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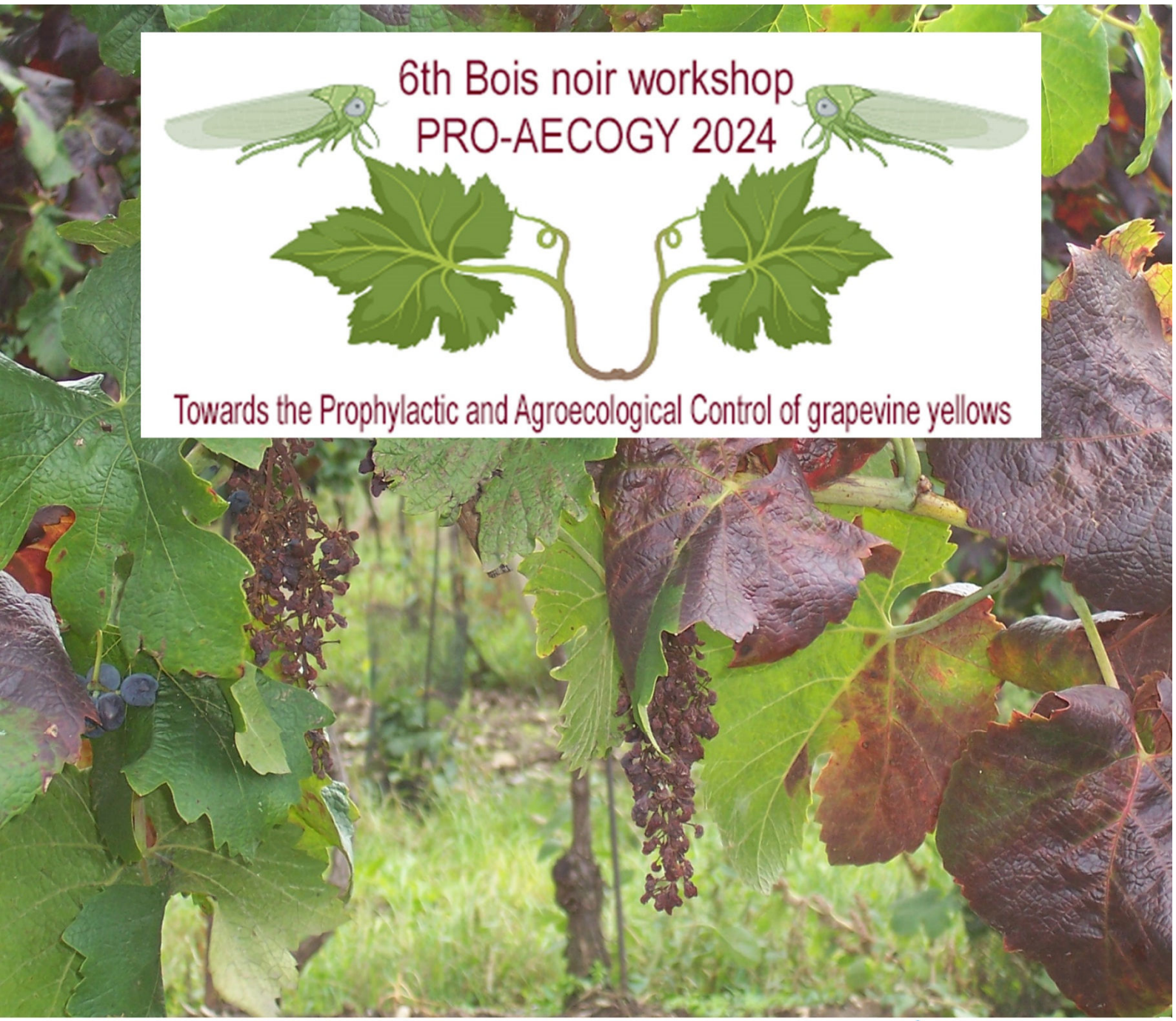

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Towards the Prophylactic and Agroecological Control of grapevine yellows

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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.

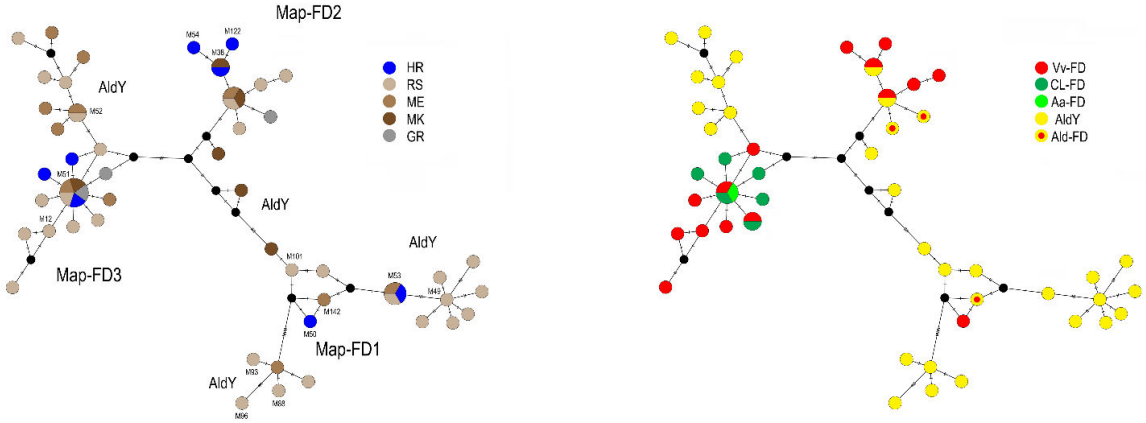

Figure 1. Median-joining networks inferred from a total of 48 *map* genotypes of FDp and related strains from Croatia and the Balkans (left), identified in grapevine and/or natural reservoir plants (right). Legends present countries of isolates carrying designates genotypes (2 letter code), and host 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).

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