Data Sheets on Quarantine Pests

Grapevine flavescence dorée phytoplasma

IDENTITY

Taxonomic position: Bacteria: Tenericutes: Mollicutes: Phytoplasmas
Notes on taxonomy and nomenclature: Several grapevine diseases known or suspected to be caused by phytoplasmas have been reported (Caudwell *et al.*, 1971; Caudwell, 1990). Flavescence dorée is the most important and best studied of these. It is an epidemic disease characterized by its rapid spread within vineyards due to vine-to-vine transmission by a cicadellid leafhopper and a crisis-recovery-relapse disease cycle. Another well known European grapevine disease, whose etiology was until recently uncertain and which was therefore treated as part of the "flavescence dorée complex" (EPPO/CABI, 1992), is "bois noir" (in France), "Mediterranean yellows" (in Italy) or "Vergilbungskrankheit" (in Germany), which is graft-transmissible but spreads only slowly and, apparently, not from vine to vine, without, until recently, known vectors. Since its phytoplasma etiology has now been established, and its agent is clearly known to be different from the phytoplasma causing flavescence dorée, its separate identity can now be asserted, under the provisional name grapevine bois noir phytoplasma. Other diseases, inducing similar symptoms in grapevine, loosely referred to as 'grapevine yellows', occur elsewhere in the world. They are transmitted with difficulty by grafting, are usually slow-spreading and have no known vectors. Their identity and etiology is not clear, and they are only incidentally mentioned in this data sheet. In general, the simple view has provisionally been taken in this data sheet that any phytoplasma found in grapevine in Europe, which is not flavescence dorée, is bois noir. This is supported by the generally reported affinity of these phytoplasmas with the aster yellows group. However, it is clear that this is potentially an over-simplification. Several different phytoplasmas of the aster yellows group may infect grapevine in Europe, in the same or different countries.

- **Grapevine flavescence dorée phytoplasma**
  Name: Grapevine flavescence dorée phytoplasma
  Common names: Baco 22A disease (English)
  Flavescence dorée (French)
  Flavescencia dorada (Spanish)
  Flavescenza dorata (Italian)
  EPPO computer code: GVFDXX
  EPPO A2 list: No. 94
  EU Annex designation: II/A2
- **Grapevine bois noir phytoplasma**
  Name: Grapevine bois noir phytoplasma
  Common names: Black wood (English)
  Bois noir (French)
  Vergilbungskrankheit(German)
  EPPO computer code: GVBNXX
  EPPO A2 list: No. 94
  EU Annex designation: II/A2, covered by the entry grapevine flavescence dorée MLO

HOSTS

The principal host of flavescence dorée is *Vitis vinifera* (grapes), but *V. riparia* can also be infected naturally (Maixner & Pearson, 1992). The causal agent has been transmitted by means of insect vectors from grapevine to *Vicia faba* and *Chrysanthemum carinatum*, then reintroduced into the woody host. In the EPPO region, *V. vinifera* is the only significant host. This is also the case for bois noir, insofar as the pathogen is only recognized on this host; in fact, it is possible that, as with some other phytoplasmas, bois noir (or other grapevine yellows) is incidentally transmitted to grapevine from other hosts, and grapevine is not the principal host of the pathogen. Recently, the bois noir phytoplasma has been detected, by a PCR-based procedure, in weeds in German vineyards (*Convolvulus arvensis*, *Solanum nigrum*) (Maixner *et al*., 1995). It is interesting to note that *C. arvensis* is also the common wild host of potato stolbur phytoplasma (EPPO/CABI, 1996b).

