EPPO Datasheet: *Xylophilus ampelinus*

Last updated: 2021-04-20

IDENTITY

Preferred name: *Xylophilus ampelinus*
Authority: (Panagopoulos) Willems, Gillis, Kersters, van den Broeke & De Ley
Taxonomic position: Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Other scientific names: *Bacillus vitivorus* Baccarini, *Erwinia vitivora* Du Plessis, *Xanthomonas ampelina* Panagopoulos
Common names: bacterial blight of grapevine, canker of grapevine
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EPPO Categorization: A2 list
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EU Categorization: RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: XANTAM



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Notes on taxonomy and nomenclature

A blight and canker disease of grapevine (*Vitis vinifera*) named in Greek 'tsilik marasi' was first described in Crete (Greece) in 1939 by Sarejanni, but only in 1969 was its causal organism identified as the very slow growing bacterium *Xanthomonas ampelina* (Panagopoulos, 1969). The disease 'mal nero della vite' described by Baccarini in 1893 and attributed to *Bacillus vitivorus* and the 'maladie d'Oléron', described in France in 1895 (Ravaz, 1895) and attributed to *Erwinia vitivora*, have been shown also to be due to *Xylophilus ampelinus* (Grasso *et al*., 1979; Prunier *et al.*, 1970). The bacterium named at the time *E. vitivora* was shown to be identical to the common, fast growing saprophytic bacterium *Pantoea agglomerans* (*Erwinia herbicola*), when a South African strain NCPPB 2036, LMG 2597 was tested by Verdonck *et al*., 1997. 'Vlamsiekte' in South Africa, and 'necrosis bacteriana' in Spain previously considered to be the same disease as the 'maladie d'Oléron', were also recognized to be due to *X. ampelinus* (Erasmus *et al.*, 1974; Lopez *et al*., 1981). A DNA and RNA structure study revealed that *Xanthomonas ampelina* belongs to rRNA superfamily III as separate branch, not related to the genus *Xanthomonas*, and placed in the newly created genus *Xylophilus* as *Xylophilus ampelinus* (Willems *et al.*, 1987). The only other member of the genus *Xylophilus* to date is *X. rhododendri*, isolated from a symptomless flower of the royal azalea (*Rhododendron schlippenbachii*) collected in Jeju Island, Republic of Korea (Lee *et al*., 2020). Symptoms may be confused with those of other bacterial blight/canker/leafspot diseases of grapevine, caused by *Xanthomonas citri* pv. *viticola* (and three other, closely related and ill-defined pathovars, namely pv. *vitistrifoliae*, pv. *vitiscarnosae* pv. *vitiswoodrowii*) reported from India and Brazil. Furthermore, a leaf spot caused by *Xanthomonas arboricola* (pathovar not identified) was reported from Japan (Sawada *et al*., 2011). *X. campestris* pv. *viticola*, however, has not yet been observed in Europe (EPPO, 2016; CABI, 2019) although it is thought to have spread from India to South America (Brazil) with planting material (Trindade *et al*., 2007, Ferreira *et al*., 2019).

X. ampelinus is a very homogeneous taxon; strains from different origins in Europe so far were very similar in enzymatic and protein patterns as well as in DND-DNA hybridization. Komatsu *et al*. (2016), however, were able to discriminate between strains from Japan and Europe using rep-PCR. Whereas in pathogenicity and virulence, these authors did not find differences between strains from the two regions. A DNA marker specific for strains isolated from the cv. Sultan was described by Manceau *et al*. (2000).

