

## GRAPEVINE YELLOWS - CURRENT DEVELOPMENTS AND UNSOLVED QUESTIONS

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Grapevine yellows (GY) are diseases associated to phytoplasmas that occur in many grape growing areas world-wide and are of still increasing significance. Almost identical symptoms of the GY syndrome are caused by different phytoplasmas and appear on leaves, shoots and clusters of grapevine. Typical symptoms include discoloration and necrosis of leaf veins and leaf blades, downward curling of leaves, lack or incomplete lignification of shoots, stunting and necrosis of shoots, abortion of inflorescences and shrivelling of berries. Those symptoms are related to callose deposition at the sieve plates and subsequent degeneration of the phloem (63,90). Although no resistant cultivars of *Vitis vinifera* or rootstocks are known so far, the various grape varieties differ considerably as far as symptom severity is concerned. It ranges from fast decline and death in highly susceptible cultivars to tolerant rootstocks as symptomless carriers of the pathogen (33).

Phytoplasmas are phloem-limited, non-helical and wall-less procaryotes (49). They represent a monophyletic clade within the class Mollicutes, which is currently divided into at least 15 subgroups on the basis of sequence analyses of various conserved genes (49). A new taxon, 'Candidatus Phytoplasma' has been established and various groups or subgroups have been described recently as 'Ca. Phytoplasma' species (49). The full genomes of two phytoplasmas are sequenced (36), providing new information on their biology and their interaction with plant and insect hosts. The available data confirm their lack of genes for autonomous metabolism and their reliance on intracellular parasitism. Phytoplasmas of several groups have been found to be consistently associated with GY (21). A variety of other phytoplasmas are occasionally detected in symptomatic or symptomless grapevines but their regular association with GY is often not clear.

Since phytoplasmas are in general vertically transmitted neither in their plant hosts nor their insect vectors, they depend on a cyclic change of host organisms (20). Their propagative and persistent mode of transmission requires specific adaptations to the vectoring insects (65), hence many of them show a high vector specificity, being transmitted by only one or a few closely related hemiptera species (115). Man acts as a vector of GY by propagating infected grapevines and spreading disease over long distance. This implies a high risk of dissemination of GY to regions where new outbreaks could be provoked if competent vectors existed or would be introduced, too.

Overviews on phytoplasmas in general (36,49), on phytoplasma vectors (115) and on GY epidemiology (20) have been recently published.

### Phytoplasmas associated with grapevine yellows

No additional phytoplasmas beside those listed by Boudon-Padieu (21) were recently found to be consistently associated to GY, although representatives of various phytoplasma groups were detected in grapevine occasionally (14,46). Stolbur (16SrXII-A) phytoplasma, the causal agent of Bois noir (BN), was reported from grapevine in Chile (14) and phytoplasmas of the same group, but also of 16SrI (aster yellows) group were identified in cv. Syrah in France, however, there was no clear association of those pathogens with symptoms of Syrah decline (95). Stolbur phytoplasma was also identified in GY diseased vines in Ukraine (83), and its presence in Serbia could be confirmed (58). Recently, new and repeated outbreaks of BN caused problems in various viticultural regions in Europe (66,91,93,98,103). These data confirm that Bois noir is an endemic disease in Europe, Asia minor and the Mediterranean. Aster yellows (16SrI-B), clover phyllody (16SrI-C) and elm yellows (16Sr-V) phytoplasmas were found sporadically in GY diseased grapevines in Italy (18), and infection of grapevine by aster yellows (16SrI-B) phytoplasma was reported from Tunisia (70). Infection of papaya and grapevine by *Ca. Phytoplasma australiense* was reported from Israel (56), however, confusion with stolbur phytoplasma which is also present in this region is not impossible. A survey in Italy revealed the presence of GY in 80% of the inspected vineyards (18). BN is widespread all over the country whereas Flavescence dorée (FD) is mainly restricted to the northern regions. According to this study FD-C isolates are spread further to the south than FD-D. In Spain, FD is restricted to some northern areas of Catalonia where containment measures after first outbreaks were successful (11,113). First vines infected by FD-C were reported from Slovenia (103) and Serbia (46,47,58). Although colonized by the vectoring leafhopper *Scaphoideus titanus* for years, southern Switzerland remained free from FD until a recent outbreak (57).

### Detection and Diagnosis:

Reliable detection of phytoplasmas in grapevine is still a challenge although a wide variety of diagnostic tools is available (16,52). Low and yearly as well as seasonally fluctuating titres of the pathogens (37) and their irregular distribution in infected vines cause problems. Visual inspection is impeded by latent infection in some rootstocks (33) and periods of symptomless infection in *V. vinifera*. For example, FD-C appears significantly earlier in the season than BN and FD-D (6). Protocols for the detection of major GY associated phytoplasmas such as FD and BN with universal or specific primers in either single or multiplex real-time PCR assays have been published recently (5,15,41,51) or will be presented during this meeting (48). A nanobiotransducer was used as a specific probe to identify PCR products amplified from FD phytoplasma (50). Detection of 16SrV phytoplasmas and differentiation of elm yellows from FD was achieved by restriction site insertion-PCR (32).

