species, ecological contexts and crop breeding peculiarities (i.e., trellis materials and structure, interplant distances etc.). By understanding this complex network of information and interactions, researchers and practitioners can develop more effective and sustainable pest management strategies that minimize environmental impact while maximizing agricultural productivity.

Looking ahead, future research should focus on refining and expanding behavioral manipulation techniques to address emerging pest threats and adapt to evolving ecological dynamics, taking into

account the rapid technological progress that makes previously considered unpractical or costly solutions, practical and effective. An important mantra should be to anticipate future challenges in terms of energy supply, material costs, smart applications etc. and not view the present situation as a limitation. In conclusion, collaborative efforts between researchers, growers, and policymakers are essential for translating scientific insights into practical solutions that promote agricultural sustainability and resilience in the face of changing environmental conditions.

ACKNOWLEDGEMENTS

We are deeply grateful to Prof. Andrea Lucchi (University of Pisa), Dr. Meta Virant-Doberlet (National Institute of Biology, Ljubljana), and Mr. Vittorio Veronelli (CBC Europe) for their invaluable contributions to the development from scratch of methods for behavioral manipulation through vibrational signals.

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Evaluation of biostimulant effectiveness in "Bois noir" control in vineyards

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INTRODUCTION

Bois noir (BN) infection dynamics in vineyards are determined by two main driving forces: new infections and recovery (Mori *et al*., 2015). Several studies showed that recovery can be induced by stressing the plants through uprooting followed by immediate transplanting, pruning or pollarding, treating the plants with resistance inducers, and grafting the plants with recovered shoots (Pierro *et al*., 2024). Several studies demonstrated that treatment of grapevine canopy by biostimulants can prevent diseases (Gutierrez-Gamboa *et al*., 2019). Recently, small-scale field trials showed that treatments with high concentration of L–α amino acids (commercial formulation Delfan plus, Tradecorp Italia, Saronno (VA), Italy) reduced the percentage of BN-infected symptomatic vines increasing the recovery rate (Moussa *et al*., 2021). The aim of this study was to evaluate the preventive and curative effects of this biostimulant in large-scale field trials.

MATERIALS AND METHODS

Field trials were carried out from 2021 to 2023 in four Chardonnay BN-affected vineyards (size ranging from 0.7 to 1.2 Ha) located in Franciacorta (North Italy). In September 2021, the vineyards were monitored for grapevine yellows symptoms. Each vineyard was divided in two blocks: one treated with the L–α amino acids (commercial formulation Delfan plus) (T), and one untreated (NT). In each year, the activities included: (i) applications with the biostimulant every two weeks, from mid-April (10 cm long grapevine shoots) to the beginning of August (7 treatments/year); (ii) mapping and sampling of symptomatic and asymptomatic vines in September; (iii) extraction of total nucleic acids and molecular identification of '*Candidatus* Phytoplasma solani' by nested PCR-based amplification of *stamp* gene; (iv) molecular characterization of phytoplasma strains by means of *stamp* gene nucleotide sequence analysis; (v) statistical analysis (Mann-Whitney U Test in SPSS software) to evaluate any differences in the curative and preventive effect based on BN-symptom observations. The curative effect (percentage of recovered grapevines) was calculated considering the health status of symptomatic plants in 2021 over the following two years. The preventive effect was calculated considering the percentage of new symptomatic plants (grapevines showing symptoms for the first time) in the two-year period 2022-23.

RESULTS AND DISCUSSION

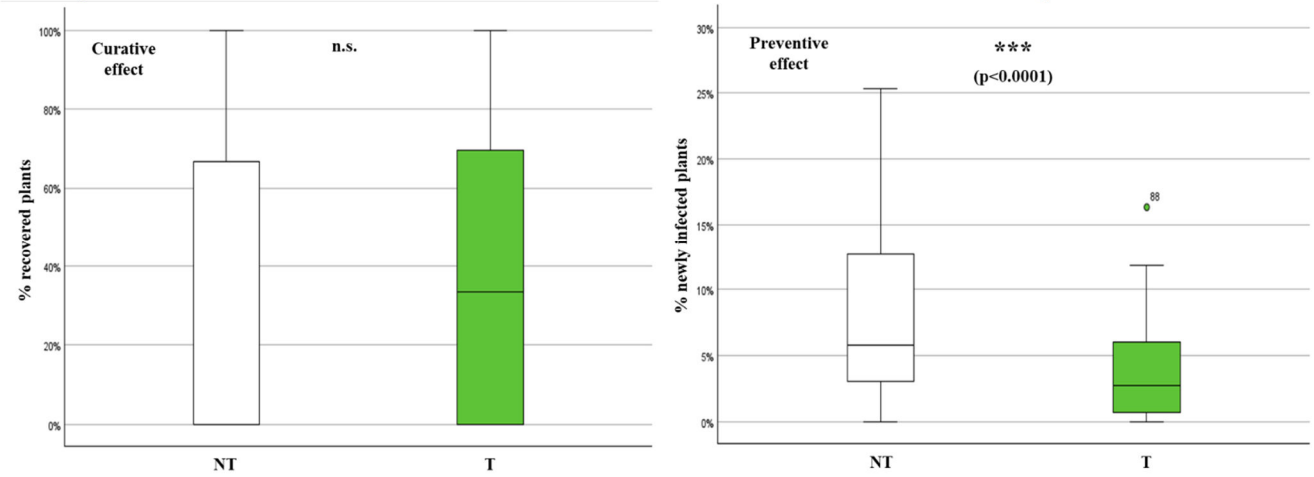
Statistical analyses showed that: (i) curative effect was higher in T block in vineyard 4 and in NT block in vineyard 2, while no differences were found between the blocks in vineyards 1 and 3; (ii) preventive effect was higher in T block in vineyards 3 and 4, while no differences were found in T and NT blocks in vineyards 1 and 2 (Table 1). Analyses of overall data highlighted no statistically significant differences in curative effect, while preventive effect was found higher in T blocks (Figure 1). Molecular and bioinformatics analyses showed the presence of eight distinct '*Ca.* P. solani' strains carrying different *stamp* gene variants (St1, St5, St8, St10, St16, St18, St19, St30). The statistical analysis showed a uniform distribution of such phytoplasma strains within the vineyard, reinforcing the evidence that the effect on BN incidence in the blocks is due to the action of the biostimulant and

not to possible differences in the '*Ca.* P. solani' strain virulence. Based on the obtained results and in accordance with previous study (Minuz *et al*., 2020), it is reasonable to hypothesize that the L–α amino acids preventive effect can be related to differences in volatiles emitted by the treated plants, affecting the attractiveness to the main vector *Hyalesthes obsoletus*. Symptom observation and molecular analyses in 2024 are necessary to confirm the effectiveness of the biostimulant in BN control.

