

High infection rate of Flavescence dorée phytoplasmas in *Orientus ishidae* (Mats.) and *Alnus glutinosa* L. in Valtellina

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This work, carried out in the summer of 2020, focused on investigating the Flavescence dorée (FD) phytoplasma diffusion in *Scaphoideus titanus* (epidemic vector), *Orientus ishidae* (secondary vector), and *Alnus glutinosa* (plant host) in viticultural (Fracia vineyard, Valgella sub-area) and forest (Castello dell'Acqua and Chiuro) areas of Media Valtellina (Sondrio, North Italy). The conducted activities were: (i) starting from June, surveys on grapevine yellows (GY) symptoms and monitoring of *S. titanus* and *O. ishidae* by chromatic traps in the Fracia vineyard, Valgella sub-area; (ii) starting from mid-July, monitoring of *S. titanus* and *O. ishidae* adults by chromatic traps, and collection of 39 leaf samples from 13 *A. glutinosa* plants in forest areas (municipalities of Castello dell'Acqua and Chiuro); (iii) total nucleic acids extraction from collected plant samples and insects; (iv) 16SrV phytoplasma identification by nested PCR-based amplification of *map* gene; (v) phytoplasma typing and phylogeny by sequencing and bioinformatics analyses of *map* gene amplicons. In Fracia vineyard, GY symptoms were observed only in two grapevines, one of which was found infected by FD phytoplasma (based on analysis by Lombardy Region Phytosanitary Service). *S. titanus* was captured only within the vineyard (7 larvae, 6 adults), while *O. ishidae* adults were found both in the vineyard (28) and, prevalently, in the forest areas (45 adults). PCRs identified FD phytoplasma in 31.1% (14/45) of *O. ishidae* adults from forest areas and 100% of the *A. glutinosa* samples, while all the *S. titanus* and *O. ishidae* specimens, captured in the vineyard, tested negative. Amplicons of the *map* gene, obtained from 14 *O. ishidae* specimens and 33 alder samples (from 13 plants), were sequenced. The nucleotide sequences were compared by alignment with the database (<https://doi.org/10.1371/journal.ppat.1007967>). FD phytoplasmas, clusters FD1 and FD2, were found in 8 *A. glutinosa* plants (strains M50, M58 and M121) and in 13 *O. ishidae* specimens (strains M38 and M50). Phytoplasmas belonging to the Alder yellows (AldY) (strains M51, M78, M106, M117) and Palatinate grapevine yellows (PGY) (strain M48) clusters were found in the remaining samples. This investigation indicated that *A. glutinosa* is an important source of 16SrV phytoplasmas but has low relative abundance in the territory and is distributed at a significant distance from vineyards. The results also indicated that *S. titanus* has a sporadic presence in the examined area and given the absence of positive specimens, suggested that its role in the epidemiology of FD is negligible. On the contrary, *O. ishidae* is widespread in the territory (including vineyards) and frequently tested positive for FD phytoplasmas. However, the distance of the alders from the wine-growing areas made the probability of transmission of FD phytoplasma to grapevines extremely low. In conclusion, this study evidenced that important sources of 16SrV phytoplasmas (alders) as well as secondary non-epidemic vectors (*O. ishidae*) are present in Media Valtellina. In the monitored vineyard, the incidence of FD is very low, and this may be due to the lack of pathogen plant hosts in the vicinity of the crops. In wine-growing areas located near woods with the presence of alders, the *A. glutinosa* - *O. ishidae* system can play an important role in maintaining a reduced but constant presence of new FD phytoplasma infections in the vineyards.