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LATEST NEWS

Flavescence dorée: the today's most significant threat to grapevine in Europe

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Flavescence dorée (FD) is currently the most damaging disease affecting grapevine (*Vitis vinifera* L.) in Europe, and the most challenging and economically impacting problem for wine industry as well as vine-nursery sector. It is urgent to develop and apply sustainable control methods against FD, based on sound scientific evidence.

Flavescence dorée (FD) is a destructive disease affecting grapevine (*Vitis vinifera* L.), whose etiological agent is a phytoplasma (Flavescence dorée phytoplasma, FDp), a small and wall-less Gram-positive bacterium belonging to the class Mollicutes. At an early stage, first FD symptoms generally include a delay or a lack of budburst, while more typical FD symptoms are going to appear later, generally one year after infection. The main symptoms that can be observed on FD infected grapevines during spring and summer are a reduced growth of the fruiting cane, an abnormal lignification of the new shoots, accompanied by leaf blade curling downwards, reddish or yellowish leaf discolouration in red and white grapevine cultivars, respectively, to end with premature leaf fall, and desiccation of inflorescences, as well as of grapes. These symptoms progressively spread in the canopy of infected plants during the vegetative season, with a reduction in chlorophyll content, as well as in the yield of grapes and of their quality, and this drastic decline can eventually result in plant death. Each of these symptoms can be more or less evident, depending on the grapevine cultivar affected. Sometimes these symptoms can be associated by mistake to other diseases, mainly to an abiotic stress, when observed separately. However, FD diagnosis can be quite certain when leaf discolouration and curling, absence of shoots lignification, and desiccation of bunches occur at the same time. Grapevine rootstocks are generally asymptomatic, even if FDp infected.

Together with "bois noir" (BN), FDp is the main phytoplasma infecting grapevines in Europe, and both BN and FD belong the so called "Grapevine yellows" (GY), according to most common characteristic symptoms they induce on infected plants. The GY phytoplasmas are taxonomically

distinct, with FDp belonging the phylogenetic 16SrV-C and -D subgroups, while BN is included into the 16SrXII-A subgroup, now attributed to the species '*Candidatus* Phytoplasma solani'.
More importantly, FD distribution is restricted to Europe, where it is currently present in several countries, although to a different extent. such as France, Italy Spain Portugal, Switzerland, Austria, Hungary, Romania, Slovenia, Croatia, Montenegro, Serbia, Slovakia, Czech Republic, while FD is now in absent in Germany, where it has been eradicated. Conversely, BN is more widespread in Europe than FD, as well as present also in other continents. Nevertheless, accordingly to its highly epidemic behaviour, just FDp is included among the quarantine pests relevant to the European Union (Reg. EU 2016/2031, Reg. EU 2017/625, Impl. Regulation (EU) 2019/2072). Despite its quarantine status, and the mandatory and constant application of several control measures, a resurgence of FD has dramatically occurred since 2018 in several European countries and areas,
including northern and central Italy. The first FD outbreak was detected in Europe at the end of the 1950s, in France. Then, the disease spread to most of the European grapevine growing countries. The recent resurgence of FD urgently calls for digging deeper into FD epidemiology and physiopathology, also by using the most advanced molecular approaches, to overcome the limits intrinsic in this complex system. For instance, FDp is an obligate plant pathogen that is not culturable *in vitro* on synthetic and cell-free media, and thus not easily amenable to molecular biology and genetic engineering studies.
Such as all phytoplasmas, and besides to be wall-less, FDp has several other characteristic features that are essential for its pathogenic lifestyle and epidemiology. FDp is an obligate intracellular plant pathogen, requiring living hosts as mandatory to successfully carry out its life cycle, and which are represented in natural conditions both by plants and insects. In infected plants, FDp is restricted to phloem sieve tubes. Therefore, in addition to grafting, FDp transmission from infected to healthy plants is mediated by phloem sap-sucking highly specific insect vectors.
FDp, as any other phytoplasma, needs to perform some phases of its life cycle into its insect vectors to be then infective on plants, in a persistent propagative and circulative manner. Briefly, when into its insect vectors, FDp is carried by the haemolymph and invade all organs and tissues. When into salivary gland cells, where it arrives about one month later its acquisition, FDp can be then injected into plant phloem sieve tubes through infected saliva and for all the remaining life of the infected vector. *Scaphoideus titanus* Ball is an ampelophagous Nearctic leafhopper, which is the main vector for the FDp transmission from grapevine plants. Moreover, its cycle is strictly dependent on grapevine, with a generation per year, although it can be sometimes found associated to other plants (*e.g. Salix viminalis* and *Prunus persica*). *S. titanus* is reported to have been introduced in Europe in the 1950s, together with grapevine plants from the North America, but more recent studies demonstrated *S. titanus* to have been already present since the 1920s.
Actually, FDp has got several other natural host plants, which are European native, such as *Alnus glutinosa* and *A. incana*, as well as *Clematis vitalba*, *Ailanthus altissima*, and some wild species belonging to the genus *Vitis*. Similarly, other insect species can be FDp vectors, such as the polyphagous planthopper *Dictyophara europaea* and the leafhopper *Oncopsis alni*, both able to transmit FDp from these alternative hosts to grapevine plants. It is now widely accepted the hypothesis considering grapevine as an accidental host for FDp, whose main and original hosts were represented from those plants that now are wrongly considered reservoirs.
On top of that complex 3-elements evolutionary relationship, FDp strains have been widely characterised by molecular methods, and its is known since long ago that strains from the FDp-D and FD-C subgroups have a different distribution. More recently, sequence and single nucleotide polymorphism analysis carried out on several FDp genes, such as *secY*, *uvrB-degV*, and *map*, gave a more detailed picture of the molecular evolution of this plant pathogen, consisting in three distinct