Table 1. Curative and preventive effects of L–α amino acids treatments

Vineyard	Block	No. symptomatic plants (2021)	% recovered plants (2022/23)	No. symptomless plants (2021)	% newly infected plants (2022/23)
1	T	86	31 a	1247	12 a
	NT	98	41 a	2256	14 a
2	T	344	58 a	3574	3 a
	NT	69	84 b	3164	4 a
3	T	293	44 a	2807	8 a
	NT	320	28 a	2530	12 b
4	T	46	93 b	5757	1 a
	NT	39	46 a	4323	3 b

Figure 1. Overall data statistical differences in L–α amino acids curative and preventive effect



ACKNOWLEDGEMENTS

This study is funded by Consorzio per la Tutela del Franciacorta

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RNAi to cope with flavescence dorée phytoplasmas vectors: insights on plant-mediated delivery and in-insect degradation of dsRNAs

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INTRODUCTION

RNA interference (RNAi) regulates gene expression in eukaryotes and can be exploited in crop protection against pests by exogenous applications of double-stranded RNAs (dsRNAs). The basic mechanism is known in arthropods, but knowledge gaps still exist. RNAi mediated gene silencing can be achieved efficiently in both flavescence dorée phytoplasmas (FDp) vectors *Scaphoideus titanus* and *Euscelidius variegatus* through cell transfection (Canuto *et al*., 2023), injection (Abbà *et al.*, 2019; Ripamonti et al., 2022) and artificial feeding (Arricau-Bouvery *et al.*, 2023). These delivery strategies are laborious, manageable only under lab conditions and hardly feasible on a field scale.

MATERIALS AND METHODS

We attempted a dsRNAs plant-mediated delivery by means of petiole absorption (Rossi *et al.*, 2023). Detached *Vitis vinifera* leaves were inserted in 1.5-ml tubes containing 200 µL of dsRNA solution until the solution was all absorbed. In order to determine the ability of dsRNAs to be absorbed and spread within plants, the presence and integrity of RNAi triggering molecules were verified in treated leaves by RT-PCR in tissue portions close and distal to the absorption site. The dsRNA-treated leaves were used to feed *S. titanus* fifth-instar nymphs for 48 h. Insects were then transferred on grapevine plants prior to RNA extraction and gene expression analysis. In order to further explore the RNAi response of FDp vectors mediated by feeding, nucleasic activities of *E. variegatus* and *S. titanus* were assayed by gel electrophoresis of dsRNAs incubated for 30, 60 and 120 minutes in gut juice obtained from both species.

RESULTS AND DISCUSSION

Our preliminary results revealed that dsRNAs were successfully absorbed and translocated from the petiole throughout the grapevine leaf. Moreover, insects fed on treated leaves showed a decreasing trend in the expression of genes targeted by dsRNAs when compared to control group fed on leaves treated with dsRNAs designed onto the GFP sequence.
The results of the degradation assays indicated that dsRNAs were not degraded by the gut enzyme content of any of the vectors, explaining, at least partially, the RNAi response triggered by dsRNAs ingestion in the insects abdomens.

ACKNOWLEDGEMENTS

This work was funded by: ReSet Project granted by Fondazione CRT (2022.1790), MIDI Project granted by Regione Piemonte and Hemint Project granted by MUR (Finanziato dell'Unione Europea NextGenerationEU missione 4, componente 2, investimento 1.1 nell'ambito del Bando PRIN 2022 codice CUP B53D23017470006 codice progetto 2022BPB5A8).

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RENOV project: ecological reservoirs and management of Bois Noir in the French vineyards

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INTRODUCTION

Since 2018, French vineyards have been alarmed by the notable increase of Bois noir (BN) observed since the last French 2002-2010 survey (Kuntzman et al., 2014). In the frame of the French national plan against vineyard decline, the RENOV project proposes to better understand the BN pathosystem, to identify levers for action with special focus on the vineyards planted in cultivar Chardonnay in French regions under sub-continental climate. Several hypotheses are posed to explain BN re-emergence: (i) new variants of "*Candidatus* Phytoplasma solani" (CaPsol) with different epidemic properties and/or virulence, (ii) change in the local abundance of CaPsol vectors, (iii) and or changes in wild reservoir host-plants, including nettle, bindweeds or other recently identified reservoirs in Europe, (iv) changes or unadapted vineyard management practices. To assess such parameters, study case plots were selected. The acceptability and obstacles for sustainable management practices and enhancement of prophylaxis, will be favored by setting up demonstration and system tests. The knowledge acquired will also be taken into account in regional control plans against Flavescence Dorée (FD), the outbreaks of which can be masked by the BN presence.

MATERIALS AND METHODS

RENOV is organized into three major scientific work packages (WP). The first WP consists in a survey of CaPsol genotypes circulating in France. In this WP, new markers for genetic analysis are being developed, including neutral markers, positively selected markers as well as bacterial variable number of tandem repeats (VNTR) (See communication by Salar et al., in the proceedings). For genotyping CaPsol, a set of BN-positive samples representative of the French viticultural regions, was selected from the French laboratories in charge of grapevines yellows diagnosis and from other laboratories in the Euro-Mediterranean basin. The second WP is to survey vineyard case study plots, in six French viticultural areas (Alsace, Burgundy, Champagne, Jura, Aude and Nouvelle-Aquitaine). Information about the plots, including management practices, are collected from winegrowers and recorded. On theses plots, samples are taken from symptomatic vines, known plant reservoirs such as *Convolvulus arvensis, Calystegia sepium, Urtica dioica* and *Crepis faetida*, in order to measure their infection by CaPsol genotypes. The presence of symptoms and their severity on grapevine is also monitored with the severity scale previously published for FD (Eveillard et al, 2016). Qgis and Qfield tools are used

for spatially map the notations, samplings and associated CaPsol genotypes. Population dynamics of known CaPsol vectors are recorded using yellow sticky traps and sweep-netting on wild reservoirs plant. Infective status and the corresponding CaPsol genotypes of all collected vector populations are also recorded and spatially mapped. Finally, the third WP involves winegrowers and/or groups of winegrowers to develop BN management strategies aimed to limit CapSol and BN incidence in the field. A specific study is also carried out to evaluate the acceptability of adapted management strategies.

ACKNOWLEDGEMENTS

This project is supported by the Plan National Dépérissement du Vignoble grant RENOV with the support of CNIV and FranceAgriMer.

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Pro-AECOGY – Diagnosis and surveillance of grapevine yellows

Chairs: Nataša Mehle and Oliver Schumpp

Validation of methods for Flavescence dorée phytoplasma *sensu stricto* identification through Test Performance Studies.

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INTRODUCTION

Flavescence dorée (FD), the most impacting grapevine yellows in Europe, is associated to *Scaphoideus titanus*-transmitted phytoplasmas also called Flavescence dorée phytoplasmas *sensu stricto* (FDp). FDp variants belong to taxonomic subgroups 16SrV-C and 16SrV-D. Up to current knowledge, the single 16SrV-D *map*-genotype variant M54 as well as the more than 150 variants of subgroup 16SrV-C are all listed on the EU quarantine list, regardless of their epidemic properties in the vineyard. FD phytoplasma epidemic nature is particularly tied to specific forms of the adhesin-like variable membrane proteins (VMP), determining Vectotypes II and III that are epidemic in European vineyards and Vectotype I (16SrV-C subgroup) that are non-epidemic in the vineyard. Vectotype I of European alders, occasionally transmitted from alder trees to grapevine by *Oncopsis alni*, poses no outbreak risks (Malembic-Maher *et al*., 2020). Distinguishing between epidemic and non-epidemic isolates is important for an effective and sustainable management. Current diagnostic tests (EPPO, 2016) target 16SrV group without differentiation, while vectotypes determination offer the advantage to directly identify vectotypes. This study aims to validate identification methods capable to distinguish FDp from other 16SrV phytoplasmas.