GEOGRAPHICAL DISTRIBUTION

Kuszala *et al.* (1993) tested samples of grapevine stems and leaves showing yellows symptoms, from all parts of the world, with polyclonal and monoclonal antibodies specific to flavescence dorée. The authentic phytoplasma was only detected in material from southern France and northern Italy. This result does not totally exclude the possibility that flavescence dorée occurs elsewhere, but it confirms that grapevine yellows from southern Italy (Sicily), central Italy (Emilia-Romagna), Switzerland, Israel, USA and South Australia is not flavescence dorée. It may be noted that both flavescence dorée phytoplasma (which is related to the elm yellows group) and another phytoplasma (of the aster yellows group, probably bois noir) have been found jointly infecting grapevines in Liguria (Italy) (Bertaccini *et al.*, 1995), and that several of the cases of grapevine yellows elsewhere in northern Italy (Friuli-Venezia Giulia, Lombardia) are also of the bois noir type (Bianco *et al.*, 1993; Osler *et al*., 1993; Carraro *et al*., 1994). The two types also exist together in certain parts of France, with possibly even a third different phytoplasma (Daire *et al.*, 1993). Prince *et al.* (1993) confirmed the division into the elm-yellows type and the aster-yellows type and also found a third type of phytoplasma (of the peach X disease group) associated with grapevine yellows in Virginia (USA) and in parts of northern Italy (Friuli-Venezia Giulia).

- **Grapevine flavescence dorée phytoplasma**
  EPPO region: France (actively spreading in Aquitaine, Languedoc-Roussillon and Midi-Pyrénées, stable in Corsica, Jura, Rhône-Alpes, contained in Bourgogne, Champagne, Centre, Pays de Loire; Descoins, 1995), Italy (Liguria, Piemonte, Veneto).
  EU: Present.
- **Grapevine bois noir phytoplasma**
  EPPO region: France (Auvergne, Bourgogne, Languedoc-Roussillon, Rhône-Alpes), Germany (Mittelrhein, Mosel-Saar-Ruwer, Nahe, Rheinhessen, Rheinpfalz), Israel (probably), Italy (Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Sicily), Slovenia (probably), Spain (Cataluña; Batlle *et al*., 1995), Switzerland (probably bois noir, definitely not flavesence dorée; Cazelles & Kuszala, 1993). Other yellows diseases occur in Bulgaria, Greece, Moldova, Romania and Tunisia (Caudwell *et al*., 1987; Caudwell, 1990). In France, a further as yet unidentified grapevine yellows is said to exist (in Bourgogne and Val de Loire), distinct from flavescence dorée and bois noir.
  Asia: Israel (probably).

Africa: South Africa (undetermined yellows, possibly not due to a phytoplasma, but to a closterovirus) and Tunisia (undetermined yellows).
North America: Mexico (undetermined yellows), USA (New York, Virginia; Wolf *et al*., 1994).
South America: Argentina (undetermined yellows), Chile (undetermined yellows).
Oceania: Australia (grapevine yellows confirmed to be due to a phytoplasma of the aster yellows group, almost certainly distinct from the European pathogens; Padovan *et al*., 1995), New Zealand (undetermined yellows).
EU: Present.

BIOLOGY

- **Grapevine flavescence dorée phytoplasma**
  Like all phytoplasmas, the causal agent of flavescence dorée is localized in the phloem of infected grapevines from where it is acquired by the vector for subsequent transmission. A single infectious insect may be enough to transmit the disease, thus starting an epidemic. As no alternate host other than the grapevine is known, it is likely that the whole biological cycle is completed in grapevine and vector.

  The principal vector, the cicadellid *Scaphoideus titanus*, was introduced into Europe from North America (Caudwell & Dalmasso, 1985). Phytoplasmas have been found in the salivary glands of infectious insects and detected serologically (ELISA) in single individuals (Boudon-Padieu *et al*., 1989). Another cicadellid, *Euscelidius variegatus*, is capable of artificially transmitting the disease from *Vicia faba* to *V. faba* and to *Pisum*, *Chrysanthemum*, *Lupinus* and *Catharanthus roseus*. Lefol *et al*. (1993) found that the phytoplasma attached well in several other insects, but it was not established whether these could act as vectors. *S. titanus* occurs commonly on cultivated vines and wild *V. riparia* in New York (USA) (Maixner *et al*., 1993). Samples of the insect gave a positive serological test for flavescence dorée, and transmission of a phytoplasma to *V. faba* was demonstrated. However, transmission to grapevine was not obtained (and other results suggest that the grapevine yellows in New York is not flavescence dorée). The significance of these American results is therefore not clear.

