Data Sheets on Quarantine Pests

Xylella fastidiosa

Certain vectors of *Xylella fastidiosa* are individually mentioned in EU Directive 77/93. Since their importance only arises in relation to *X. fastidiosa*, they are covered in this data sheet, together with the vector situation for this pest in general.

IDENTITY

- ***Xylella fastidiosa***

Name: *Xylella fastidiosa* Wells *et al.*
Taxonomic position: Bacteria: Gracilicutes
Common names: Pierce's disease, California vine disease, Anaheim disease (grapevine), leaf scorch (almond), dwarf (lucerne), phony disease (peach), leaf scald (plum), leaf scorch (elm, oak, plane, mulberry, maple), variegated chlorosis (citrus) (English)
Maladie de Pierce (grapevine), chlorose variégée (citrus) (French)
Notes on taxonomy and nomenclature: The bacterium causing Pierce's disease of grapevine has only recently been grown in culture and named as *Xylella fastidiosa* (Wells *et al.*, 1987). It now appears that a number of xylem-limited fastidious bacteria from different hosts can be cultured by the same techniques, and can be referred to the same species. These include some which were already known by transmission experiments to be the same as the grapevine pathogen and some which previously were considered distinct and still appear separated biologically (see Hosts) (Raju & Wells, 1986). One of these is peach phony bacterium (Hopkins *et al.*, 1973), also causing plum leaf scald, which was previously listed and described separately by EPPO (OEPP/EPPO, 1986). Another is the bacterium responsible for citrus variegated chlorosis in South America. The grapevine-virulent strains do not infect peach, whereas the strains virulent in peach do not cause disease in grapevine (Hopkins, 1988b). The citrus strains seem more serologically related to the peach strains than to the grapevine strains. Strains tested so far fall into these two broad categories. This data sheet refers principally to the fairly well characterized grapevine and peach pathogens, with as much detail as is available on the recently described citrus pathogen. It only very briefly attempts to cover the diseases caused by *X. fastidiosa sensu lato* on other hosts. It is expected that, as host-range and cross-inoculation studies are carried out, a concept will emerge of host-specialized forms (pathovars) within *X. fastidiosa*.
Bayer computer code: XYLEFA
EPPO A1 list: Nos 137 & 166
EU Annex designation: I/A1 - as *Xylella fastidiosa* on grapevine, peach phony rickettsia on peach and variegated chlorosis on citrus

- **Vectors**

Numerous species of Cicadellidae and Cercopidae (Insecta: Hemiptera: Homoptera) are known to be vectors of *X. fastidiosa*. *Carneocephala fulgida* Nottingham, *Draeculacephala minerva* Ball and *Graphocephala atropunctata* (Signoret) are among those most frequently mentioned in the literature as vectors to grapevine.

EU Annex designation: I/A1 - as Cicadellidae (non-European) known to be vectors of Pierce's disease, such as *Carneocephala fulgida*, *Draeculacephala minerva* and *Graphocephala atropunctata*

HOSTS

The principal host is grapevine (*Vitis vinifera*) and also the American species *V. labrusca* and *V. riparia*. Other American species used as rootstocks (*V. aestivalis*, *V. berlandieri*, *V. candicans*, *V. rupestris*), and hybrids derived from them are resistant, as is *V. rotundifolia* (Goheen & Hopkins, 1988). Almonds (*Prunus dulcis*) and lucerne (*Medicago sativa*) are minor cultivated hosts of the grapevine-infecting bacterium, and numerous wild plants and weeds can carry it without symptoms (e.g. wild grasses, sedges, lilies, various bushes and trees) (Raju *et al*., 1983; Hopkins & Adlerz, 1988).

Peaches (*Prunus persica*) are another major host (phony disease), attacked by a distinct form of *X. fastidiosa* also found in *P. salicina* (causing leaf scald). All cultivars, forms and hybrids of peach are attacked, whether on their own roots or other rootstocks. Plums (*Prunus domestica*), almonds (*P. dulcis*), apricots (*P. armeniaca*) and the wild *P. angustifolia* were reported susceptible to phony disease before the association with *X. fastidiosa* was established. This reported range partly overlaps that of the grapevine-infecting strain and further studies are now needed to clarify the host ranges of the different strains. Various perennial weeds of orchards, such as *Sorghum halepense*, may act as reservoirs for the peach-infecting strain (Yonce & Chang, 1987).