MATERIALS AND METHODS

Design of the study. Eleven participant laboratories from 9 different countries tested an identical series of double-blind samples following the provided working protocols and using their own reagents and machines.

Samples. The tested samples consisted of total nucleic acid (TNA) extracts, instead of fresh plant leaf samples, to avoid problems of homogeneity between laboratory extraction methods and stability over shipping. To evaluate analytical specificity, the samples consisted of 15 target isolates of FDp from different plant hosts, different geographical origins and different genotypes and 15 plants contaminated by other phytoplasmas of 16SrV group or phytoplasmas responsible of grapevine yellows or healthy grapevines. Each sample was tested in duplicate. To evaluate the analytical sensitivity, the repeatability and the reproducibility, a 5-level dilution series of grapevine infected by FDp diluted in healthy grapevine was analyzed 6 time.

Tested protocols. After intra-laboratory adaptation and selection, three molecular protocols were submitted to the interlaboratory trial: 1) a nested-PCR 16SrV *map* adapted from Arnaud *et al.* (2007), followed by sequence analysis (Method1); 2) a nested-PCR *VmpA-R1* adapted from Rossi *et al*. (2019)

and Malembic-Maher *et al*. (2020), followed by sequence analysis (Method2); 3) a real-time PCR *Vmp-RK-A23B1* (Foissac et al., this congress) (Method3).

Processing of the results. The following parameters were calculated: analytical specificity, representing the ability of the methods to correctly identify the target phytoplasma; analytical sensitivity, representing the last level at 100% positive results; repeatability and reproducibility (adapted from EPPO, 2021).

RESULTS AND DISCUSSION

Analytical specificity varied across the methods. The real-time PCR *Vmp-RK-A23B1* (Method3) exhibited the highest analytical specificity for FDp (98%), while the nested-PCR 16SrV *map* followed by sequence analysis (Method1) showed the lowest analytical specificity (87%). However, for the *Vmp I* cluster, Method3 generated 32.7 % of false positive results. The best analytical sensitivity is obtained with Method1 and Method3 with 100% detection of FDp at $1x10^{-1}$. The repeatability of the evaluated methods ranges from 78 to 100% depending on the laboratory. The best average repeatability is obtained with Method3. The reproducibility also varied across the methods from 81 to 96%, with Method3 demonstrating the highest reproducibility.

The performance characteristics of the real-time PCR are most interesting for the identification of FDp. However, the primers and probe designed for the identification of Vectotype I (phytoplasmas of 16SrV group but not FDp) showed low specificity as they target the poorly variable *vmp*B gene. The *Vmp-RK-A1* simplex qPCR method could hence be carried out separately when confirmation of the presence of a Vectotype I variant is needed.

Less accurate results were obtained for the two nested-PCRs followed by sequencing analysis. As it can be expected for such methods, their reproducibility was also lower than that of the real-time PCR. These methods remain useful as they allowed the distinction between different *map* types and vectotypes (here only based on R1-*vmp*A repeated domain), and the specific identification of the other non-epidemic phytoplasmas that can be detected in grapevine. However, critical points have to be underlined and taken into consideration by users for their proper implementation: risk of micro-contaminations, choose of reliable sequencing service providers, experience and/or training in sequence analysis.

By sharing protocols, conducting test performance studies and aligning diagnostic procedures, laboratories involved in routine diagnosis can ensure the accuracy, reliability and reproducibility of diagnostic methods. And, by enhancing our ability to accurately identify FDp, we can significantly improve disease surveillance and sustainable control measures.

ACKNOWLEDGEMENTS

This work was carried out in the frame of EUPHRESCO project FLADO VIGILANT. The authors would like to thank Fiona Constable and Bianca Rodrigues Jardim (Australian Government) for providing of DNA extracts of some non-target phytoplasmas.

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A DNA-capture approach for detection and genome-wide sequencing of Flavescence dorée phytoplasma

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INTRODUCTION

Flavescence dorée phytoplasma (FDp) is a non-cultivable quarantine bacterial pest causing outbreaks in European vineyards where it is transmitted by the leafhopper *Scaphoideus titanus* (Tramontini et al., 2020). FDp can be detected by real-time PCR assays and FDp strains can be genetically characterized by Multilocus sequence typing (Arnaud et al., 2007). However, the genetic characterization of FDp is often limited due to the low amount of FDp DNA in grapevine nucleic acid. Recently, a DNA capture approach was described to enrich DNA of the citrus pathogen "*Candidatus* Liberibacter asiaticus" prior to Illumina sequencing (Cai et al. 2019). We describe the design of a SureSelect (Agilent) RNA probe system to capture FDp DNA. The FDp enrichment probe consisted of RNA probes covering all coding sequences (CDS) of the FD92 FDp genome (Carle et al., 2011). RNA probes were preferred to take advantage of the high strength of RNA/DNA hybridisation expected to compensate the lower capture efficiency due to the low GC % in the FDp genome (21.7%). This approach was applied to DNA extracts of field -collected or insect-inoculated FDp-infected grapevines. We present here the outcome in terms of enrichment and sequencing coverage for pure extracts and for serial dilutions mimicking decreasing rates of infection.

MATERIALS AND METHODS

The samples VISa and VISb were Cabernet-Sauvignon grapevine plants inoculated with FDp genotype M54 using infectious *S. titanus* under greenhouse-controlled conditions (Eveillard et al., 2016). The sample VIT was Cabernet-Sauvignon infected with M54 collected in vineyards in Faleyras (Gironde, France). Total nucleic acids were extracted from 1.5g of petioles according to standard procedure (Maixner et al., 1995), treated with 1 µg/µl RNAse A for 30 min at 37°C and purified on Promega Wizard® SV Gel and PCR Clean-Up System columns. DNA concentration was measured with Qubit-4 fluorimeter. The number of FDp genome copies per µl of DNA extract was measured by qPCR (Eveillard et al. 2016). Serial dilutions in healthy grapevine DNA were prepared. Probes consisted of (i) 560 CDS and (ii) 16S and 23S rRNAs of the genome of strain FD92 of genotype M54 and vmpA and B cluster II which predominates in European vineyards, (iii) *vmp*D and *vmp*E adhesin gene sequences specific to strain FD-CAM05 of genotype M50 and (iv) *vmp*A, *vmpB* and *imp* genes representative of the FDp strains diversity. A total of 48649 RNA probes long of 120 nucleotides covering 495524 bp with tiling coverage of 3X were synthetized by Agilent. Samples were processed according to "SureSelectXT HS target enrichment system for Illumina multiplexed sequencing platforms" Agilent manual version D0 (August 2020). Paired-end Illumina sequencing 2 x 150 bp was performed on a NextSeq 2000 at the Plateforme Génome Transcriptome de Bordeaux (PGTB). Sequences were trimmed to eliminate adaptor sequences. Sequences with length shorter than 50 bp or quality below phred20 were eliminated. Finally, sequences were mapped to the FD92 FDp genome using Bowtie 2 and mapping results analyzed with Samtool coverage under Galaxy.