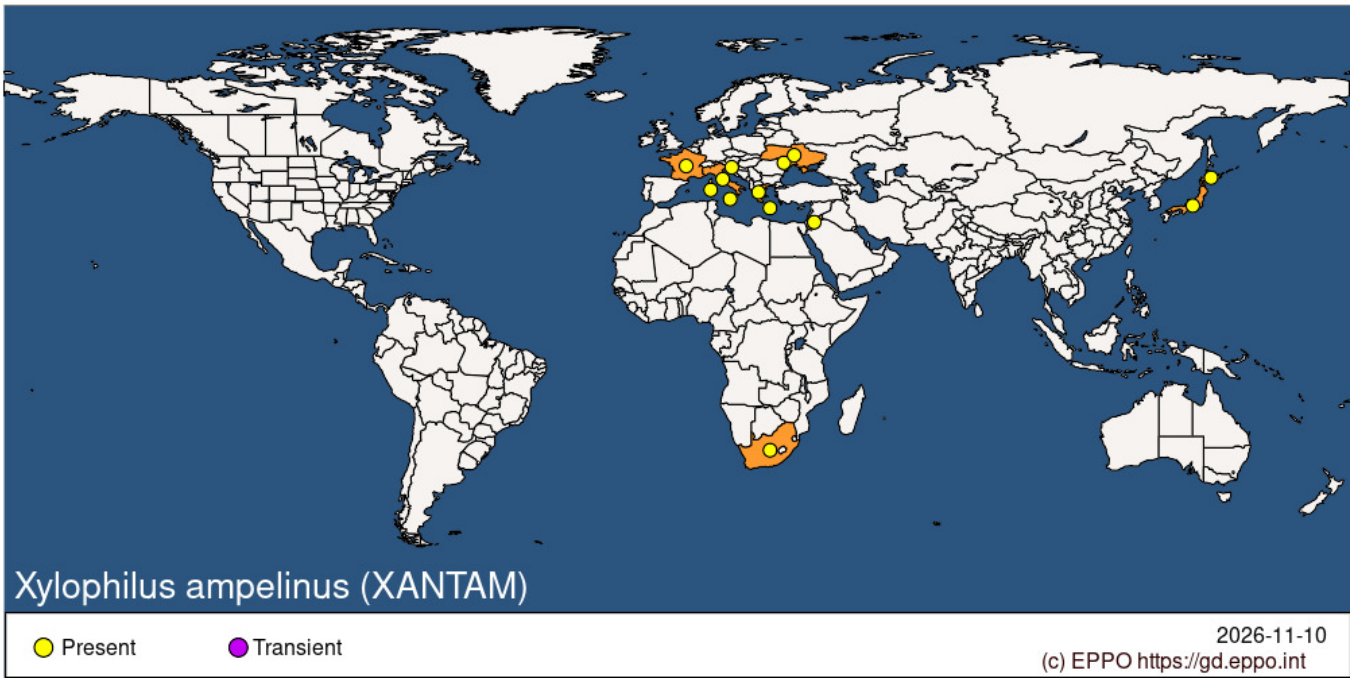
HOSTS

European grapevine (*Vitis vinifera*) is the only known host so far (Panagopoulos, 1988), and the bacterium can infect scions and rootstocks. The susceptibility of American rootstocks of other *Vitis* spp. (which are very commonly used in Europe) is not yet known.

Host list: *Vitis vinifera*

GEOGRAPHICAL DISTRIBUTION

Bacterial blight and canker of grapevine caused by *X. ampelinus* has been observed in several grapevine growing areas of the Mediterranean basin, Russia, South Africa and Japan. In the past, reports of symptoms resembling those of 'maladie d'Oléron' (which in France was shown to be caused by *X. ampelinus*) have been made from Argentina, Portugal, Switzerland, Tunisia, Turkey, and former Yugoslavia but the presence of *X. ampelinus* has not been confirmed with modern diagnostic methods (except for Slovenia). In Spain, the disease was first discovered in 1978 and it has been of some importance in the Cariñena area, Aragón (Lopez *et al.*, 1981). However, it was reported as no longer found in the 2010s. Given the erratic occurrence of the disease over the years, its frequent and sometimes long-lasting latency, its frequent confusion with other diseases and the absence of systematic surveys in many areas, there is uncertainty about its geographical distribution. *X. ampelinus* may be present in more grapevine growing countries than is currently known, remaining undetected, as was the case for many years for *Xylella fastidiosa* (Moralejo *et al*., 2020), another systemic pathogenic bacterium of grapevine which is difficult to diagnose solely on the basis of symptoms.



EPPO Region: France (mainland), Greece (mainland, Kriti), Italy (mainland, Sardegna, Sicilia), Jordan, Moldova, Republic of, Slovenia, Ukraine
Africa: South Africa
Asia: Japan (Hokkaido, Honshu), Jordan

