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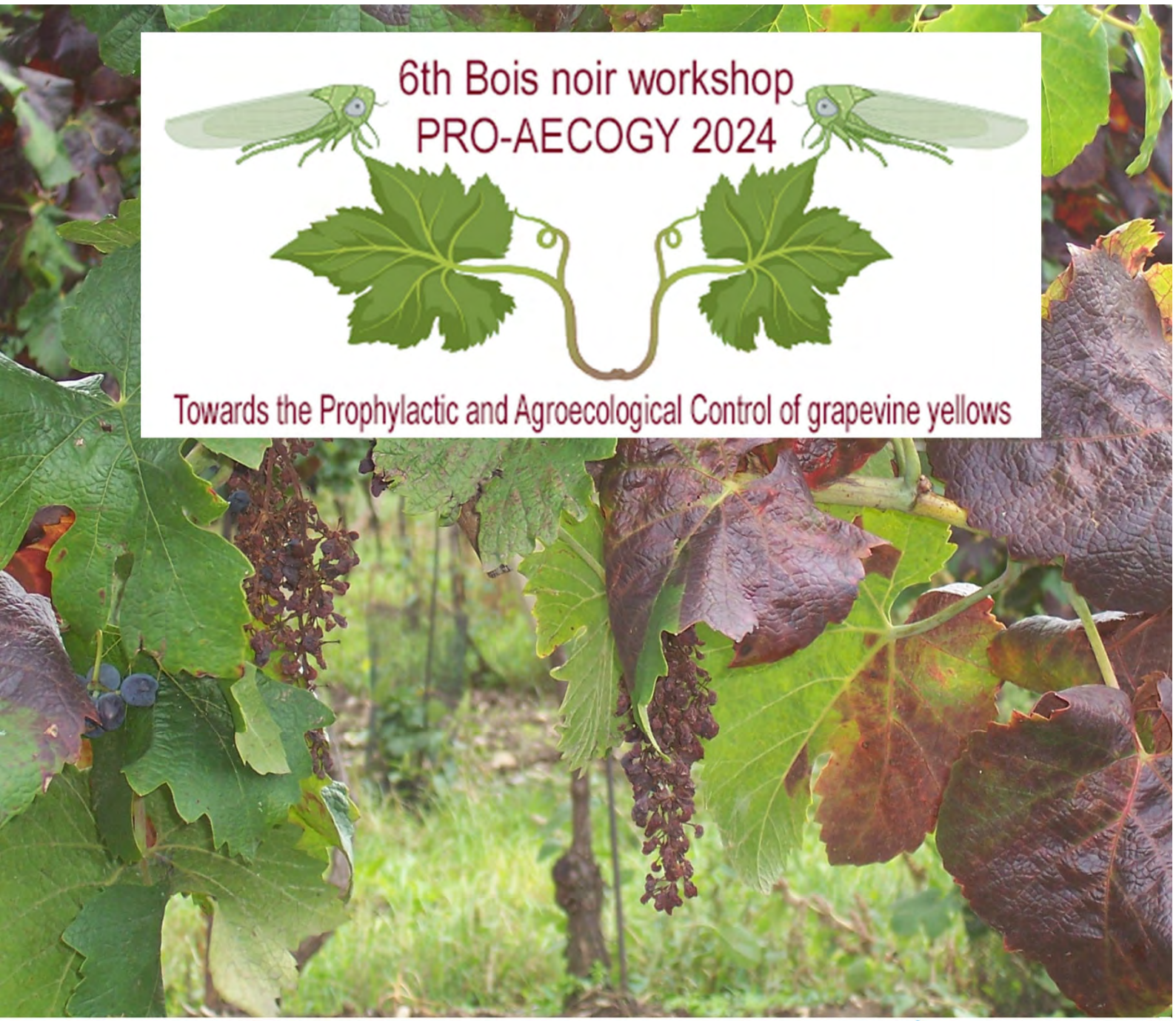

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Towards the Prophylactic and Agroecological Control of grapevine yellows

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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.