### Diversity and Taxonomy:

The phytoplasma groups are defined on the basis of phylogenetic analysis of the 16SrDNA sequences, forming distinct subclades within the phytoplasma clade (64). Further differentiation within those groups requires the analysis of more variable DNA sequences. Such discrimination of strains is necessary mainly to distinguish phytoplasmas within the same groups with distinct biological features, e.g. different host plants, vector specificity or geographic distribution. FD is associated to phytoplasmas of the elm yellows (16SrV) group, one of the most diverse clusters (64). Analyses of the ribosomal protein genes (9,64), *secY* gene (7,9,64), or MAP gene (9) have been used to differentiate phytoplasmas of the 16SrV group in general or FD and other grape-pathogenic 16SrV phytoplasmas in particular.

Although the stolbur (16SrXII-A) group appears to be less variable than the elm yellows group, different strains could be distinguished by the analysis of *Tuf*-gene sequences (60). Three of those strains are associated with BN in grapevine, but they exhibit a distinctive specificity for alternative host plant species, both in the field and under experimental conditions (77). A fourth strain was recently detected in the planthopper *Reptalus panzeri* (13). *Ca. Phytoplasma australiense* (CPA; 16SrXII-B) is a widespread pathogen in Australia and New Zealand where it causes diseases in various cultivated plants including grapevine. While heteroduplex mobility assay of the *Tuf*-gene revealed only low variation between isolates (39), four different strains of this phytoplasma could be differentiated by combined sequence analyses of the *Tuf*- and the ribosomal protein-genes (109). Similar to FD-associated phytoplasmas (9), the differentiation of CPA strains was improved by the combination of different markers.

### **Epidemiology**

The epidemiological behaviour of GY is considerably influenced by the biology of the insect vectors. All known or suspected vectors of GY are phloem feeding Auchenorrhyncha (20). Knowledge of vectors, their biology and behaviour and their range of host plants is a prerequisite for the understanding of GY epidemiology and the development of well-adjusted control strategies. Surveys of hemiptera in vineyards and attempts to identify vectoring insects are still ongoing research activities (4,22,35,54,87,100,101). Due to the high sensitivity of molecular diagnostic tools, phytoplasmas can be detected in various hemiptera after feeding on infected plants. Subsequent transmission trials are therefore indispensable to prove the vectoring ability of a particular species. While the principal transmission capacity can be tested under laboratory conditions by feeding on artificial medium (111), herbaceous plants or in-vitro grapevines (62) transmission to grapevine is required to take into account the specific vector-grapevine relationship and to show the ability of a particular species to act as a vector of GY under field conditions. Two principal epidemic systems can be distinguished in GY: Disease cycles including polyphagous vectors and alternative host plants (BN) or less complex but highly efficient cycles where only grapevine and a vector are included in the system (FD).

### Flavescence dorée:

*Scaphoideus titanus*, the vector of FD is still extending its range in Europe. After reaching the western border of the continent in Portugal (92) this nearctic species (10) is now widely distributed (81) occurring to the east as far as Slovenia (103), Serbia and Croatia (67). It started to invade the Pannonic plains recently (102), reached viticultural areas in central Switzerland (57) and southern Austria (117), and is present in most parts of Burgundy (21,85). In years with favourable climatic conditions it is likely to be able to complete its life cycle even further to the north, for example to viticultural areas in Germany (74).

While *S. titanus* colonizes a variety of woody plants including wild and cultivated grapevine in North America (10), it is restricted to grapevine in Europe (4). Adult vectors have been found sporadically on *Clematis vitalba* in the vicinity of vineyards and this plant proved to be infected by FD-C phytoplasma at different locations in Italy (8). Whether it plays a role in FD epidemiology by serving as a reservoir of the pathogen has to be further investigated (31).

Detailed studies have been carried out on different aspects of the plant-vector-phytoplasma interrelations that determine infection pressure and the degree of spread and dissemination. The structure of vineyards, cultural intensity and spraying regime influence population density and flight activity (1,67,68). The specific susceptibility of grapevine cultivars and their disease history affect the acquisition efficiency and thereby the rate of infestation of the *S. titanus* populations and the disease progress (30). The main period of flight activity extends from late afternoon to early morning (67). Data on vector development and vector-phytoplasma interaction were integrated in a logistic model expressing infection pressure as a function of growth degree-days (27). The observation that FD phytoplasma has a detrimental effect on its experimental vector *Euscelidius variegatus* (25) as well as on its natural vector *S. titanus* (28) by reducing life span and fecundity is understood as a hint on a short co-evolution of the north American leafhopper with the pathogen of presumably European origin (28). FD is one of the phytoplasmas with a high vector specificity, being transmitted under natural conditions only by *S. titanus* (20). This was confirmed by experiments where the gut barrier was overcome by injection of phytoplasma into the abdomen of Auchenorrhyncha species. Only four cicadellids were able to transmit the pathogen thereafter (24,26).