RESULTS AND DISCUSSION

The selected samples were highly infected. According to qPCR, VIS and VIT grapevine DNA extract contained 0.754 % and 0,46 % of FDp DNA (Table 1). After SureSelect enrichment, 90.34 % and 84.69 % of the sequence reads corresponded to FDp for VIS and VIT pure extracts. The enrichment

was of 120 for VIS and of 184 for VIT. The enrichment was even higher with all the 1/16 serial dilutions of both VIS and VIT, indicating that the capture system was saturated in the case of pure extracts. The coverage of mapping to the FDp genome was higher than 72 % for pure extracts, the 1/16 dilutions of VIS and VIT and for the 1/256 dilution of VIS. It must be noted that the probes are covering 75 % of the 647 kbp of the FD92 FDp genome. Therefore, higher coverage indicated that intergenic sequences bordering the RNA probes were partly captured. For higher dilutions of VIS and VIT, the coverage was limited to 24.3 % and 45.3 % respectively. Depth of 329 and 224 however indicate that some parts of the genome were more efficiently captured. These results indicate the efficiency of the SureSelect RNA probe capture system to enrich grapevine DNA in FDp DNA and give sufficient data for a genome-wide genetic characterization of FDp strains. In order to reduce costs, the enrichment was also evaluated with capture probes diluted at 1/8 in comparison with pure probes, on serial dilutions of infected grapevine extracts (VITb) prepared as described above. For FDp DNA loads higher than 0,04 %, enrichment, coverage and depth showed high values for both conditions (Table 2).

Table 1: Statistics of Illumina sequencing after enrichment capture on FDp SureSelect probes.

Samples	Total reads (million)	Reads mapped (million)	Coverage (%, ≥30X)	Depth (X, ≥30X)	% of FDp reads after capture (A)	% of FDp DNA in initial extract (B)	Enrichment A/B
VISa pure	46,84	42,31	84.5	11162	90,34	0.758 (1)	119
VISa dil 16	25,04	11,05	82.2	3059	44,14	0.034 (2)	1284
VISa dil 256	22,61	1,40	72.2	436	6,21	0.0021 (2)	2891
VIS dil 4096	24,75	0,40	24,3	329	1,64	0.00013 (2)	12214
VIT pure	55,34	46,86	85.4	12468	84,69	0,466 (1)	182
VIT dil 16	21,44	3,88	78.0	1229	18,10	0.0073 (2)	2487
VIT dil 256	19,49	0,48	45.3	224	2,48	0.00046 (2)	5451

(1) Evaluated by qPCR, (2) Calculated from the dilution factor in healthy grapevine DNA

Table 2: Statistics of sequencing after enrichment capture on FDp SureSelect probes pure vs 1/8 diluted.

Samples	Total reads (million) pure/diluted	Coverage (%, ≥30X) pure/diluted	Depth (X, ≥30X) pure/diluted	% of FDp reads after capture (A) pure/diluted	% of FDp DNA in initial extract (B)	Enrichment A/B pure/diluted
VISb pure	78,07/33,4	82.9/81,4	18722/8545	89/92,8	0.51 (1)	174/182
VISb dil 16	15,3/22,5	83,2/84,6	4123/5981	42,1/67,62	0.041 (1)	1027/1649
VISb dil 256	31,5/19,5	72,5/45,3	656/224	6,9/2,5	0.0045 (1)	1537/551

(1) Evaluated by qPCR

ACKNOWLEDGEMENTS

We thank J-S. Bey, D. Lacaze and T. Lusseau for plants and insects. This study was supported by the Plan National Dépérissement du Vignoble grant Co-Act2 and the ANR grant BEYOND.

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Enhancing Control and Surveillance of Flavescence Dorée in Bordeaux and Champagne Vineyards using spatio-temporal models

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INTRODUCTION

Controlling highly infectious diseases like Flavescence Dorée (FD) in vineyards presents significant challenges in epidemiological and ecological modeling. Current strategies, relying on planting disease-free stocks, yearly symptom surveillance, infected plant removal, and insecticide application, struggle to eradicate or slow down disease spread. Mathematical models, including spatio-temporal stochastic models, are increasingly vital in designing control strategies as agencies seek stronger evidence for selected approaches (Parnell et al., 2017, Adrakey et al., 2017). We leverage these models to develop methodologies facilitating FD disease management, ensuring control in endemic areas and preparing for potential new incursions using spatio-temporal models within the Bayesian framework. Our approach focuses on understanding factors influencing spread and estimating FD transmission likelihood within plots and across broader landscapes. Initially, we characterized field and landscape risk factors impacting FD infection in Bordeaux vineyards (Adrakey et al., 2022). Then, we estimated the distance at which FD spreads at field level using a mechanistic SIR model (Adrakey et al., 2023) and finally we extend the knowledge gained from field level analysis to a much larger landscape using surveillance data from Trelou-sur-Marne area in Champagne region to underscore the risk of incursion and spread within a landscape with multiple fields.

MATERIALS AND METHODS

Spatial statistical model. Between 2012 and 2016, GDON des Bordeaux organization monitored and georeferenced grapevine fields in the Bordeaux vineyard (area of 347 districts and 84000 ha), with 10 % achieved each year. A geographic information system was designed by collating the characteristics of the 34581 fields inspected over 5 years: FD-infection status, date of survey, altitude, organic or conventional practice, area, age, density of plantation and cultivar, the later extracted from the Casier Viticole Informatisé (CVI) (Fig 1). Landscape characteristics obtained from the CVI and land cover map were expressed as percentages of urban, forest, vineyard areas, vineyards with Merlot or with organic practice in radii from 50 to 5000 m around each field. The effects of these field and landscape factors on the probability that a field is infected by FD were estimated using spatial generalized linear models fitted with INLA (Integrated Nested Laplace Approximation) method.

Spatially explicit mechanistic SIR model. In 3 adjacent fields situated near Bordeaux (France) and planted with 5961 stocks of Merlot and Cabernet-Sauvignon, FD symptomatic and removed plants were precisely mapped in 2018 and 2019. A larger area of 300 m radius around the target fields was also monitored from 2014 to 2018 by recording FD-infected fields and the number of infected plants per field, without their exact location. A spatial SIR (Susceptible, Infected, Removed) model with a discrete time step of one year was used to describe the probability of infection of each plant in the focal fields. The model considered a time varying rate of primary infection, differences in cultivar susceptibility for secondary infection and a dispersal kernel representing the statistical distribution of the infected hosts after inoculum dispersal from a focal plant source. Model inference relied on data augmentation with a Bayesian approach that accounts for the missing information related to plants removed before the initial survey in order to construct the full trajectory of the epidemic. Similar analysis was conducted in Champagne region with the main exceptions of taking into account the susceptibility of grapevines and at landscape scale represented by multiples fields on data collected between 2020-2022.

RESULTS AND DISCUSSION

Effects of field and landscape factors on FD infection. Our analysis emphasizes the importance of monitoring periods, with FD detection probability four times higher in September than in August. At the field scale, altitude and cultivar choice significantly affect FD infection. Fields with susceptible cultivars like Cabernet Sauvignon, Cabernet Franc, or Muscadelle exhibit twice the odds of FD infection compared to those with less susceptible Merlot. Field infection is also influenced by immediate surroundings within a radius of 150 to 200 m, corresponding to landscapes of 7 to 12 hectares. Increased forest and urban land proportions, along with susceptible cultivar compositions, elevate FD infection probabilities, highlighting landscape-scale impacts on FD epidemiology. Our model's predictive performance suggests its utility in targeting areas for valuable future surveys.