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REFERENCES

Aryan, A., Brader, G., Mörtel, J., Pastar, M., & Riedle-Bauer, M. (2014). An abundant '*Candidatus* Phytoplasma solani' tuf b strain is associated with grapevine, stinging nettle and *Hyalesthes obsoletus*. *European Journal of Plant Pathology*, *140*, 213-227.

Atanasova, B., Jakovljević, M., Spasov, D., Jović, J., Mitrović, M., Toševski, I., & Cvrković, T. (2015). The molecular epidemiology of bois noir grapevine yellows caused by '*Candidatus* Phytoplasma solani' in the Republic of Macedonia. *European Journal of Plant Pathology*, *142*, 759-770.

Cargnus, E., Pavan, F., Mori, N., & Martini, M. (2012). Identification and phenology of *Hyalesthes obsoletus* (Hemiptera: Auchenorrhyncha: Cixiidae) nymphal instars. *Bulletin of Entomological Research*, *102*(5), 504-514.

Hren, M., Boben, J., Rotter, A., Kralj, P., Gruden, K., & Ravnikar, M. (2007). Real-time PCR detection systems for Flavescence dorée and Bois noir phytoplasmas in grapevine: comparison with conventional PCR detection and application in diagnostics. *Plant pathology*, *56*(5), 785-796.

Imo, M., Maixner, M., & Johannesen, J. (2013). Sympatric diversification vs. immigration: deciphering host-plant specialization in a polyphagous insect, the stolbur phytoplasma vector *Hyalesthes obsoletus* (Cixiidae). *Molecular Ecology*, *22*(8), 2188-2203.

Johannesen, J., Foissac, X., Kehrli, P., & Maixner, M. (2012). Impact of vector dispersal and host-plant fidelity on the dissemination of an emerging plant pathogen. *PloS One*, *7*(12), e51809.

Jović, J., & Toševski, I. (2023). Multiplex PCR for Discriminating Host Plant Associations of *Hyalesthes obsoletus* (Hemiptera: Cixiidae), a Key Vector and Driver of '*Ca*. Phytoplasma solani' Epidemiology. *Agronomy*, *13*(3), 831.

Kosovac, A., Radonjić, S., Hrnčić, S., Krstić, O., Toševski, I., & Jović, J. (2016). Molecular tracing of the transmission routes of bois noir in Mediterranean vineyards of Montenegro and experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and associated *Hyalesthes obsoletus* (Cixiidae). *Plant Pathology*, *65*(2), 285-298.

Kosovac, A., Johannesen, J., Krstić, O., Mitrović, M., Cvrković, T., Toševski, I., & Jović, J. (2018). Widespread plant specialization in the polyphagous planthopper *Hyalesthes obsoletus* (Cixiidae), a major vector of stolbur phytoplasma: Evidence of cryptic speciation. *Plos One*, *13*(5), e0196969.

Kosovac, A., Jakovljević, M., Krstić, O., Cvrković, T., Mitrović, M., Toševski, I., & Jović, J. (2019). Role of plant-specialized *Hyalesthes obsoletus* associated with *Convolvulus arvensis* and *Crepis foetida* in the transmission of '*Candidatus* Phytoplasma solani'-inflicted bois noir disease of grapevine in Serbia. *European Journal of Plant Pathology*, *153*, 183-195.

Langer, M., & Maixner, M. (2004). Molecular characterisation of grapevine yellows associated phytoplasmas of the stolbur-group based on RFLP-analysis of non-ribosomal DNA. *Vitis*, *43*(4), 191-199.

Maixner, M. (1994). Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, *33*(2), 103-104.

Maixner, M., Albert, A., & Johannesen, J. (2014). Survival relative to new and ancestral host plants, phytoplasma infection, and genetic constitution in host races of a polyphagous insect disease vector. *Ecology and Evolution*, *4*(15), 3082-3092.