### Bois noir:

The Cixiid planthopper *Hyalesthes obsoletus* is the principal vector of Bois noir (Legno nero, Schwarzholzkrankheit) (2,78,105) and has been found in areas affected by this GY (19,82,87,102,103). However, other Cixiid and Cicadellid species are known or suspected vectors of stolbur phytoplasma, too (3,4,20). Alternative vectors are searched for, in particular where incidence of BN and occurrence or population density of *H. obsoletus* appear not to be correlated like in some regions of Spain (99) or Austria (96). Some additional species like *Goniognathus guttulinervus* (53,54) in Sardinia and *Reptalus panzeri* in Hungary (87) and Italy (19)

were found recently to carry stolbur phytoplasma beside the already known species (20) but their ability to transmit the phytoplasma is still not proved. Stolbur phytoplasma was also detected in different Cicadellid species in Spanish vineyards (101) and some of them were able to inoculate feeding medium or in-vitro grown grapevines under laboratory conditions (62).

Alternative host plants of stolbur phytoplasma play a vital role in the epidemiology of BN as key factors for both the population density of the polyphagous vector and the frequency of vector infection (19,43,60,87,104,106,114). A wide variety of mainly herbaceous plants have been surveyed for the presence of stolbur infection and colonization by *H. obsoletus* (42,43,62,80,82,87,96,97,102,103,106). *Urtica dioica* and *Convolvulus arvensis* are major host plants of BN phytoplasma with high epidemic significance. *Vitex agnus-castus* is a shrub that frequently hosts *H. obsoletus* in Israel, but its function as source of the phytoplasma is not yet clear (107). With other herbaceous plants that harbour the phytoplasma such as *Taraxum officinalis* (42,96) it needs to be proved that they could serve as a source of inoculum. The predominant host plant has an influence on the spatial pattern of BN. It is often scattered over the whole vineyard where *C. arvensis*, a common weed inside vineyards, is the major source of inoculum. With *U. dioica* on the other hand, which more frequently grows outside the vineyards, diseased vines are often concentrated at the borders and incidence decreases to the inside of vineyards (17,23,43,82). High levels of phytoplasma infestation of *H. obsoletus* populations and a short minimum inoculation access period on grapevine might compensate for the low propensity of this vector to grapevine (23).

The two most important alternative host plant species harbour different strains of the BN phytoplasma (60). Type I is associated to *U. dioica* and seems to be the predominant one in northern Italy (13,19) where this plant is the major natural host of *H. obsoletus*. This type was previously rare in Germany but is now the reason for outbreaks of BN at the same time as the 'traditional' type II is still in an endemic phase (77). Given that also host specific populations of *H. obsoletus* were observed, it is likely that different epidemiological cycles of BN exist in the field, consisting of different host plants and adapted phytoplasma strains and vector populations (60). First results of an ongoing characterization of *H. obsoletus* populations indicate genetic differences between populations of different geographic origin rather than between host specific populations (69). The reasons for the currently increasing exploitation of nettle in areas where *H. obsoletus* was previously restricted to *C. arvensis* are still enigmatic. In Spain, only type II has been found in *H. obsoletus* while type I was predominant in grapevine but could be detected in other potential vector species (101). An additional, so far unknown variant of the phytoplasma has been detected in *Reptalus panzeri* in Italy (19).

#### Australian grapevine yellows:

Lack of knowledge of the vectors of *Ca. Ph. australiense* (CPA) that is associated with AGY hampers the understanding of its epidemiological behaviour and the development of control strategies. AGY infected vines showed a clustered but variable distribution in the vineyards and no evidence for vine to vine transmission could be found (38). Indigenous plants in Australia and New Zealand were monitored for the presence of CPA in order to identify sources from which it is probably transmitted to cultivated plants including grapevine. *Coprosoma robusta*, a widespread native plant in New Zealand appeared to be frequently infected by CPA (12). Studies in areas of Australia where CPA causes disease in strawberry identified various wild plants as hosts of tomato big bud (16SrlI) and CPA phytoplasmas (110). One widespread and frequently infected plant, *Gomphocarpus physocarpus* (cottonbush), is also a host of stolbur phytoplasma in Europe (44). Due to the specific spatial distribution of AGY in Australian viticultural areas it had been hypothesized that natural hosts are most likely elements of swamp land vegetation (72). A survey of those areas identified several plants infected with CPA, with *Mairana brevifolia* (Yanga bush) as the most important species (73). With respect to vector identification it is an interesting coincidence that leafhoppers of the genus *Batracomorphus* have been found on both *M. brevifolia* in Australia (71) and *C. robusta* in New Zealand (12).