X. fastidiosa in the wide sense also attacks: *Acer rubrum* (Sherald *et al.*, 1987), *Morus rubra* (Kostka *et al.*, 1986), *Platanus occidentalis* (Sherald, 1993) (wilt and leaf scorch), *Quercus rubra* (Chang & Walker, 1988), *Ulmus americana* and *Vinca minor* (stunt). Strains from *Ulmus* and from *P. occidentalis* are not reciprocally infectious (Sherald, 1993). The bacteria involved are not known to be transmissible to grapevine. Until their relationships and pest significance have been clarified, they can all be regarded as potentially dangerous for the EPPO region.

X. fastidiosa causes a newly recognized disease, citrus variegated chlorosis, in Brazil (Lee *et al*., 1991; Beretta *et al.*, 1992). The disease affects mostly oranges (*Citrus sinensis*); it has been observed especially on cultivars Pera, Hamlin, Natal and Valencia. It occurs on trees propagated on all commonly used rootstocks in Brazil: *C. limonia*, *C. reshni* and *C. volkameriana*. The disease has not been observed on *C. latifolia* or mandarins (*C. reticulata*), even when the trees were planted in severely affected orange groves. Some weed species are also hosts and act as reservoirs of infection.

It has also been suggested (Hopkins, 1988a) that *X. fastidiosa* is associated with citrus blight in Florida (USA) and artificial inoculation has reproduced symptoms (EPPO/CABI, 1996). Control of known *X. fastidiosa* vectors reduces the rate of spread of citrus blight (Adlerz *et al.*, 1989). However, Koch's postulates have not been satisfied in this case. In fact, no relationship has been found between citrus variegated chlorosis and citrus blight in Brazil (declinamiento). The diagnostic tests used to detect trees suffering from blight indicate that variegated chlorosis is a separate disease. The water flow in trunk xylem is normal, the zinc levels in the trunk wood are not elevated and amorphous plugs are not present. The proteins reported to be unique to blighted trees are not present, and there is no cross reactivity of affected trees with antisera developed for citrus blight proteins. Wilt symptoms do not occur on affected trees as they often do on blighted trees.

In the EPPO region, grapevine and citrus are clearly the most significant potential hosts, though peach is also important. Many other hosts could carry the bacterium, without necessarily being significantly affected themselves.

GEOGRAPHICAL DISTRIBUTION

The distribution given is for the grapevine, peach and citrus pathogens. Apparently some strains of *X. fastidiosa sensu lato* occur farther north in North America.

EPPO region: Absent. Unconfirmed interceptions on grapevine material imported from the USA (EPPO Reporting Service 500/02, 505/13).

Asia: India (on almonds; Jindal & Sharma, 1987; this isolated record from the Old World is based on a test described in the 1970s; it requires confirmation by more modern methods). Taiwan (pear leaf scorch is caused by a bacterium similar to *X. fastidiosa* but with questionable serological relatedness) (Leu *et al.*, 1993).

North America: Mexico, USA (Alabama, Arizona, California, Florida, Georgia, Louisiana, Mississippi, Missouri, Montana, North Carolina, South Carolina, Texas); oak scorch found in Kentucky and as far north as New York and West Virginia.

Central America and Caribbean: Costa Rica, probably most countries in Central America.

South America: The citrus disease has been reported from Argentina (Brlansky *et al.*, 1991) and Brazil (São Paulo, Minas Gerais, Rio de Janeiro; rapidly spreading since the first report in 1987). The grapevine disease has been reported from Venezuela (Jimenez, 1985). Plum leaf scald has been recorded in most areas where *Prunus salicina* is grown in South America (EPPO Reporting Service, 503/08).

EU: Absent.

Distribution map: See CMI (1980, No. 262).

