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Technical article

Flavescence Dorée: the importance of territory monitoring

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 652601

In Europe, Flavescence Dorée (FD), one of the more serious grapevine yellow diseases (GY), was first detected in the 1950s in France, soon to be followed by reports of similar diseases from other European countries (Stolbur for instance). The disease, caused by a phytoplasma, which is a quarantined organism indexed on the A2 EPPO list (EC directive no. 2009/297EC), is one of the most damaging diseases in European vineyards, with significant economic consequences in the major wine-producing countries. The principal vector for FD is a leafhopper strictly associated with grapevines which transmits the phytoplasma. FD has a very severe impact on vineyards, including yield loss and vine plant dieback. Without effective control measures, the disease spreads rapidly and can affect a complete vineyard in just a few years. Despite mandatory controls in place in Europe, the disease is still spreading and permanent monitoring is required to detect newly infected areas..

1. Spread to Europe and further spread of FD and its vector

FD's main vector, *Scaphoideus titanus*, was introduced into Europe from North America in the 1950s (Papura et al., 2012). Its introduction was attributed to the introduction of vine plants from the North American continent. However, *S. titanus* may have already been present as early as 1927, but on such a small geographical scale and population level that is was never recorded in any inventories. The invasion of European vineyards by *S. titanus* is continuing. The insect spread from France to most European vineyards and is now widely present in winegrowing regions throughout Europe - from Portugal to Serbia and from northern France to southern Italy. Distribution of *S. titanus* in Europe is wider than the phytoplasma, the insect being present in regions so far safe from FD (e.g. northern Spain or the Alsace region in France). The first FD outbreak was detected in France in 1957 by Caudwell, and the disease then spread rapidly to other European wine-making regions. Today the Flavescence Dorée phytoplasma is present in all the main wine-producing countries in Europe, namely Austria, Croatia, France, Hungary, Italy, Portugal, Slovenia, Spain, Switzerland and Serbia (Fig. 1). In some of these countries, the phytoplasma is limited to certain geographical areas. The spread of the disease in Europe is strongly linked to the spread of *Scaphoideus titanus*, mainly based on the dispersion of introduced populations and linked to human activities (Pavan et al., 1997; Bertin et al., 2007; Papura et al., 2009). Indeed, the spread of *Scaphoideus titanus* might not be at an end: populations of *S. titanus* could become established in northern Europe or China because of favourable climatic conditions in such areas (Maixner, 2005; Steffeck et al., 2007).

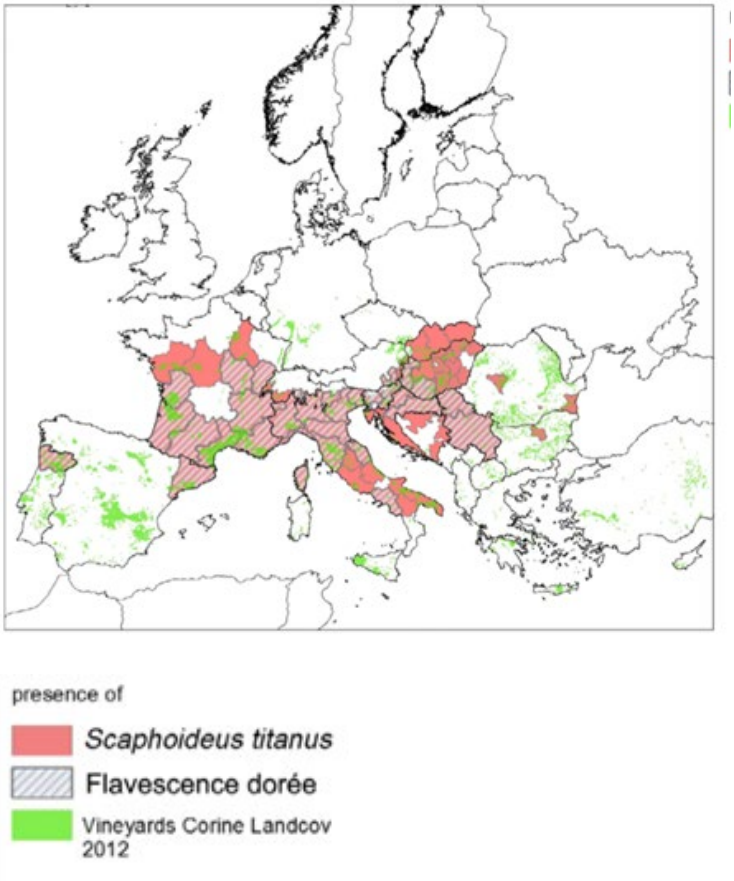


Figure 1: *Scaphoideus titanus* and Flavescence Dorée presence in Europe (EFSA 2016)