  In south-west France, *S. titanus* has one generation a year; eggs laid in the bark of 2-year-old grapevine wood from the end of July are the overwintering stage. There are five larval instars, which develop from mid-May to mid-July. Adults appear towards the end of July and are present usually until the beginning of September. The acquisition period is generally about 7-8 days, occasionally 4 days, and there follows a long latent period, so that transmission takes 38-42 days in all. The larval stages and adults are capable of acquiring the phytoplasma, but males are more efficient than females in transmitting the disease. Infectivity is thought to be retained throughout the insect's life but there is no evidence of adult-to-egg transmission (Schwester *et al*., 1969).
- **Grapevine bois noir phytoplasma**
  Bois noir is not transmitted by *S. titanus*. In Spain, bois noir (identified by molecular methods) occurs in vineyards where *S. titanus* is abundant (Batlle *et al*., 1995); in this case, the authors fear the introduction of flavescence dorée (possibly from adjoining Roussillon, in southern France), because it would be spread by the vector, whereas the bois noir which is present is (presumably) not spreading. In Northern Italy, *S. titanus* failed to transmit a yellows from Friuli-Venezia Giulia, but did transmit a yellows from Veneto (Carraro *et al*., 1994). Vidano *et al*. (1989) found *S. titanus* to be abundant in affected vineyards in Piemonte; they also found *Hyalesthes obsoletus* transmitting phytoplasmas to various wild plants and weeds (see below). *S. titanus* also occurs in Slovenia (Gabrijel, 1987).

Until recently no other specific vector of bois noir had been identified, though it seemed probable that a vector existed. Di Terlizzi *et al*. (1994) found that vines near the edge of the vineyard were commonly infected in Puglia (southern Italy), suggesting that the disease enters the plot from outside, presumably on a vector. Maixner (1994) and Maixner *et al*. (1995) have now found bois noir phytoplasma in the planthopper *Hyalesthes obsoletus*, by a PCR-based procedure. *H. obsoletus* feeds on various wild plants and weeds, including in particular *Convolvulus arvensis* (in which the phytoplasma was also detected, see Hosts), but rarely on grapevine. When infected planthoppers were placed on grapevine seedlings, these developed yellows symptoms, so *H. obsoletus* appears definitely to be a vector of bois noir. However, because it does not normally feed on grapevine, vine-to-vine transmission rarely occurs. It is interesting to note that *H. obsoletus* is also the main vector of potato stolbur phytoplasma, which it similarly transmits incidentally to potato from *C. arvensis* (EPPO/CABI, 1996b). Both stolbur and bois noir are members of the aster yellows group, and it will clearly be very interesting to investigate how close the relationship between these two phytoplasmas is (they could conceivably even be identical).

Bois noir also differs from flavescence dorée in affecting a different set of grapevine cultivars.

DETECTION AND IDENTIFICATION

Symptoms

- **Grapevine flavescence dorée phytoplasma**

A full description of the symptoms of flavescence dorée is given by Caudwell (1964) and Belli *et al*. (1973). These generally become apparent during summer, but diseased grapevines are identifiable from spring onwards by their reduced growth and, sometimes, by the absence of shooting. Only a group of shoots is affected on each grapevine, or the whole vine may show symptoms.

On shoots

The shoots of susceptible grapevines, when infected early, fail to lignify, are thin, rubbery, and hang pendulously. They later become brittle and there may be a necrosis of the apical and lateral buds. During winter, the non-lignified branches blacken and die. If infected later in the season, lignification is interrupted. These late-infected shoots also blacken in winter but survive and grow a little in the following spring. In the more resistant cultivars, non-lignification is much more pronounced, being limited to certain internodes. Numerous small black pustules form along the diseased branches of susceptible cultivars. At the end of summer, especially in southern regions, longitudinal fissures appear in the bark at the base of badly diseased branches.