BIOLOGY

The life-cycle of *X. ampelinus* has not been completely elucidated. Primary infections occur mainly on 1- or 2-years old shoots and on leaves and flowers. Infections of fruits have not been observed. The pathogen is readily transmitted with pruning and harvesting tools (EFSA, 2014). During pruning the bacterium enters healthy tissues mainly through pruning wounds, especially in wet and windy weather, but also through natural openings (stomata), causing leaf spots. The bacteria then spread to other shoots and/or leaves in the early summer via bleeding sap and from those

organs to the trunk. Bacteria are emitted in bleeding sap during the whole bleeding period (Grall & Manceau, 2003; Grall *et al*., 2005). The disease progress is associated with warm moist conditions and spread of the bacterium is favoured by overhead sprinkler irrigation. The bacterium survives in old wood, often in high numbers, and may be transmitted from nursery to nursery via infected cuttings. Endogenous colonization of canes only takes place during dormancy. From initial disease foci, local spread in vineyards tends to occur along the rows. Transmission of the pathogen via soil and root material was reported, when flooding was used to control grapevine phylloxera (*Daktulosphaira* (*Viteus) vitifoliae*) (Panagopoulos, 1988).

Inside the plant the bacterium spreads mainly via the xylem, where it is often present in biofilms. In later stages phloem and cambial tissues are also infected. The bacterium may often be present as a latent (symptomless) infection for several years and symptom expression is highly variable from year to year (Panagopoulos, 1987; Grall & Manceau, 2003). *X. ampelinus* may be spread by (latently) infected grafting and planting material (cuttings and rootstocks) in (international) trade and cultivation (Panagopoulos, 1987). Insect vectors are not known.

Remarkably, under the Hokkaido winter conditions in Japan *X. ampelinus* apparently did not survive on plant surfaces or in xylem tissues, but epiphytically on the underside surface of the bract and bud wool (Komatsu & Kondo, 2015a). These authors also showed that the distribution of the pathogen in the plant is uneven in space and over time.

Cultivar susceptibility, weather conditions and agricultural practices can therefore have a very important influence on possible disease outbreaks and their severity (Panagopoulos, 1987; Grall & Manceau, 2003).

DETECTION AND IDENTIFICATION

Symptoms

On shoots and branches

On infected shoots, bud break is delayed or does not occur. Symptoms are observed in early spring to June. Infection usually occurs on the lower two to three nodes of shoots that are 12-30 cm long, and spreads slowly upward. Initially, linear reddish-brown streaks appear, extending from the base to the shoot tip; then, more or less lens-shaped cracks and cankers develop, sometimes as deep as the pith. Branches may be swollen, due to cambial hyperplasia that are soft and resemble soft cheese, showing brown discoloration and longitudinal cracks in later stages of their development. Shoots subsequently wilt, droop and desiccate. On very young shoots, discoloration is less common and the whole young shoot dies back. In cases of severe infection, a large number of adventitious buds develop, but these quickly die back. Infected shoots are shorter, giving the vine a stunted appearance. Cross-sections of stems will reveal browning of the vascular tissues. Symptoms on stalks of grape bunches are similar to those observed on shoots. Large, developing cankers on branches may kill them and eventually lead to death of the whole plant.

On leaves

Leaves may be penetrated by the bacterium via the petiole and then the veins, in which case a large sector of the leaf or the whole leaf becomes necrotic and dies. Alternatively, leaves are penetrated directly via the stomata, with development of angular, reddish-brown spots, often surrounded by a yellow halo. The centre of the spot may dry and drop out, giving a ‘shot hole’ symptom appearance. When infection occurs through the hydathodes, reddish-brown discolorations develop on the leaf tips. Light-yellow bacterial ooze may be seen on infected leaves when humidity is high.

On inflorescences and flowers

Cracks may appear on the rachis. Infected flowers which have not reached maturity turn black and die back.

On roots

Roots may also be attacked, resulting in retardation of shoot growth, either when the plant is grafted or when it is grown on its own rootstock.

Detailed description of symptoms can be found in Panagopoulos (1969 and 1988) and Grasso *et al*. (1979).

Similarities to other species/conditions

On shoots, symptoms of bacterial blight and canker may be confused with those caused by the fungus *Eutypa lata*. Furthermore, symptoms may be confused with those of several other diseases such as cane and leaf spot caused by the fungus *Phomopsis viticola*, heavy infections by the fungus *Sphaceloma ampelinum* (but without brown discoloration of the xylem vessels), grapevine flavescence dorée phytoplasma, grapevine fan leaf virus, as well as with frost and hail damage (shot holes). Failure of spurs to sprout, dead branches and /or vascular browning can also be confused with symptoms caused by the wood fungi: *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, *Fomitiporia mediterranea*, *Botryosphaeria* spp. or *Verticillium* spp. (Panagopoulos, 1988; EPPO, 2009; CABI, 2020).