BIOLOGY

- ***Xylella fastidiosa***

X. fastidiosa proliferates only in xylem vessels, in roots, stems and leaves. The vessels are ultimately blocked by bacterial aggregates and by tyloses and gums formed by the plant. The bacterium is efficiently acquired by vector insects, with no latent period, and persists in infective adult insects indefinitely (Severin, 1949) (see below). In tests made during the 1940s in California (USA), 75 of 100 tested plant species proved to be hosts from which vectors could acquire the pathogen by feeding (Freitag, 1951). Most of these hosts have only very mild or no symptoms when infected. Recent experiments show that the bacterium multiplies at the point of inoculation but does not move systemically in some of these host species (B. Hill and A. H. Purcell, personal communication).

Winter climate is probably the key factor in delimiting the areas where *X. fastidiosa* can persist from one season to the next. Pierce's disease and phony disease only occur in areas with a mild winter, presumably in relation to survival of the bacterium in dormant plants (Purcell, 1989). Experimental cold therapy of diseased grapevines suggests that freezing temperatures can eliminate the bacterium directly from plants (Purcell, 1980). Wet winters promote survival of high vector populations and favour disease spread in regions with dry summers.

X. fastidiosa is not seed-borne. It tends to multiply rapidly in infected grapevines, causing their death within a single season, or over a few years. Infected vines are conspicuous and easily destroyed. Thus it does not tend to persist in latent form in grapevines, and its populations are maintained by latent multiplication in wild plants.

On citrus, the disease has been discovered too recently for much detail to be available on biology. The pathogen behaves in the same way as a xylem-inhabiting bacterium. It takes 9-12 months incubation before symptoms appear in nursery trees. Thus, the trees and propagative budwood are symptomless carriers of the bacteria during this period. No vector has yet been specifically identified. A survey of two different citrus areas in São Paulo has shown that several species of sharpshooter occur on citrus, which may explain the rapid spread of the disease within citrus groves in Brazil.

- **Vectors**

Virtually all sucking insects that feed predominantly on xylem fluid are potential vectors (Purcell, 1989). Leafhoppers (Cicadellidae) in the subfamily Cicadellinae (sharpshooters) and spittle bugs or froghoppers (Cercopidae) are by far the most common species of known vectors within the natural range of *X. fastidiosa* in North America. *Cicadella viridis* (Cicadellinae) and the meadow spittle bug, *Philaenus spumarius* (Cercopidae), are common and widespread in central and southern Europe. In California (USA), all tested members of the subfamily Cicadellinae (including *Carneocephala fulgida*, *Draeculacephala minerva* and *Graphocephala atropunctata*) were vectors of the grapevine strain. *Homalodisca coagulata, H. insolita*, *Oncometopia orbona*, *Graphocephala versuta* and *Cuerna costalis* are reported vectors of the peach strain (Turner & Pollard, 1959; Yonce, 1983). All these are xylem-feeding suctorial insects which acquire the bacterium rapidly on feeding (less than 2 h). The bacterium adheres to the mouthparts and is released directly from them when the insect feeds again (Purcell *et al*., 1979). It multiplies in the vector but does not circulate in its haemolymph, nor does it require a latent period before transmission (unlike a phytoplasma). Transmission is usually from wild, generally symptomless, hosts to cultivated hosts (grapevines, peaches) rather than between cultivated hosts, though the latter can occur. Feeding preferences of *Graphocephala atropunctata* for different grapevine cultivars have been noted (Purcell, 1981).

The biology of the vectors is important in understanding the epidemiology of the disease. In California (USA), species such as *D. minerva* and *C. fulgida* inhabit permanent pastures alongside vineyards, or live on weeds within them. Irrigation and weed control practices which produce foci of preferred host plants, including *Cynodon dactylon* and *Echinochloa crus-galli*, increase vector populations and the spread of the bacterium (Purcell & Frazier, 1985). Other species, such as *G. atropunctata*, multiply on grapevine but overwinter on a variety of other wild hosts.