2. Symptoms and impacts of FD

Grapevines infected with Flavescence Dorée develop symptoms that are indistinguishable from other grapevine phytoplasmas that belong to the group of grapevine yellows (GY). Symptoms may be typical for GY but some of them may easily be confused with those for other diseases or abiotic agents. Once affected by Flavescence Dorée, grapevines show symptoms characteristic of grapevine yellows. Sometimes, the first symptoms may be observed at early vine stages: the first visible symptom may be either a delay or lack of budburst (Caudwell 1964), but such observations have always to be confirmed later by monitoring of typical FD symptoms during the summer.
In spring, symptoms of reduced growth of the fruiting cane, slight leaf blade curling and premature leaf fall can be observed, but the more evident symptoms appear later and are much more visible in September. On the infected grapevine, a lack or absence of lignification in the new shoots can be observed, with leaves curling downwards, cracking when folded

Symptoms of FD on leaves and bunches.

in the hand, and becoming reddish for red cultivars or yellowish for white cultivars. Desiccation of inflorescences and grapes is observed and a premature leaf fall due to limb detachment from the petiole can also occur in summer. Inside the plant, phytoplasmas reduce photosynthetic activity and nutrient transport, decreasing grape quality or even causing total desiccation of bunches, involving significant yield losses (up to 100%).
Symptoms may be more or less visible according to the cultivar. Furthermore, rootstocks are asymptomatic (healthy carriers of FD phytoplasmas).
FD symptoms can be confused with other symptoms, such as deficiencies or physiological disorders. In case of doubt, the presence of the three typical symptoms (leaf discolouration and curling, no lignification of the shoots and desiccation of bunches) must be checked. Laboratory analysis can then be carried out. As FD symptoms are similar to Stolbur symptoms, a PCR analysis can determine which phytoplasma is responsible for the symptoms observed. The PCR method enables diagnosing and identifying phytoplasmas inside grapevine organs (leaf blade and petiole) by analysing a DNA fragment.

When infected vine plants are not yet dead - what is very rare - FD phytoplasma infection will manifest itself in the quality of the grapes and the wine caused by delayed or poor ripening, with associated changes in the concentrations of sugar and other compounds. Nevertheless, compared with the impact on quantity caused by yield losses, reduction in quality is a minor problem.
In nurseries, Flavescence Dorée can have a significant impact on production. When FD phytoplasmas are detected in a nursery plantation, the latter will lose the Plant Passport for the production batch, and increased eradication and containment measures will be required. In infected areas, nurseries are required to implement FD control measures, such as monitoring mother vines and controlling the vector population.

Although it is only reported in a limited fashion, FDp infection may sometimes have an impact on grapes or wine quality. In some cases, grapes produced by infected plants may show delayed or uneven rip ening, and may see their concentration in sugar or in other compounds affected, resulting ultimately in lower quality. For example, FDp infection has been suggested to negatively impact the quality of wines produced from grapes harvested on infected Merlot plants. The Panel considered these reductions in crop quality as a minor impact as compared to the direct quantita tive impact on production and therefore selected not to analyse the impact on crop quality in detail. Given its current regulatory status, FDp has the potential to have significant impact on grapevine nurseries activities and production. On the one hand, detection of FDp infection in a nurser y will result in the loss of the Plant Passport for the complete production lot and will also re quire increased eradication and containment measures. On the other hand, in affected production areas, nurseries are required to implement significant FDp surveillance and *S. titanus* vector control efforts that may negatively impact their competitiveness. However, specific data on the number of nurseries and of grapevine plants for planting having lost

their Plant Passports in recent years as a consequence of FDp infection was generally not available to the Panel. Therefore, due to the lack of data and detailed information on this aspect, the Panel is not in a position to precisely evaluate the impact of FDp on grapevine nurseries production. Anecdotal information obtained from experts indicates, however, that loss of nurse ry production lots through FDp infection has occurred in some MSs but apparently only on a limited scale. Overall, the Panel concludes that although it is significant in terms of the necessity to implement additional control measures to protect nurseries in affected areas, the impact on nurseries production is likel y to be limited within the PRA time scale but cannot be adequately quantified at this stage. For the same reasons, evaluation of this impact under scenarios A1 and A2 cannot be precisely quantified.

3. FD disease: a 3 parts-relationship

To exist, Flavescence Dorée needs the simultaneous presence of 3 factors: the infection agent – i.e. the phytoplasma, the vector and a host plant.

