First report of "flavescence dorée" phytoplasma identification and characterization in the leafhopper species *Graphocephala fennahi, Japananus hyalinus, Hishimonus hamatus*

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During a survey conducted in Canton Ticino (Southern Switzerland) and focused on edges and woods surrounding vineyards infected with "flavescence dorée" (FD) 5,137 leafhoppers and planthoppers were captured, by means of yellow sticky traps exposed from June 22nd to October 21st, 2017. In particular, ten traps were placed in a plot in Stabio, twelve in a plot in Bedano and eight in a vineyard in Rovio and changed every week. Among the captured insects, 53 were *Graphocephala fennahi*, 38 *Japananus hyalinus*, and 17 *Hishimonus hamatus*. *G. fennahi* specimens were caught during all the vegetative season, while the captures of *J. hyalinus* were limited from the end of June until the end of August, and those of *H. hamatus* between the second week of July and late October. The specimens of the three species were detached from the traps for DNA extraction and subsequent molecular analyses for the identification of 16SrV group phytoplasmas. After a first screening conducted with a group specific real time PCR, the positive samples were amplified by nested PCR both on the ribosomal gene region and on the *secY-map* genetic *locus*. RFLP analysis was performed on the ribosomal gene amplicons, whereas double-stranded sequencing was conducted for the purified *secY-map* PCR products. The molecular analyses evidenced the presence of phytoplasmas in three samples: one specimen of *H. hamatus* out of eight analysed samples; one pooled sample of *J. hyalinus*, grouping five insects, out of ten; one pooled sample of *G. fennahi*, grouping three insects, out of 14. The RFLP analyses showed that all the phytoplasmas present in the three samples belonged to the 16SrV-C ribosomal subgroup. The *secY-map* sequencing revealed that *H. hamatus* was infected with the M12 genotype and *J. hyalinus* with the M50 one. On the opposite, at least two different phytoplasma genotypes were present in the positive pooled sample of *G. fennahi*, as various double peaks could be observed in its chromatograms. Phytoplasmas with both M12 and M50 *secY-map* genotypes can be transmitted by the vector of FD *Scaphoideus titanus* and they are probably involved in FD outbreaks in some European regions. So far M12 had been found in grapevines from North-Western Italy; M50 had been detected in grapevines and in alders among plants, and in *S. titanus, Orientus ishidae* and *Oncopsis alni* among insects, in Italy, France, Hungary and Germany. In this study, the entomofauna present on the vine canopy was not addressed, however *H. hamatus* was already sporadically identified in Switzerland on the vineyard floor vegetation and on the vine canopy. Moreover, specimens of all the species caught were observed on plants facing towards the vineyards. In conclusion, the finding and characterization of FD phytoplasma genotypes in the three insect species examined suggest that these leafhoppers could play a role in the epidemiological cycle of FD, if, in the future, their ability to acquire and transmit the phytoplasma to other plants and in particular to grapevine is demonstrated. The low density of captures and positivity suggests that this role could be marginal.