FD dispersal distance. At plot level in Bordeaux vineyards, inference indicates that heavy-tailed dispersal kernels, characterized by frequent long-distance dispersal events, best explain FD spread. I also show that On average, 50% (resp. 80%) of new infection occur within 10,5 m (resp. 22,2 m) of the source plant (Adrakey et al., 2023). These values are in agreement with estimates of the flying capacity of S. titanus using mark-capture techniques (Lessio et al. 2014). In Champagne vineyards, analysis indicates that Chardonnay proves to be 1.8 times more susceptible than Meunier, Pinot Noir, and Pinot Blanc. At landscape level, inference show that on average, 50%, 80%, and 95% of new infections occur within a radius of 33.6, 58.1, and 86.3 m from the source if the epidemic is triggered by a Chardonnay in a plot of the same grape variety. Simulation of removal scenarios suggests that despite complete symptomatic plant removal, the disease persists over years due to cryptic infections. Moreover, the choice of infection threshold (the level of epidemic tolerance or the probability of infection beyond which a vine is considered infected) significantly impacts management strategy efficacy, with higher thresholds increasing the likelihood of omitting infected plots and lower thresholds requiring more resources for management.

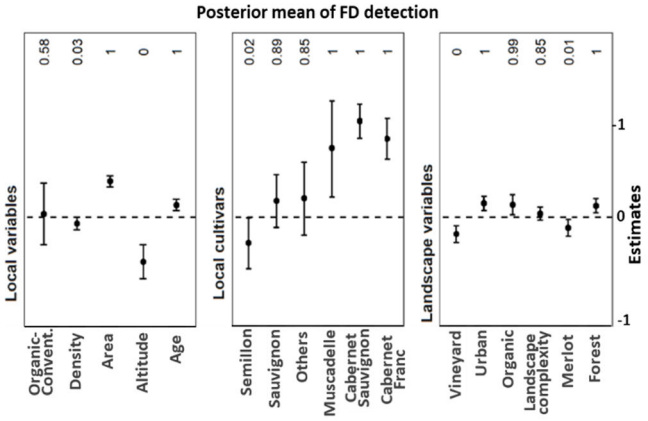

Fig 1: Effects of the local and landscape explanatory variables. For each variable, the posterior mean (dots) and 95 % credible intervals (solid lines) are displayed, together with the posterior probability of the effect being positive. The dashed lines correspond to the value 0. For estimating the local effect of cultivars, the poorly susceptible Merlot was chosen as the reference (value 0).

ACKNOWLEDGEMENTS

We thank S. Bentejac, M. Le Goff and C. Labit (GDON des Bordeaux), D. Vergnes (FREDON NA) for providing data. Thanks to G. Gibson (Heriot-Watt Univ.), A. Rush, J.S Ay, S. Dembele, L. Ramalanjaona, L. Riley and L. Michel (INRAE) for participating in the studies. The studies received financial support under research contracts Co-Act, Co-Act2 and RISCA (Plan National Dépérissement du Vignoble). We also thank the financial support of the CIVC, through INRAE.

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Pro-AECOGY – Breeding grapevine for resistance traits to phytoplasmas and insect vectors

Chairs: Critina Marzachi and Sandrine Eveillard

Unlocking Phytoplasma Strategies: A Path to Enhanced Crop Resistance and Biotechnological Breakthroughs

Saskia A. Hogenhout and past and present team members[1]

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Phytoplasmas, spread by insect vectors, often trigger profound developmental alterations in plants, such as witches' brooms, phyllody, neoteny, and extended lifespan (Zhang et al., 2004; Hogenhout et al., 2008; Sugio et al., 2011a; Tomkins et al., 2018; Huang et al., 2020; Huang et al., 2021). Our research uncovered that these pathogens secrete effectors that specifically target critical plant transcription factors (TFs) involved in developmental regulation (Bai et al., 2009; Sugio et al., 2011b; MacLean et al., 2011; Sugio et al., 2014; MacLean et al., 2014; Pecher et al., 2019; Huang et al., 2021; Liu et al, 2023; Correa Marrero et al., 2024). These effectors facilitate the degradation of TFs, frequently creating short circuits between plant pathways and bypassing the ubiquitination process, leading to disrupted plant growth and distinct developmental anomalies (Sugio et al., 2011b; MacLean et al., 2014; Huang et al., 2021; Liu et al, 2023).

Our research also uncovered that plants displaying symptoms are more appealing to insect vectors responsible for transmitting phytoplasmas across vast distances (Sugio et al, 2011b; MacLean et al. 2014; Orlovskis & Hogenhout, 2016; Al-Subhi et al., 2021; Huang & Hogenhout, 2022). While the effector-induced degradation of TFs significantly contributes to making these plants attractive to insects, we found that intriguingly the specific developmental symptoms of the plants are not always required for attracting insect vectors (Orlovskis & Hogenhout, 2016; Huang & Hogenhout, 2022).

The effectors are found in diverse phytoplasmas that colonize a broad range of plant species, including economically important crops (Sugio et al., 2012; MacLean et al., 2014; Music et al., 2019; Huang et al., 2021). The effector genes reside on pathogenicity islands that can form extrachromosomal units and have horizontally transferred between distantly related phytoplasmas (Bai et al., 2006; Hogenhout et al., 2008; Toruño et al., 2010; Sugio et al., 2012; Music et al., 2019). This underscores the significant fitness advantage that effector actions provide to phytoplasmas.

A key discovery in our study is the identification of susceptibility factors that are required for phytoplasma symptom induction (MacLean et al., 2014; Huang et al., 2021). Introducing specific amino acid modifications in susceptibility factor genes or deleting them markedly enhances resistance to phytoplasmas and their insect carriers (MacLean et al., 2014; Huang et al., 2021). Considering the broad conservation of susceptibility factors across plant species (Huang et al., 2021; Liu et al., 2023) and the advent of gene editing tools applicable to diverse crops, our research has uncovered a novel pathway for managing these pathogens in a range of agricultural systems.

Furthermore, the unique mechanisms of action uncovered in phytoplasma effectors point to potential new strategies for targeted protein degradation (Huang et al., 2021; Hogenhout et al., 2022; Liu et al., 2023). These strategies hold wide-ranging implications, offering not only enhancements in plant health and agricultural productivity but also paving the way for innovative biotechnological tools applicable to humans, animals, and other organisms (Hogenhout et al., 2022).

In summary, phytoplasmas have evolved sophisticated mechanisms to control their plant hosts and insect vectors. By identifying susceptibility factors, we have revealed a promising strategy for enhancing resistance against these economically significant insect-vectored pathogens across various crops. Furthermore, decoding how phytoplasma effectors interact with plant proteins has led to the

discovery of new cellular processes, opening avenues for innovative biotechnological applications extending beyond plant biology.