A. The phytoplasma

Phytoplasmas are wall-less intracellular bacteria living in phloem sieve tubes. FD phytoplasmas can be transmitted from one host to another only by vector insects in which they can multiply and circulate, or by grafting.
The phytoplasma causing FD exhibits genetic diversity: several phytoplasma strains can cause FD and are widespread throughout Europe. Today, 3 genetic groups of the FD phytoplasma have been identified in Europe (Malembic-Maher, 2009):
- FD1, mostly localised in the south-west of France and, more rarely, elsewhere
- FD2, the major group present in Europe
- FD3, mainly reported in Italy

Other host-plants that can serve as phytoplasma reservoirs are Alnus glutinosa, Clematis vitalba and wild Vitis species (Malembic-Maher et al., 2007; Filipin et al., 2009). In Europe, the generally accepted hypothesis is that FD-related phytoplasmas were hosted in such plants prior to the grapevine.

B. The vector

i. Life cycle

Scaphoideus titanus is a univoltine species. Eggs are laid in late summer under the bark of old wood, then, after a diapause stage of 6 to 8 months depending on climatic conditions and vineyard characteristics, the eggs hatch. Hatching period duration is related to the diapause, which does not require cold temperatures to break (Chuche and Thiery, 2012).
Hatching period duration varies according to the regions, with long hatching periods being typical of vineyards with mild winters. Temperature regulates the beginning and the length of the hatching period as well as the sex ratio (Chuche and Thiery, 2014). After hatching, 5 nymphal instars follow each other in 5 to 8 weeks according to the climatic conditions before adult appearance. Nymphs usually stay on the plant where they hatch but sometimes jump from one plant to another (Maixner et al., 1993). They feed preferentially on suckers at the base of the trunk or on the lower and inner leaves. Adults appear generally from July, are highly mobile and fly from vine to vine. In order to mate, *Scaphoideus titanus* emits vibratory communication signals. Females, if mated, are able to start laying eggs 10 days after emergence (maturity at 6 days after emergence).

ii. Feeding behaviour

Scaphoideus titanus feeds on grapevine leaves. It is generally admitted that *Scaphoideus titanus* is mostly feeding in the phloem vessels, but it can probe sap either in the phloem or in the xylem. Nymphs prefer to feed on the small veins of the leaf blade and adults feed more on the larger veins or petioles (Chuche and Thiery, 2014). From the first nymphal instar, *S. titanus* can acquire the phytoplasmas when feeding on infected plants, and then remains infected for the rest of its life. An incubation delay of one month is required for the vector to become infectious. During this period the phytoplasmas circulate and multiply in the leafhopper to reach the salivary glands where the multiplication rate is increased. Once the concentration of phytoplasmas in the salivary glands is sufficient, the infectious agent may be transmitted to a healthy plant for every intake.

C. The host plants

In Europe, *S. titanus* is strongly associated with *Vitis vinifera* but can be occasionally found on other plants such as *Salix viminalis* and *Prunus persica* (Chuche and Thiery, 2014). The entire life cycle of the insect takes place on grapevines but feeding can occasionally occur on other plants. *S. titanus* may have varietal preferences: in multi-varietal vineyards different population levels have been observed for each variety (Schvester et al, 1962; Posenato et al, 2001).
Although *S. titanus* is associated with the grapevine, FD phytoplasmas can be found in other species such as *Alnus glutinosa, Clematis vitalba*, *Ailanthus altissima*, etc. Other vector species, such as the planthopper *Dictyophara europaea* and the leafhopper *Oncopsis alni,* can transmit the phytoplasma from these species to grapevines. But this phenomenon seems very occasional, the transmission probability being low as these vectors are very rare feeders on grapevines, unlike *S. titanus* (Maixner et al., 2000; Arnaud et al., 2007; Filippin et al., 2009).
When a vine plant is infected, phytoplasmas colonise all parts of the plant (including the leaves) via the phloem and constitute therefore a source of infection. By feeding on the grapevine and moving from grapevine to grapevine, *S. titanus* spreads the disease. Therefore, the infection rate in year N is strongly correlated with vector populations in year N-1 (Morone et al.,