DETECTION AND IDENTIFICATION

Symptoms

- ***Xylella fastidiosa***

On grapevines

The most characteristic symptom of primary infection is leaf scorch. An early sign is sudden drying of part of a green leaf, which then turns brown while adjacent tissues turn yellow or red. The desiccation spreads and the whole leaf may shrivel and drop, leaving only the petiole attached. Diseased stems often mature irregularly, with patches of brown and green tissue. In later years, infected plants develop late and produce stunted chlorotic shoots. They rarely survive more than a year or two, despite any signs of recovery.

On peaches

Young shoots are stunted and bear greener, denser foliage than healthy trees. Lateral branches grow horizontally or droop, so that the tree seems uniform, compact and rounded. Leaves and flowers appear early, and leaves remain on the tree longer than on healthy trees. Affected trees yield increasingly fewer and smaller fruits until, after 3-5 years, they become economically worthless.

On citrus

Trees can start showing the symptoms of variegated chlorosis from nursery size up to 7-10 years of age. These younger trees become systemically affected by *X. fastidiosa*. Trees more than 15 years old are not usually totally affected, but rather have one or two major scaffold branches showing symptoms. Affected trees show foliar chlorosis resembling zinc deficiency with interveinal chlorosis. The chlorosis appears on young leaves as they mature and may also occur on older leaves. Newly affected trees show sectoring of symptoms, whereas trees which have been affected for a period of time show the variegated chlorosis throughout the canopy. As the leaves mature, small, light-brown, slightly raised gummy lesions (becoming dark-brown or even necrotic) appear on the underside, corresponding to the yellow chlorotic areas on the upper side.

Fruit size is greatly reduced; it may take 550 affected fruits to fill a field box, compared with 250 normal fruits. The sugar content of affected fruit is higher than in non-affected fruit, and the fruit has a hard rind, causing damage to juicing machines. Blossom and fruit set occur at the same time on healthy and affected trees, but normal fruit thinning does not occur on affected trees and the fruits remain small but open earlier. Since more fruits remain, total production is not greatly reduced. On affected trees of cv. Pera and other orange cultivars, fruits often occur in clusters of 4-10, resembling grape clusters. Affected trees show stunting and slow growth rate; twigs and branches die back and the canopy thins, but affected trees do not die.

- **Vectors**

Vector feeding causes no visible damage. Xylem feeders are prodigious feeders, consuming hundreds of times their body volumes per day in xylem sap. Most non-xylem-feeding leafhoppers produce a sugary or particulate excrement, but that of xylem feeders is watery, drying to a fine whitish powder where abundant. The excrement of froghopper nymphs takes the form of persistent bubbles or "froth" that surrounds the body of the insect, presumably to provide protection from natural enemies.

Morphology

- ***Xylella fastidiosa***

X. fastidiosa is a fastidious Gram-negative xylem-limited bacterium, rod-shaped with distinctive rippled cell walls. It is non-flagellate, does not form spores and measures 0.1-0.5 x 1-5 µm. The peach strain was given by Nyland *et al.* (1973) as 0.35 x 2.3 µm. See also Bradbury (1991).

- **Vectors**

Adult sharpshooters are distinguished from other leafhopper subfamilies by the possession of highly "inflated" or "swollen" faces caused by the massive musculature required by the cibarial (pharyngeal) pump to suck in large quantities of xylem (Young, 1968). Sharpshooters are typically among the largest of leafhoppers. The smaller species (e.g., *Carneocephala fulgida*) may be about 4 mm in length, while larger species such as *Homaladisca coagulata* may exceed 15 mm. Nymphs generally resemble adults in form but usually differ markedly in coloration and markings. The subfamily is quite diverse, especially in the American tropics, with over 210 genera (Young, 1977). Coloration varies from uniformly green (e.g., *Draeculacephala* spp.) or other cryptic coloration to combinations of bright-red, orange, yellow and blue tropical species. Sharpshooters typically have broad plant host ranges. Even those species that are found on a single or few host species are capable of prolonged survival in captivity on unusual hosts. The rhododendron sharpshooter, *Graphocephala fennahi*, is a North American species now established in Europe, where it occurs not only on *Rhododendron* but on many other woody plants. Female sharpshooters and spittlebugs insert their eggs into plant tissues. The eggs of some sharpshooter species remain dormant over winter.