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The unique defense response of Tocai Friulano cultivar to Flavescence dorée

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INTRODUCTION

Flavescence dorée (FD) is a Grapevine Yellows disease (GY) associated to FD phytoplasma (FDp) and it is transmitted in vineyard by the leafhopper vector *Scaphoideus titanus*. Grapevine varieties completely resistant to FD have not been uncovered yet, however inter and intraspecific differences in susceptibility have already been observed, such as poor presence of the disease in vineyard, no severe symptoms and recovery ability of the plant. Among the *Vitis vinifera* varieties, Tocai friulano is one of most scarcely susceptible. Recently, Casarin et al. (2023) demonstrated that the low susceptibility of this cultivar originated from its ability to avoid the spread of FDp and FD symptoms to the whole canopy and to the permanent tissues of the attacked plants. Specific defense mechanisms were shown to occur in cane portions close to the symptomatic ones, where jasmonic- instead of salycilic- mediated signalling was up-regulated and active stilbenoids, such as viniferin, were accumulated. Analyses of the secondary phloem showed the disappearance of FDp in 2-year-old arms from July to November, which did not happen in Pinot gris, a highly susceptible variety.
In the current work, further studies were conducted to better define the phase in which FDp disappears from the infected arches of Tocai friulano plants during the vegetative season. Moreover, the investigations were extended to the trunk and arches of other grapevine cultivars with different susceptibility to FD, in order to understand if any of these implemented the same defense strategy as Tocai friulano.

MATERIALS AND METHODS

The most part of the study was carried out in the vineyard described in Casarin et al., 2023. Five symptomatic plants of Tocai friulano and five of Pinot gris were selected and the presence of FDp was verified through the analysis of the secondary phloem of 2-year-old arms and trunk collected at the beginning of July, August, September and November. In parallel, in 2021 and 2022 GY symptom observation was carried out also in a vineyard in Piedmont, Northwestern Italy, where four varieties with variable susceptibility to FD (Moscato bianco, Arneis, Sauvignon blanc, Chardonnay) were grown. Three plants for each cultivar were chosen for the sampling of the phloem from arches in August, September, and November. Moreover, during the dormancy, trunk longitudinal sections were collected from ten plants of Tocai friulano, Moscato bianco, Arneis, Sauvignon blanc and Chardonnay that showed symptoms of GY during the vegetative season for more than one year. FDp detection was carried out following protocols previously published (Casarin et al., 2023).

RESULTS AND DISCUSSION

The monthly sampling performed during the vegetative season revealed that the disappearance of the FDp from symptomatic arches in Tocai friulano occurred between the beginning of August and the first days of September (Figure 1). This period coincides with the summer dormancy, characterized by a color change of shoots from green to brown, concomitant to callose deposition in the plasmodesmata, lignin deposition and accumulation of starch reserves. It could be supposed that these events, leading to a certain block of connection with the shoot, could be related to the confinement of FDp in symptomatic shoots and its disappearance from the arches.

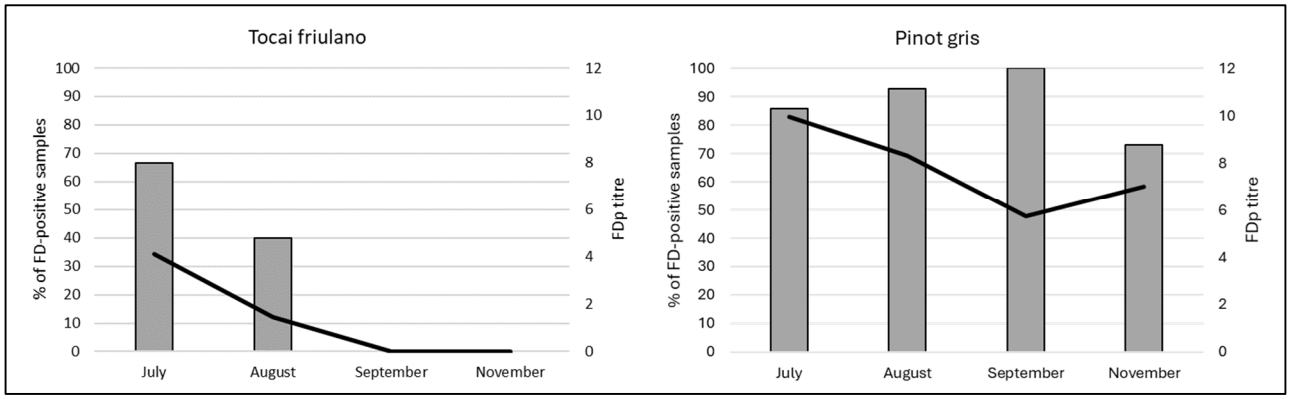

Figure 1: Percentage of FD-positive samples (grey bars) and average FDp titre (black line) detected in portions of 2-year-old symptomatic arches collected from Tocai friulano and Pinot gris plants in July, August, September and

The analyses were extended to other four cultivars and revealed that the ability to eliminate the FDp from infected arches, avoiding the spreading of the pathogen to the whole plant, was a unique feature owned by Tocai friulano. Indeed, all the other four varieties harbored a detectable FD titre in most of the samples collected from symptomatic 2-year-old arms and from the trunk (Figure 2 and 3).

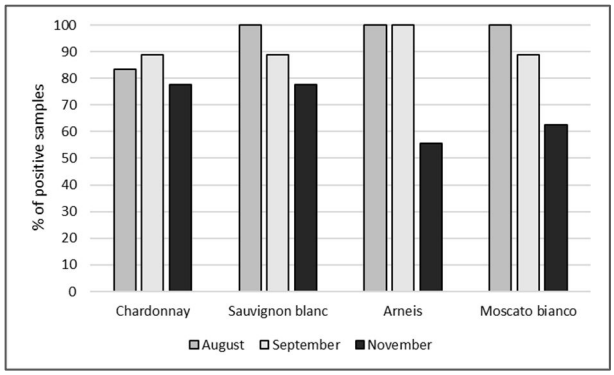

Figure 2: Percentage of FD-positive samples detected in 2-year-old arm portions collected from FD-symptomatic plants of four different cultivars in August, September, and November.

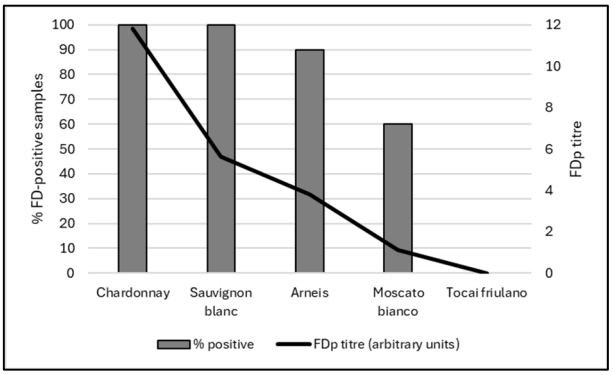

Figure 3: Percentage of FD-positive samples (grey bars) and FDp titre (black line) detected in samples collected from trunk of FD-infected plants of different cultivars during dormancy.

Interestingly, the number of positive samples and the FDp titre measured in woody organs appeared to be correlated with the degree of susceptibility to FD of the cultivars. Indeed, higher values were detected in the very susceptible Chardonnay, while lower values were obtained from samples collected from Moscato bianco, known for its partial resistance to FD (Ripamonti et al., 2021).
The results suggested that none of the observed varieties was able to confine the FDp in the one-year-old canes like Tocai friulano did, but highlighted interesting varietal features.

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Looking for plant targets of flavescence dorée phytoplasma effectors

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INTRODUCTION

Like other bacterial pathogens, phytoplasmas produce proteins termed 'effectors' that target specific host proteins and alter host-cell structure and function. Several phytoplasma effectors have already been well documented (Wang et al., 2024). However, they are not shared by all phytoplasmas, and currently only limited data are available regarding pathogenicity factors deployed by flavescence dorée phytoplasma. In a previous study, we predicted candidate effectors from the assembled genome of flavescence dorée phytoplasma, and set out to determine and compare their expression level and subcellular localization in plant and insect cells. A major objective was to provide assumptions for each effector about the targeted host, *i.e.* the plant or insect host (Garcion et al., 2019).