FDp cluster (*i.e.* FDp1, FDp2, FDp3). Furthemore, multilocus sequence typing (MLST) has been recently able to differentiate closely related strains into each cluster, and this molecular typing is in accordance with their insect vectors and host plant species.

The recent resurgence of FD, despite the control measures in place since years ago as mandatory, can be related also to the emergence of new FDp lineages, as found to occur in Tuscany, and more generally in central Italy. The starting point has been the demonstration of an event, such as the coinfection of grapevine plants by different FDp strains, to naturally occur in vineyards. FDp multistrains infections have been shown to be not so uncommon, and demonstrated to drive genetic recombination events of FDp homologues genes to generating new FDp lineages.

The role of several FDp membrane proteins (called "variable membrane proteins", Vmps) was demonstrated by expressing Vmps into the *in vitro* culturable *Spiroplasma citri*, closely related to FDp, and the leafhopper *Euscelidius variegatus*, already adopted as vector for the FDp experimental transmission. The FDp membrane protein VmpA, expressed by this phytoplasma during the colonization of insect midgut and salivary glands, was demonstrated essential for FDp adhesion to insect cells. Moreover, the variability found in *vmpA* and *vmpB* genes appears to be correlated with ecological adaptation of different FDp strains and lineages to the transmission by different insect vectors.

In conclusion, nowadays no genetically resistant grapevine varieties and cultivars have been found so far, although different cultivar susceptibility/tolerance levels are known, as well as the differential ability of some grapevine varieties to spontaneously recover from FDp infection. Therefore, the traditional FD management still relies on preventive control strategies and measures, such as the prompt and specific molecular identification of FDp infected plants and vectors, eradication of grapevine plants and replacement with healthy grapevine propagation materials, but mostly the compulsory application of insecticide treatments against FDp insect vectors, whose repeated use caused the selection of resistant insect populations as well as a reduction of insecticide effectiveness. These approaches to contain FDp infection are definitely very poor in terms of sustainability, because having a dramatic environmental and economical negative impact.

In the frame of the efforts done in Europe towards a more sustainable agriculture, it is mandatory to burst those studies aiming to unveil the ecological factors driving FDp evolution in vineyards, including the impact of the changing environmental conditions and the many selective pressures acting on FDp and its vectors, and altering the genetic structure of their populations. Similarly, it is pivotal to identify those genetic determinants that regulate and control plant susceptibility to FD, as well as those biotechnological approaches to switch on grapevine defenses against FDp.

On all these topics, a Conference on the current situation of FD in Italy and Europe, as well as on the most significant and cutting-edge perspectives, was successfully organised by Accademia dei Georgofili, and held in Florence on the last 9th of May. The main aim was to contribute to increase the general knowledge and awareness on this devastating grapevine disease, and several italian internationally recognised scientists highlighted the most challenging and current threats posed by FD to stakeholders and policy makers, in a fruitful and participated discussion. Moreover, other technical meetings have been planned as a spin-off of this main event, and are ongoing at the many italian sections of Accademia dei Georgofili, in order to make this dissemination action as widespread as possible.

The Abstracts of the Conference are available at https://www.georgofili.it/Media?c=69f68306-3e47-42a2-a3b3-e56651f43b5f

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