#### Other grapevine yellows:

No vectors of other GY are known so far. Field studies in Virginia identified a number of potential vector species of Virginia grapevine yellows and described their seasonal movement (116).

## **Control**

Management of FD includes the eradication of infected plants that serve as sources for infection as well as the control of the vectoring leafhopper *S. titanus* (89). Recent investigations deal with the identification of cultural factors that influence population density or infestation of vector populations and also with the prediction of infection pressure (27,29,68). The objective is to make FD control more compatible with integrated control programs (45). Biological control of *S. titanus* is not yet applicable but the efficiency of dryinid parasitoids from North America is evaluated in France (79)

Due to the more complex epidemic cycle of BN that includes alternative host plants as sources of inoculum and a non-amplephagous vector whose life history puts it out of reach of insecticides, control of BN is considerably more difficult and less efficient than control of FD (40). Since chemical control failed to decrease BN incidence (34,88,105) regular insecticide treatments to contain BN (93) are hardly justified. Control of host plants within affected vineyards but also in adjacent areas is a measure to decrease infection pressure, but weed control should not be carried out during the flight of adult vectors that otherwise are forced to move onto grapevine (76,84). Since *H. obsoletus* is attracted by sparse vegetation on open soil, a well managed green cover reduces the attractiveness of vineyards and the risk of contamination of grapevine (59). *H. obsoletus* is susceptible to the entomopathogenic fungus *Metarhizium anisopliae* (61), but the efficiency of this biocontrol agent in the field is not yet tested. The high attractivity of *V. agnus-castus* for *H. obsoletus* was used in a control experiment in Israel, where belts of these bushes were planted around vineyards and treated with insecticides (107). Though fewer

adults emerged from roots of treated plants, populations of *H. obsoletus* and incidence of BN in belted vineyards were higher than in control plots.

### Open questions

Although considerable information has been gathered on the etiology, epidemiology and control of phytoplasma diseases of grapevine, a number of problems are still not solved. New outbreaks of GY occur without a sufficient explanation of the underlying reasons, e.g. the synchronous outbreaks of BN in different European regions where it is not known what triggers this development.

Graft transmissibility and the vegetative propagation of grapevine imply the risk of dissemination of GY by propagation material. This threat can be minimized by hot water treatment (112) which is particularly useful as a quarantine measure where highest phytosanitary standards are required to prevent dissemination of GY. The significance of infected planting material is controversially discussed particularly where epidemic outbreaks of GY occur and young plantings are often severely affected. Risk assessments are necessary to clear up the specific traits of particular GY and the risks posed by the planting of infected vines. Vector behaviour and biology, modes of transmission and types of epidemic cycles have to be considered. In areas already affected by a particular GY, it is necessary to weigh up the risk by the infection pressure in the field and by the contamination of planting material against each other. For example, rapidly increasing infection of BN in young plantings is often attributed by growers to contaminated planting material. However, taking into account the transmission pathway from wild reservoir plants to grapevine, the high infection pressure in young plantings and the dynamic behaviour of the disease, the low probability of infected planting material (85,86,94) should be a minor problem. Single vines infected by FD, on the other hand, imply a high risk for areas where the vector *S. titanus* is already present. Detailed risk analyses for the different GY are required for practical purposes as well as plant health standards in order to apply preventive measures adequately.

Another still open question is the role of infected alternative host plants for the epidemiology of GY. Like various insects that feed on phloem of infected plants carry phytoplasmas without being able to transmit them, many plant species are occasionally infected but their epidemiological significance remains unclear. Frequent infection of a particular plant species might indicate its role as an alternative host and source of infection like *C. arvensis* with BN, or it could be due to regular inoculation by vector feeding without the subsequent chance of acquisition like in *Solanum nigrum*. Since alternative hosts can play an important role in GY epidemiology identification of such plants is still an important task. It is not yet understood why *H. obsoletus* uses different host species specifically and what causes the exploitation of new plant species by this vector. Knowledge of the factors that drive the host plant selection could help to understand the reasons for disease outbreaks and dynamics.

Little is known yet about the reasons for the considerable varietal differences in susceptibility of grapevine cultivars to GY (27,38,55,75,108). Identification of the environmental and intrinsic factors that lead to less effective inoculation, reduced susceptibility as well as recovery could provide hints on the phytoplasma grapevine interactions involved in disease development and, thereby, might help to induce resistance to GY in grapevine.

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