In this study, we carried on the functional analysis of these candidate effectors and looked for their putative targets in grapevine using the yeast two-hybrid system. In this technique, the candidate effector is fused to the GAL4 DNA-binding domain that binds to a specific DNA sequence, strategically engineered in the promoter of reporter genes. The putative interacting protein is fused to the GAL4 transcription activation domain. Both fusion proteins are expressed in a suitable strain of the yeast *Saccharomyces cerevisiae* (Fields & Song, 1989). Interaction of the candidate effector with its target leads to transcriptional activation of reporter genes and growth on defined media.

MATERIALS AND METHODS

The yeast two-hybrid library was created using the kit "Make your own 'Mate & Plate' library system" (Takara Bio). Briefly, RNA was extracted from *Vitis vinifera* cv. 'Cabernet Sauvignon' young healthy leaves using the Monarch total RNA isolation kit. Poly-adenylated mRNAs were isolated from total RNA using the Dynabeads™ mRNA purification kit (Invitrogen). cDNAs were produced using oligo-dT primers, amplified by PCR and simultaneously transformed in yeast strain Y187 and cloned in pGADT7-Rec vector by following the protocol indicated in Yeastmaker Yeast Transformation System 2 User Manual. Yeast strain Y2H Gold was used to perform the yeast two-hybrid screen and assays, according to the instructions of the Matchmaker Gold yeast two-hybrid system user manual (Clontech). Yeast media employed in this study were from Takara Bio.

RESULTS AND DISCUSSION

Five candidate effectors were selected for serving as baits in yeast two-hybrid screens. For three of them, previous subcellular localization studies showed an association with undetermined punctuate structures, or vesicle-like structures that could be linked to the secretion system in plant or insect cells (Garcion et al., 2019). Unfortunately, none of the two-hybrid screens performed for these three candidates allowed to disclose any putative target. Indeed, although the yeast two-hybrid system is a popular technique that is widely used for characterization of pathogens effectors, it may not always succeed in identifying interacting proteins, and several reasons could explain such a lack of results. For instance, the screened cDNA library may not contain the coding sequences of the targets, due to a low level of expression in our plant material. Another hypothesis is that the fusion of the candidate of interest with the GAL4 DNA binding domain, as required by the yeast two-hybrid system, prevents the interaction of the candidate effector with its targets. Another possibility would be that these three

candidates retain in yeast cells their capacity to be addressed to specific cell compartments, whereas the yeast two-hybrid system employed here requires that the "hybrid" fusion proteins interact inside the nucleus of yeast cells to activate reporter genes.

A fourth candidate effector showed sequence similarity to the candidate effector SAP68 of aster yellows phytoplasma strain witches' broom. Because SAP68 was demonstrated to interact with some Arabidopsis TEOSINTE BRANCHED 1-CYCLOIDEA-PROLIFERATING CELL FACTOR (TCP) transcription factors (Correa Marrero et al., 2024), we reasoned that our fourth effector might similarly interact with grapevine TCP transcription factors. However, when we performed the screen of the cDNA library, we obtained a large number of positive clones (> 5000). Sequence analyses of a set of random clones indicated that they corresponded to functionally unrelated genes, and that no gene appeared more frequently than others in the selected set. No TCP transcription factor was detected in the set of sequenced clones. From this experiment, it was concluded that our candidate effector likely behaves as a "sticky" protein in yeast cells, preventing the identification of a plausible target with confidence.

We obtained more satisfying results with a fifth effector. Previous characterization indicated that a green fluorescent protein (GFP) fusion to this effector was localized in the nucleolus of plant cells, suggesting that it recognized a nucleolus-specific component or mechanism (Garcion et al., 2019). A yeast two-hybrid screen of the grapevine cDNA library allowed to recover one clone coding for a putatively interacting gene product with no known function in grapevine. Related genes have been reported in *Arabidopsis thaliana*, but further experiments suggested that the function of these homologs may not be strictly identical between grapevine and Arabidopsis. Current experiments aim at confirming the interaction with other techniques than the yeast two-hybrid system (*i.e.* bimolecular fluorescence complementation or pull-down).

The knowledge of the targets of phytoplasma effectors will help to provide a fundamental understanding of how phytoplasmas manipulate the physiology of their host plants to their own benefit, especially in the case of a phytoplasma that has recently spilled-over from its original ecological niche. Furthermore, such data will contribute to the efforts of plant pathologists and breeders to improve crop varieties with altered effector targets and increased resistance (McLellan et al., 2022).

ACKNOWLEDGEMENTS

We thank Lucie Dutilh and Lina Fawaz for their help at the bench, and Denis Lacaze, Jean-Said Bey and Thierry Lusseau for assistance in growing plants. The authors acknowledge the support of the French National Research Agency (ANR) under the grant 20-PCPA-0010 (VITAE).

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Insights into physiological responses of different grapevine varieties to Flavescence Dorée infection: an integrated approach

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INTRODUCTION

Flavescence dorée (FD) is a severe and widespread quarantine disease that affects grapevines in various European viticultural regions (Boudon-Padieu *et al.*, 2002). It is caused by a phytoplasma (FDp), and grapevine varieties exhibit different levels of susceptibility to the disease. Cabernet Sauvignon (CS) is highly susceptible whilst Merlot (M) is less susceptible (Eveillard *et al.,* 2016). Previous studies have shown that phytoplasma infections lead to significant transcriptomic and metabolomic changes in grapevines (Bertazzon *et al.,* 2019; Dermastia, 2019; Margaria *et al.,* 2014; Prezelj *et al.,* 2016). The purpose of this study is to better decipher the differences of response to FDp infection of various grapevine cultivars, and identify biomarkers associated with reduced susceptibility. This was achieved under controlled conditions integrating transcriptomics, metabolic profiling and physiological studies.

MATERIALS AND METHODS

For all experiments, grapevine plants were inoculated with FDp strain FDPEY-05 (Papura *et al*., 2009) in a high-confinement greenhouse as described in Eveillard *et al.* (2016). FDp-infected or non-infected *Scaphoideus titanus* were placed for a one-week transmission on the fifth leaf from the apex. One week post-inoculation (wpi) and ten wpi, leaves were collected from each plant and immediately frozen in liquid nitrogen. Plant DNA extractions and phytoplasma absolute qPCR quantifications were performed as in Eveillard *et al*., 2016. For transcriptomic analysis, RNA extraction and reverse transcription were performed as in Dufour *et al.* (2016) followed by a high-throughput qPCR method (Fluidigm ©) with the pathways-targeted NeoViGen and Biostim chips (Bodin *et al*., 2020; Bodin *et al*., 2023; Dufour *et al*., 2016). For metabolic profiling, ethanolic extraction was performed on lyophilized samples. Soluble sugars, organic acids, free total amino acids, starch, proteins and cellular residues were quantified through enzymatic methods and via spectrophotometric/fluorescent assays. Physiological data, *i.e.* stomatal conductance and chlorophyll a fluorescence, have been acquired with the LI-COR 600 System in automatic mode. All statistical analyses were performed using R Studio (version 4.3.2.).