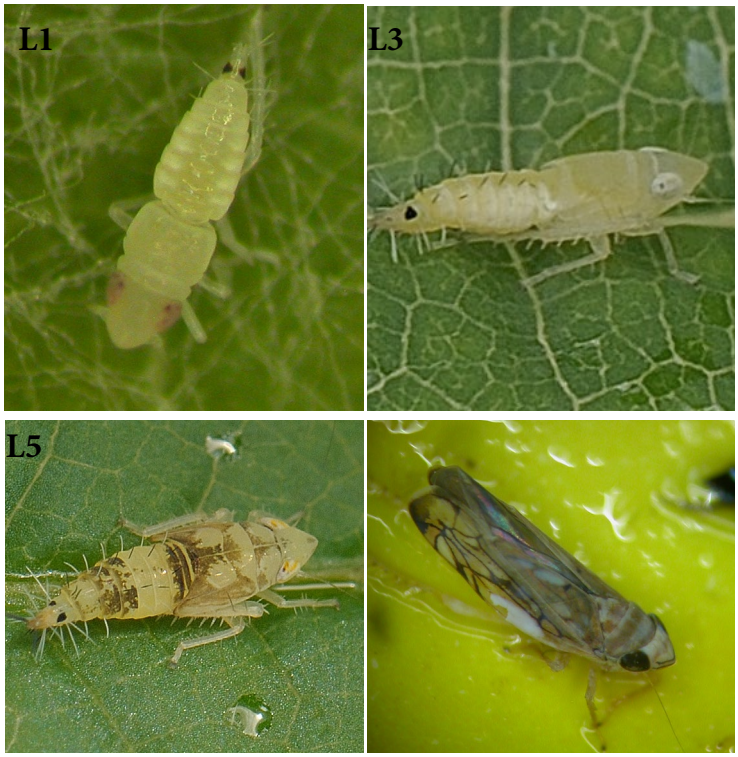
2007). Without insecticide treatment, *S. titanus* populations in vineyards can reach a level of thousands of individuals per hectare (Schvester, 1969) leading to a rapid spread of the disease, with an increasing number of infected vines - up to a 10-fold increase every year!

4. Monitoring the territory to detect vector or disease presence

For those regions not affected by Flavescence Dorée, continuous territory monitoring is crucial to prevent any spread of infection from an FD outbreak. The monitoring work needs to be done both in the nursery, to avoid propagation material contamination and thus the spreading of FD, and in the vineyard. Actions can be taken by monitoring the vector to prevent any introduction and, in cases where the vector is already present, by monitoring the territory for the early detection of FD symptoms.

A. Recognize the vector and detect its presence

Scaphoideus titanus is difficult to detect and to recognise because the nymphs are small and mobile. Furthermore, the specific leafhopper can be confused with other leafhoppers or insects living on grapevines. Early stages of *S.titanus* nymphs are at first white to translucent in colour, and then become darker with age. Nymphs are identifiable thanks to two symmetrical black points in the dorsolateral position near the abdomen's posterior end. Nymphs, when disturbed, show a typical behaviour: they tend to jump away. This behaviour can be used to differentiate *S.titanus* nymphs from other leafhopper juvenile forms that might be present at the same time on the grapevine leaves, such as Empoasca vitis (when disturbed moves laterally on the leaf surface) and Zygina rhamni (when disturbed tends to move along a straight line on the leaf surface). *Scaphoideus titanus* adults range in size from 4.8 to 5.8 mm, are brown in colour and have stripes on the head.r and stripes on the head .



Nymphs of *Scaphoideus titanus* and adult (IFV South-West, INRA Bordeaux)

Monitoring the vector is helpful in detecting any new presence of *S. titanus*. Monitoring can start on *S.titanus* nymphs but requires trained technicians. To be sufficiently accurate, visual control should be performed on the underside of some 100 to 200 leaves and on grapevine basal shoots and leaves, taking care not to disturb the vegetation excessively - because the leafhopper tends to jump away. Finding *Scaphoideus titanus* does not mean that there is FD in the area but means that there is a risk for a future FD outbreak. The plot needs to be monitored during the season and insecticides can be applied as a preventative measure to prevent infection.
Monitoring of *S.titanus* can be done more easily on adults by positioning sticky traps in the vineyards, inside plots and close to areas with wild vines.
Monitoring the FD vector can help to prevent FD infection and, in cases of infection, to take necessary measures rapidly. In vine areas close to winegrowing regions with a confirmed presence of Flavescence Dorée disease, monitoring vectors is extremely important.

