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Quarantine

New insights into the emergence of the grapevine "flavescence dorée" epidemics in Europe

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Abstract

A survey of genetic diversity of "flavescence dorée" (FD)-related phytoplasmas in grapevines, alders and clematis as well as alder-feeding leafhoppers was conducted in France, Hungary, Germany, Italy and Serbia. Genotyping was based on the housekeeping gene *map* and on the *vmp* genes encoding surface variable membrane proteins. Transmission assays of the phytoplasmas were performed with alder and/or grapevine-feeding leafhoppers. The study demonstrated that European alders constitute an original reservoir of FD phytoplasma by hosting a high diversity of FD-related phytoplasma genotypes, also present in non viticultural areas. The alder phytoplasmas grouping in Vmp-I cluster were transmitted by the Macropsinae *O. alni*, but were not compatible with the FD phytoplasma vector on grapevine the Deltocephalinae *Scaphoideus titanus*. The alder phytoplasmas in Vmp-II and -III clusters were transmitted by the Deltocephalinae *Allygus* spp. and *Orientus ishidae*. Such pre-existing phytoplasmas were compatible with *S. titanus* transmissibility and can be responsible for the emergence of FD phytoplasma epidemics in grapevine. VmpA proteins of cluster II better adhered to *Euscelidius variegatus* and *S. titanus* insect cells and midguts than those of cluster I. Such adhesins might play a key role in the adaptation to new vectors.

Keywords: 16SrV-C and -D phytoplasmas, genetic diversity, vectorial competence, Vmp adhesins

Introduction

"Flavescence dorée" (FD) epidemics had been associated to the introduction of the leafhopper vector *Scaphoideus titanus*, when Europe imported American *Vitis* rootstocks. However, the geographical and ecological origin of this phytoplasma remained unclear despite evidences for a plant host-range not restricted to grapevine. FD-related phytoplasmas were described in *Clematis* sp. and *Alnus* sp. in the vicinity of vineyards and it was demonstrated that autochthonous Auchenorryncha feeding on these plants were able to occasionally transmit these phytoplasmas to grapevine (Maixner *et al*., 2000; Filippin *et al*., 2009). More recently, it was evidenced that the introduced leafhopper *Orientus ishidae* was able to transmit FD phytoplasma to grapevine (Lessio *et al*., 2016), but the source plants for acquisition remained to be elucidated. This study brings new insights into the ecological cycle of 16SrV-C phytoplasmas between the vineyards and their environment and into the emergence of the grapevine FD phytoplasma epidemics in Europe.

Materials and Methods

Plant and insect samples were collected in Hungary, France, Germany, Italy and Serbia in the surroundings of FD-phytoplasma infected and FD-phytoplasma free vineyards and in non-viticultural areas. Plants were *Clematis vitalba* and *Vitis vinifera* exhibiting yellows and *Alnus glutinosa* without typical symptoms. Leafhoppers *S. titanus* were collected on infected grapevine stocks and various Cicadellidae were collected on alder trees. For genetic characterization, the *map*

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gene was amplified and sequenced as described in Arnaud *et al.* (2007). Primers used for the amplification and sequencing of *vmpA* and *vmpB* genes (Arricau-Bouvery *et al.*, 2018) were defined from the sequences of 16SrV selected strains. Phylogenetic reconstructions using maximum parsimony were performed by MEGA7. For the transmission experiments, alder leafhoppers were sorted by species and placed on *Vicia faba* or *A. glutinosa* shoots until death. Plants were regularly tested for symptoms and phytoplasma presence. Infected *V. faba* plants obtained after transmission were then incubated with *S. titanus* and *E. variegatus* larvae for phytoplasma acquisition followed by transmission to new broad bean plants. Adhesion assays were performed on cells of *E. variegatus* in culture and on midguts of *E. variegatus* and *S. titanus* as described (Arricau-Bouvery *et al.*, 2018). Retained fluorescent latex beads coated with different amounts of VmpA protein of FD92 and PGYA (Palatinate grapevine yellows) phytoplasma strains were counted for each experiment.

Results

The *map* sequence was obtained for 736 samples with 132 genotypes identified. Grapevines and *S. titanus* individuals from FD phytoplasma outbreaks hosted 11 genotypes which belonged to the clusters map-FD1, -FD2 and -FD3 (Arnaud *et al.*, 2007). *Clematis* was infected by 3 genotypes related to map-FD1 and -FD3. In contrast, 128 genotypes were detected in the alder trees and leafhoppers. Alders were infected at 86%, half of them with a mixture of genotypes. Interestingly, 8% of the phytoplasmas were identical with grapevine FD phytoplasma genotypes and could be detected in FD phytoplasma-free areas. Among the Cicadellidae tested, only three species resulted infected with 16SrV phytoplasmas. The Macropsinae *O. alni* was infected at 21% and transmitted 14 times to *V. faba* and *A. glutinosa*. These genotypes could not be subsequently transmitted by the Deltocephalinae *E. variegatus* and *S. titanus* when tested. The Deltocephalinae *Allygus mixtus/modestus* and *O. ishidae* were infected at 60% and 52% by FD phytoplasma genotypes, mainly M38 and M38/M50 respectively, which they transmitted to *V. faba* and *A. glutinosa*. The genotype M38 (map-FD2) was subsequently transmitted by *S. titanus* and *E. variegatus*; the M50 (map-FD1) was compatible with both. *VmpA* and *vmpB* genes had a variable number of 234 nt repeats with high sequence variability among phytoplasmas. The topology of phylogenetic trees was similar among *vmp*s but strongly differed for *map* gene by discriminating 3 clusters: Vmp-I grouped all the alder and PGY phytoplasmas transmitted by the Macropsinae, while Vmp-II and -III grouped the map-FD phytoplasmas from grapevine and alder transmitted by the Deltocephalinae. Interestingly, repeated domains evolved independently in cluster -I, whereas they evolved by duplications in clusters -II and -III. Latex beads coated with higher ratio of VmpA-II showed enhanced adhesion to the epithelial cells of *E. variegatus* and were better retained in both *E. variegatus* and *S. titanus* midguts.

Discussion

This study demonstrate that FD phytoplasma is endemic in European alders. Its emergence as an epidemic pathogen for grapevine is restricted to some genetic variants pre-existing in alder. MLSA studies suggested multiple emergences from wild environment in Europe (Krstic *et al.*, 2018; Plavec *et al.*, 2019). The compatibility of the phytoplasma to *S. titanus* resulted from the adaptation of Vmps to other Deltocephalinae living on perennial wild plants. It was demonstrated that VmpA acts as an adhesin with cells of the vectors (Arricau-Bouvery *et al.*, 2018). Its organization, similar to adhesion-related proteins, allows the fast duplication of pre-adapted repeated domains. This suggests a key role of Vmps in the life-style of woody host phytoplasmas that rely on the adaptation to new insect vectors to expand their plant-host range. The presence of the polyphagous Deltocephalinae *O. ishidae* could expand the ecological cycle of FD-related phytoplasmas.

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