RESULTS AND DISCUSSION

No phytoplasma was detected in the leaves of the grapevines that were exposed to non-infected insects. Phytoplasma titers at 1 wpi were non statistically different among the grape varieties tested. However, titers were significantly higher in CS than in M at 10 wpi.

Transcriptomic analysis revealed specific gene expressions for each plant condition. Indeed, principal component analysis (PCA) separated infected and healthy plants on the first dimension and varieties on the second dimension. For example, infected-M showed overexpression of genes involved in jasmonic acid pathways, while healthy-M modulated the genes related to salicylic acid and ethylene pathways.

Results using the LI-COR 600 system also suggested different responses depending on the susceptibility of grapevine varieties. Indeed, in M and CS, chlorophyll fluorescence tended to increase

in M but decreased in CS in infected leaves. The stomatal conductance significantly decreased in infected leaves at 10 wpi, as compared to leaves exposed to non-infected insects.
Finally, preliminary enzymatic assays indicate that, at 1 wpi, carbohydrate levels increased in infected CS as compared to healthy ones, while they decreased in infected M.
Altogether, these results are in line with studies showing significant transcriptomic differences between healthy and phytoplasma-infected plants, as well as between highly susceptible and poorly susceptible varieties (Bertazzon *et al.,* 2019; Margaria *et al.,* 2014). Additionally, the reduction in chlorophyll fluorescence in infected cultivars, directly linked to photosystem II activity, is in agreement with findings indicating a decrease in chlorophyll content (Teixeira *et al.*, 2020).

ACKNOWLEDGEMENTS

We thank our colleagues T. Lusseau, D. Lacaze and T. Mauduit for production of insect and plant material. We thank also J. Masson and M. Perrin for supplying *in vitro* plantlets. This research was funded by the 'Conseil Interprofessionnel du Vin de Bordeaux', 'France Agrimer', the Nouvelle Aquitaine region, 'Plan National Dépérissement du Vignoble' and "Grand Programme de Recherche – Bordeaux Plant Science" of the University of Bordeaux.

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Exploring grapevine genetic resistance traits against Flavescence dorée

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INTRODUCTION

Grapevine genotypes show different susceptibility to Flavescence dorée (FD) in the field, but complete resistance has not been confirmed upon under controlled conditions. Nevertheless, several *Vitis* cultivars (cvs) have been ranked from susceptible to tolerant to FD infection. This natural tolerance needs to be further explored, extending investigations into the high genetic variability of grapevine, to highlight plant FD-resistance traits, in cvs never ranked before, as a starting point for future breeding programs.

Some *Vitis* accessions, including some rootstocks and *V. vinifera* subsp. *sylvestris*, are partially resistant/tolerant to FD. Among these, are Brachetto and Moscato bianco (Ripamonti et al., 2021), although both show a lower palatability for *Scaphoideus titanus* than that of the susceptible Barbera. In addition, the vector has reduced fitness when feeding on these tolerant cvs (Ripamonti et al., 2022).

MATERIALS AND METHODS

As grapevine challenge under controlled conditions is expensive and time consuming, a modified protocol was developed, based on mass inoculation of plants derived from *in vitro* culture exposed to *S. titanus* infected under experimental conditions. Two selection criteria were explored. *Vitis* varieties associated with the progenitor Moscato bianco were selected in an attempt to identify accessions that disjunctively exhibit pathogen resistance and vector repellency. The second criterion for choosing the cultivated varieties under analysis was the frequency of genome introgression of genetic elements from *V. vinifera sylvestris*. This information was available for many Piedmontese grape varieties, thanks to preliminary results (Schneider et al., unpublished). Vector feeding behavior on the selected varieties, explored by electropenetrography, was also described to disentangle pathogen resistance due to the plant response to phytoplasma rather than to repellency/antibiosis toward the vector.

RESULTS AND DISCUSSION

Our preliminary results revealed that FD tolerance is more easily detected in cultivars derived from tolerant parentals, but in the possibility that pathogen resistance may be due to the plant response to vector feeding must be explored case by case.

ACKNOWLEDGEMENTS

The authors wish to thank Flavio Veratti for his skilled technical help, Elena Zocca for her indispensable assistance in plant production and greenhouse management. The work was supported by the Regione Piemonte (Project MIDI).

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Effector proteins in '*Candidatus* Phytoplasma solani'

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INTRODUCTION

Effectors of the Sec pathway are believed to be important components for the interaction of phytoplasmas with their hosts. Only a limited number of phytoplasma effectors have been characterized in detail despite *in silico* analyses suggest several and diverse numbers. Within "*Candidatus* Phytoplasma solani" (CaPsol) , among distinct host cycles involving host-plants such as nettle, bindweeds, *Crepis*, lavender, *Vitex*, distinguishable CaPsol types have been confirmed for nettle and bindweed. This differentiation is mirrored in marker genes like *tuf*, *sec*Y, *stamp* and *vmp*. Here, we show the differential portfolio of effectors in CaPsol and discuss their potential.

MATERIALS AND METHODS

Grapevine and *Catharanthus roseus* leaf vein material from infected and healthy plants was collected from greenhouse and vineyard plants in Tulln and Vienna. *Hyalesthes obsoletus* was collected in Eastern Austria and used for infection of *C. roseus* kept in greenhouses. RNA was extracted, depleted for ribosomal RNA and enriched for bacterial sequences. DNA data have been collected previously. In addition, DNA from infected *C. roseus* was subjected to nanopore sequencing.

RESULTS AND DISCUSSION

Phenotypically, infection with different CaPsol strains tend to show differentiation of symptoms in *C. roseus*, from absence of virescence to extreme greenish flowers, differentiation in flower size and different degrees of decay. Partly, the differences are certainly due to severeness of infection – hence CaPsol titer should correlate with severeness of symptoms. However, this is not necessarily the case and both expression of effectors as well as titers indicate a role of specific effectors for the development of disease.

Six potential effector proteins (PoStoSP04, PoStoSP06, PoStoSP13, PoStoSP14, PoStoSP18, and PoStoSP28) are widely distributed in different CaPsol accessions and have been functionally analyzed in more detail by Strah et al., 2024. However, beyond that, other expression factors are of limited distribution in CaPsol, or, show higher variation in primary amino acid sequence, indicating a role for specific host interaction and symptoms expression. Here, an inventory of candidate effectors for these differentiations will be presented, and their genomic context will be discussed. Certainly, the functions go beyond the important findings of Dermastia et al., 2021, 2023, and indicate roles in addition to the carbohydrate metabolism and reactive oxygen species response during phytoplasma infection. How far these effector proteins also target a number of processes in the host plants or are of specific function in the host vector, requires further research.

ACKNOWLEDGEMENTS

This work was supported by the Slovenian Research Agency (ARRS) grants J4-2544 and J1-7151, young researcher fellowship to Špela Tomaž, fellowship of The Jožef Stefan International Postgraduate School to RS,

Austrian Science Fund (FWF) grants I-2763- B29and I-5042B, and the National Sustainability Programme I (NPU I) of the Ministry of Education, Youth and Sports of Czech Republic, grant LO1415.

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6th Bois noir workshop
PRO-AECOGY 2024

Towards the Prophylactic and Agroecological Control of grapevine yellows