B. Prevent infection by removal of vector reservoir

Wild vines and other species represent a reservoir for *Scaphoideus titanus*, and for FD phytoplasmas. FD phytoplasmas have been reported on such wild species as Clematis and Alnus, and may be transmitted occasionally to grapevines.
This so-called "wild compartment" thus presents a known risk of epidemics. On the other hand, such a wilderness may provide natural 'services' for the benefit of the vineyard: a reservoir of biodiversity, natural regulation of certain pests by auxiliaries, etc. Thus, it is important to consider and understand the relationships between epidemic risk and what natural regulation is offered in such semi-natural areas. In effect, beyond the perimeter of the vineyard itself, extending monitoring and disease control into these wild compartments is not easy.

C. Monitoring the territory

i. The importance of territory supervision/monitoring

The objective of monitoring is first to produce an inventory of the sanitary status of vineyards and agricultural fields and then to check for the appearance and spread of any new harmful organisms.
Control of visible symptoms on Vitis vinifera cultivars has to be implemented at the vineyard level, by winegrowers on their own vineyards, or on a larger scale for a regional vineyard with

collective exploitation. It is important that all stakeholders in the vine and wine sector are involved in the monitoring process and are aware of the damage caused by Flavescence Dorée. Areas without FD need to be protected from infection and monitoring is a key element in preventing outbreaks. Winegrowers need to be trained on symptom recognition as well as having specialised technicians available to carry out monitoring of the territory on a large scale. Where FD is known to be present, a dedicated plan should be in place to organise the monitoring of the territory, paying specific attention to rootstocks as these can carry FD phytoplasmas without showing any symptoms.
In cases where FD is detected in areas previously marked as uninfected, control and eradication measures must be applied in compliance with European, national and regional regulations. Indeed, as a result of the FD quarantine status, reporting of symptomatic plants potentially infected with the Flavescence Dorée phytoplasma is mandatory in every winegrowing region of Europe. Collection and analysis of symptomatic grapevine samples can complete the visual observations and is the only way to distinguish the FD phytoplasma from the Stolbur phytoplasma.

ii. Whom to inform in case of symptoms detection?

If FD symptoms are detected in a vineyard area, local technicians should be asked to confirm the potential FD case(s) before reporting to official authorities.

iii. How to be sure?

FD and Stolbur show similar symptoms on vines. In order to distinguish between these two diseases, and even from other possible confusing causes of the symptoms observed, an analysis has to be performed by accredited laboratories.

iv. Whom to inform in case of vector introduction?

If a vector is detected in a vineyard area, an entomologist or specialised technician should be asked to confirm the leafhopper species before reporting to official authorities.

5. The use of healthy planting material

There are three possible mechanisms in the spread of FD:

- Movement of infected propagation material
- Infected vector transport (or flights)
- Transfer from the wild compartment. In areas free from FD, nurseries can implement special actions to prevent FD phytoplasma infection such as hot water treatment and a conscientious monitoring of mother-vines.

In Europe, depending on the country or region, there are specific rules regarding nurseries such as mandatory hot water treatment and, in some cases, prohibition to carry out nursery activity in FD outbreak regions.

A. Management in nurseries

In nurseries, monitoring should be focused on controlling symptom development on the mother plants, both mother plants of rootstocks (healthy carriers) and scions, by regular observations. Any symptomatic plants should be registered and uprooted. In case of detection, local competent authorities should be informed. The Flavescence Dorée vector, *S.titanus* should be also monitored on mother-vines by preventive monitoring of nymphs and adults. Furthermore, diagnostic tools for phytoplasma detection should be used.

B. Hot water treatment

Hot water treatment (HWT) allows the eradication of the FD phytoplasma from propagation material and can be used to prevent infected material entering the area. Hot water treatment needs to be applied on scions or grafted rootlings in controlled conditions: plants are immersed in a bath of hot water for 45 minutes at 50°C. Combination of time and temperature is the most important factor in HWT effectiveness and needs to be such as to suppress phytoplasmas without affecting vine development. If the combination of 50°C and 45 minutes is not respected, buds can present localised cellular degeneration and even total alteration if the temperature rises to 60°C.
Purchasing hot water treated material is strongly recommended for European growers, in particular for regions where FD is not yet present. In presence of the vector, a single infected plant can generate widespread infection.

Conclusion

Flavescence Dorée is a serious disease and, if not properly managed, spreads rapidly and causes significant economic loss for the wine sector. In Europe, areas spared from FD do exist. But the vector is spreading fast and, with it, so is the disease. In regions free from FD, monitoring vineyards is essential to prevent introduction of the Flavescence Dorée vector. In the presence of the vector, a single infected plant can generate widespread infection. Raising awareness among the sector's stakeholders by education on the disease's epidemiology, symptoms and risks, is important in the prevention of FD outbreaks.

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Article written within the framework of WINETWORK project
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