On leaves

The leaves show colour aberrations and downward-rolled margins. In white-fruited cultivars there is a yellowing of the portion of the lamina exposed to the sun that confers a metallic lustre to the leaf surface. Later in the season, well defined creamy-yellow spots a few mm in diameter appear along the main veins. These spots enlarge and form continuous yellow bands along the veins, which gradually extend over large parts of the leaf surface. Red-fruited cultivars develop a similar pattern of colour changes of the leaves, but the discolorations are reddish. The central portion of the discoloured areas becomes necrotic and dries out. These brittle rigid leaves are frequently detached in wind but they appear to withstand autumn frosts well and fall later than healthy leaves.

On fruit

Fruit setting is reduced on grapevines infected early in the season, and the inflorescences dry out and fall off. In later infections, bunches become brown and shrivelled, while the peduncles dry out. In cultivars such as Baco 22A, berries fall off at the slighest disturbance.

- **Grapevine bois noir phytoplasma**

  The symptoms caused by other "grapevine yellows" pathogens are practically the same as those of flavescence dorée. "Bois noir", or "black wood", a name used in Bourgogne (France), refers to the blackening of non-lignified shoots in winter, which is a symptom of flavescence dorée (see above). Accordingly, the names given to the disease in different places have no particular significance in relation to diagnosis from symptoms. One point may be noted: symptoms of bois noir and of some other yellows reappear on the same vines in successive years, although there may be an interruption for one or more years. Flavescence dorée occurs apparently randomly in vineyards (this is perhaps associated with vine-to-vine transmission by the vector).

Morphology

- **Grapevine flavescence dorée phytoplasma**

  Phytoplasmas can be found in the phloem of infected grapevines.

- **Grapevine bois noir phytoplasma**

  Phytoplasmas have been found in grapevine infected with probable bois noir in Puglia (southern Italy) and in *Catharanthus roseus* infected via dodder from grapevine (Di Terlizzi *et al.*, 1994). Serological relationships suggest that this phytoplasma is more closely related to the aster yellows group than the elm yellows group (which includes flavescence dorée). Maixner *et al.* (1994) detected phytoplasmas in vines affected by bois noir in Germany, and found by RFLP analysis that these were closely related to potato stolbur phytoplasma (aster yellows group) and not to flavescence dorée. Credi (1994) detected pleomorphic phytoplasmas in mature sieve-tube elements of vines showing yellows symptoms in Emilia-Romagna (Italy), an area where authentic flavescence dorée does not occur.

Detection and inspection methods

- **Grapevine flavescence dorée phytoplasma**

  Serology (ELISA, ISEM) can be successfully applied using polyclonal or monoclonal antibodies for identifying the causal agent in herbaceous hosts and leafhopper vectors (Boudon-Padieu *et al.*, 1989). Molecular probes are being developed (Daire *et al.*, 1992), and used to detect the phytoplasma in its vector (Bertaccini *et al.*, 1993). These various detection methods indicate that grapevine flavescence dorée phytoplasma belongs to the elm yellows group of phytoplasmas. However, these methods have not yet proved reliable for grapevine itself, for which visual inspection remains the routine practice for grapevine certification (OEPP/EPPO, 1994a), with the possibility of testing by grafting on indicator cultivars (Bacco 22A, Chardonnay or Aramon; OEPP/EPPO, 1994b).

- **Grapevine bois noir phytoplasma**

  Specific PCR probes for flavescence dorée did not react with samples from bois noir-infected vines, but less specific probes did react, confirming that a related phytoplasma might be involved (Daire *et al.*, 1993). RFLP analysis (Davis *et al.*, 1993) confirmed that material from northern Italy thought to be flavescence dorée was similar to authentic flavescence dorée from France (related to elm yellows), and distinct from "southern European grapevine yellows" from southern Italy (which is probably bois noir, related to aster yellows). Various techniques are thus becoming available to detect and identify bois noir specifically. Chen *et al.* (1993) compared monoclonal antibodies, DNA probes and PCR for the detection of the North American grapevine yellows phytoplasma, which is very similar to bois noir. In grapevine certification (OEPP/EPPO, 1994a), bois noir is

routinely detected only by visual observations, but tests can be done by grafting on the indicator cultivars Chardonnay or Riesling.