Morphology

X. ampelinus is a Gram-negative rod with one polar flagellum. In culture, at 25°C, growth is very slow (and upon isolation on general media sometimes absent or overgrown by fast growing saprophytic bacteria that also may form yellow colonies); non-mucoid, smooth, yellow, round, entire colonies up to 2 mm in 7-10 days growth on yeast-peptone glucose agar.

Detection and inspection methods

Inspection in the field

Visual inspection with symptom assessment in surveys or other field operations (e.g. advisory service) should be followed when possible by laboratory diagnosis.

Laboratory detection and diagnosis

Serologically *X. ampelinus* can be detected using immunofluorescent microscopy or (DAS-) ELISA. Monoclonal and polyclonal antisera are commercially available. Fatty acid analysis, conventional PCR and real-time PCR methods for detection and identification are described in the EPPO Standard on *Xylophilus ampelinus* (EPPO, 2009, also see Manceau *et al*., 2005 and Dreo *et al*., 2007). A pathogenicity test in the greenhouse, using young shoots according to Panagopoulos (1969) is detailed in the EPPO Standard. Enrichment of the bacteria in plant samples before isolation or PCR may be beneficial, especially in the case of suspected latent infections (Serfontein *et al*., 1997).

Details about detection and identification methods (including methods for extraction of bacterial cells and DNA, biochemical, serological and molecular and pathogenicity tests for latent and symptomatic infected material, flow chart, media, chemicals and reference material are provided in the EPPO Standard PM 7/96, 2009 on *Xylophilus ampelinus*.

PATHWAYS FOR MOVEMENT

Dispersal is limited to the vineyard and immediate surrounding area. Viticultural practices, mainly pruning and possibly also (sprinkler) irrigation/flooding contribute to the disease dispersal within the vineyard, and adjacent ones. Bacteria can be spread with water (including rain splash) when plant sap is exuding from wounds (Panagopoulos, 1988; Grall & Manceau, 2003). In international trade, *X. ampelinus* is liable to be carried on infected grapevine planting and grafting material (cuttings, rootstocks and grafted plants), especially due to the fact that the bacterium can be present in a latent form in the wood. In conclusion, plants for planting are a likely pathway of spread to suitable grapevine growing areas in the world. This was strongly suspected for the occurrence of the disease in South Africa (Du Plessis, 1940). No alternative hosts, carrier plants or insect vectors have been found.

PEST SIGNIFICANCE

Economic impact

Significant crop damage, including yield and quality losses, are caused by the development of cracks and cankers on stems, leading to the dieback and desiccation of infected flowers and eventually death of whole vines. Severity of the disease appears to be dependent on cultivar and strain (Peros and Ridé, 1997).

Severe infection of susceptible cultivars can lead to serious harvest losses. In South Africa, 70% and 80% losses were reported in 1940 and 1980 respectively in some areas (Du Plessis 1940; Botha *et al*., 2001). Since then, however, the disease has only appeared sporadically in South Africa and, when preventive copper sprays were used, it was no longer of no economic importance.

In France, from the end of the 1960s to the 1990s, serious damage has been reported, particularly on Alicante Bouschet and Ugni Blanc cultivars in Charente, and on Grenache and Maccabeu in Languedoc. Vines growing on their own roots in the irrigated areas around Narbonne were most severely affected. In outbreaks in the early 21st century Ugni Blanc, Colombard, Grenache and Clairette Muscat were the most sensitive cultivars in the Die, Cognac and Armagnac regions (Manceau *et al*., 2005).

In Greece, the disease is widespread in Crete, especially in Iraklion county, where it occurs mainly on the very susceptible cultivar Sultanine. It has spread to some other Aegean islands. On the mainland, where it was previously limited to the Kynegos area in the South Peloponnesos on cv. Corinthe noir, it has appeared in two of the major grape-growing counties in West Peloponnesos.

As there are few surveys/studies on the disease, its impact is difficult to assess and due to the possible confusion with other pathogens it may be underestimated.

Control

Control can be obtained by preventive measures such as application of good viticultural practices, careful pruning under dry weather conditions, disinfection of tools, destruction of infected shoots and avoiding overhead sprinkler irrigation. Infected vines should be removed and destroyed immediately after disease detection. Chemicals have failed to control the disease but copper sprays, eventually in combination with mancozeb or maneb (in countries where these active ingredients are authorized) applied early in the growing season and/or at an early stage of disease development can be used in a preventive way (Panagopoulos, 1987; Bugaret *et al*., 2002; Komatsu and Kondo 2015b). Infected shoots should be destroyed.

Management of bacterial blight can also be achieved by rapid, reliable detection and identification of *X. ampelinus* and use of pathogen-free propagative and planting material.

Hot water treatment of canes, at 52 °C for 45 minutes, was shown to eliminate *X. ampelinus* efficiently in grapevine cuttings (Roberts, 1993; Psallidas & Argyropoulou (1994)). This treatment also eliminates *Xylella fastidiosa* and the phytoplasmas associated with flavescence dorée and bois noir, other bacterial pathogens of grapevine (EFSA, 2015b, EPPO, 2012).

Lastly, phytosanitary (quarantine) measures can be implemented to reduce the risk of long-distance dissemination of the pathogen (FAO, 2020; EPPO, 2002, 2008, 2018).

Phytosanitary risk

As *X. ampelinus* can be moved on grapevine propagation and planting material, there is an obvious danger that the disease will spread into areas previously not affected by the bacterium. It is difficult to isolate the pathogen from propagation material both when latent and when in the infectious phase. Enrichment of the pathogen before isolation or detection methods was successfully applied by Serfontein *et al*. (1997). Further spread could lead to severe economic losses, especially since no efficient control measures are known. A general problem with this disease is that the symptoms may be easily overlooked or confused with other disorders, as was also the case for *Xylella fastidiosa* infections in Europe (Moralejo *et al*., 2020). In addition, *X. ampelinus* can be difficult to detect in

propagation material, especially when infections are latent or at an early stage. In countries where this bacterium is present, it often took a long time (from several years to more than 10 years) after first symptoms observation to isolate and characterize the true pathogen (Dreo *et al*., 2005; Seljak *et al*., 2005). In Sicily, bacterial blight was diagnosed in 1973, but according to growers it had been already present since 1954 (Grasso *et al*., 1979). In Japan, disease symptoms were confused with those of *Phomopsis viticola* for several years and only when fungal treatments were unsuccessful the bacterium was eventually isolated and identified (Komatsu and Kondo, 2015a). Moreover, the disease has an erratic character, prevalent in some years, absent or very minor in others. Its distribution therefore may well be underestimated, as in many grapevine-growing countries no systematic surveys for *X. ampelinus* are performed. Declarations of eradication and absence should therefore be regarded with caution.

To date *X. ampelinus* has been reported from 5 of the 21 grapevine-producing countries of the EU. Disease occurrence presently (2021) is sporadic, even when occurring in susceptible cultivars. However, there are no ecological studies, as performed e.g., for *Xylella fastidiosa* (EFSA 2015a; Godefroid *et al*., 2018; Purcell, 1997) that map the (worldwide or European) possibilities of occurrence and model damage/losses upon introduction.

PHYTOSANITARY MEASURES

Direct inspection of imported planting material is not very reliable, due to the occurrence of latent infections and therefore, when material is imported from areas where the disease is known to occur, nursery inspections and laboratory testing are necessary (EPPO 2009, 2018). For the design of surveys, including those for *X. ampelinus*, see FAO, 2020. Plants for planting should originate from an area where *X. ampelinus* has not been found or originate from mother plants which have been laboratory tested against *X. ampelinus* (EPPO, 2002 For example, this could be achieved within the framework of a certification scheme, during which the absence of *X. ampelinus* is verified by appropriate testing during the multiplication process (EPPO, 2008).

The presence and possible importance of *X. ampelinus* needs to be checked in countries or regions where the maladie d'Oléron was previously reported. Over the past forty years, disease outbreaks have been sporadic and many years without noticeable symptoms may pass between outbreaks in an infected vineyard/area.

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ACKNOWLEDGEMENTS

This datasheet was extensively revised in 2021 by Dr Jaap D. Janse, independent consultant, bacteriologist. His valuable contribution is gratefully acknowledged.

How to cite this datasheet?

EPPO (2026) *Xylophilus ampelinus*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int

Datasheet history

This datasheet was first published in the EPPO Bulletin in 1984, revised in the two editions of 'Quarantine Pests for Europe' in 1992 and 1997, as well as in 2021. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', ‘Hosts’, and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

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Co-funded by the European Union