MEANS OF MOVEMENT AND DISPERSAL

Spread of flavescence dorée occurs through infected grapevine planting material and through the vector. Local spread is efficiently accomplished by *Scaphoideus titanus* at a rate of 5-10 km each year in south-west France. Bois noir moves relatively little, since its vectors rarely feed on grapevine. Long-distance dissemination of either phytoplasma could be through infected propagative material. Symptomless budwood can host both the eggs of the vector (for flavescence dorée) and the disease agent.

PEST SIGNIFICANCE

Economic impact

Where allowed to spread uncontrolled, epidemic flavescence dorée has catastrophic consequences. Between 1949 and 1954 in Armagnac and Chalosse (France), all Baco 22A grapevines became infected. The disease continues to be of great economic importance in France particularly for the cultivars Chardonnay and Baco 22A, and is acquiring relevance in northern Italy, where it is causing serious problems to Chardonnay, Pinot blanc and other susceptible cultivars (Refatti *et al*., 1992). Recently, flavescence dorée was shown to be present in many parts of France but not to be spreading (Descoins, 1995). In Languedoc-Roussillon, disease incidence is, however, increasing and compulsory treatments were made in 1995 against the vector *S. titanus* on 25 000 ha of vines. In contrast, bois noir and the various grapevine yellows, which do not spread epidemically, are of small economic importance, but some of the yellows of central and southern Italy and Australia have a clear-cut negative impact on the grapevine industry (Magaray & Wachtel, 1986; Credi *et al*., 1987).

Control

Selection of suitable planting material can be used to control the disease. Differences in sensitivity are known among cultivars of *V. vinifera*, some being resistant to infection and others recovering completely the year after the appearance of symptoms. Control of the insect vector is facilitated by the fact that, in Europe, *S. titanus* is confined to grapevine as a host and has just a single generation per year (Caudwell *et al.*, 1987). Sprays against overwintering eggs or during the spring hatching period have been found to be effective.

Phytosanitary risk

Further spread of flavescence dorée could cause much more severe problems. The recent record of the cicadellid vector *Scaphoideus titanus* in south-western Slovenia (Gabrijel, 1987) represents a serious threat for Croatia and neighbouring countries. Grapevine flavescence dorée phytoplasma is an A2 quarantine pest for EPPO (OEPP/EPPO, 1983), because of the risk of spread of the vector-transmitted pathogen to countries where yellows diseases of grapevine are unknown, or where only bois noir occurs. It is also of quarantine significance for COSAVE.

Bois noir and the other European yellows do not present a significant phytosanitary risk, since they already occur widely, they hardly spread on grapevine, they are of minor economic importance, and the pathogens involved are phytoplasmas of the aster yellows type which may be even more widely distributed in wild plants and weeds such as *Convolvulus arvensis*. The existence in grapevine in Northern Italy of one form of

phytoplasma related to peach X-disease phytoplasma may be a cause for concern, since the latter is an A1 quarantine pest (EPPO/CABI, 1996a).

PHYTOSANITARY MEASURES

EPPO (OEPP/EPPO, 1990) recommends, to ensure freedom from disease, that grapevine nurseries should be established in, and propagating material should be collected from, areas where flavescence dorée does not occur. Alternatively, mother plants should be inspected during the growing season and be particularly well protected against the vector. Control of the vector is achieved by: (i) eliminating eggs through burning pruning wood and treating before bud burst with parathion-activated oils; (ii) one or two chemical applications against instars 30 and 45 days after first hatching, followed by another treatment against adults (Caudwell & Martelli, 1992). An EPPO certification scheme for grapevine is available (OEPP/EPPO, 1994a) and should give phytoplasma-free planting material with a high level of security.

Phytoplasmas may be eliminated from infected wood by treating dormant canes with water at 45°C for 3 h or 50°C for 40-60 min (Caudwell *et al*., 1992). However, the effectiveness of these treatments for plant quarantine purposes has not been confirmed.

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