Cercopidae may be distinguished from leafhoppers, which possess one or more rows of spines along the hind tibia, by the smooth hind tibia with a few or a circlet of stout spines at the apex of the tibia. The body shape of froghoppers is generally stouter than that of leafhoppers.

Detection and inspection methods

X. fastidiosa can be detected microscopically in vessels in cross-sections of petioles, and by electron microscopy (French *et al*., 1977) or SDS-PAGE (Bazzi *et al.*, 1994). Methods such as grafting to susceptible indicator plants or vector tests (Hutchins *et al*., 1953) are still available, and may have their place in certification schemes in which woody indicators are routinely used. *X. fastidiosa* can also be isolated onto suitable selective media (Raju *et al*., 1982; Wells *et al*., 1983; Davis *et al.*, 1983). Serological methods are less sensitive (10- to 100-fold) than culture but are the easiest means of detecting and identifying the bacterium, by ELISA or use of fluorescent antibodies (French *et al*., 1978; Walter, 1987; Hopkins & Adlerz, 1988; Sherald & Lei, 1991). Strains differ in quantitative reaction to antisera and in ease and efficiency of culture. Recently DNA hybridization probes and polymerase chain reaction (PCR) primers specific to *X. fastidiosa* have been developed (Minsavage *et al.*, 1994). *X. fastidiosa* can also be detected in its insect vectors (Yonce & Chang, 1987).

A dot immunobinding assay (DIBA) has recently been developed, and used in particular for *X. fastidiosa* in citrus.

MEANS OF MOVEMENT AND DISPERSAL

X. fastidiosa is dispersed by its vectors, which could ensure spread on a local scale. Vectors could also be carried internationally on plants, or fruits, of grapevine, peach or other plants. However, the bacterium is not passed through the eggs of the vectors and does not persist past moulting in immatures. The bacterium could be spread in planting material, but this has not been considered a major risk for grapevine in North America, where such material does not survive long enough to present a hazard (Goheen & Hopkins, 1988). However, the bacterium has presumably reached the limit of its natural distribution in America, so that infected planting material presents a relatively minor risk of introduction and establishment. The situation in the EPPO region is quite different, since large areas of susceptible grapevines are at risk, and the bacterium could readily be introduced on grapevine planting material or in a wide range of species of symptomless plant hosts from which vector spread may be possible.

The pathogen has spread on citrus in Brazil both with infected planting material and probably by insect vectors.

PEST SIGNIFICANCE

Economic impact and control

In the USA, within the main areas where *X. fastidiosa* occurs naturally (coastal plains of the Gulf of Mexico), *Vitis vinifera* and *V. labrusca* cannot be cultivated because they are rapidly infected due to high rates of natural spread. As a consequence, only selections of *V. rotundifolia* (muscadine) and specially bred resistant hybrids can be cultivated. The same situation exists throughout tropical America. In California, however, *X. fastidiosa* occurs only in "hot spots". *V. vinifera* has to be cultivated outside these hot spots. There have been considerable losses in the past, before this situation was clarified. Control is based essentially on the principle of locating and delimiting hot spots (Goheen & Hopkins, 1988), by trapping insect vectors and testing wild hosts serologically. Vector habitats can be eliminated as a preventative measure, but this is not possible in all situations. Control has been attempted by antibiotic treatment of grapevines against *X. fastidiosa* and by insecticide treatment against its vectors, but with only partial success. These methods are little used in practice.

Pierce's disease is thus a major constraint on grapevine production in the USA and tropical America. However, it does not occur in all grapevine-producing areas of the USA and has apparently no tendency to spread to uninfested areas. The distribution of *X. fastidiosa* thus appears to be limited by climatic constraints, affecting the bacterium itself or its vectors.

By contrast, in peaches, phony disease does not kill trees or cause dieback, but it does significantly reduce the size and number of fruits. An analysis of biophysical effects on peach trees has been made by Anderson & French (1987). The disease was extremely important in the south-eastern USA in the 1940s, when 5-year-old orchards were often found to be 50% affected and older orchards entirely so. However, the efficient control methods now available (insecticides, destruction of infected trees, elimination of wild host plants around orchards) allow a high degree of control, except in areas where incidence is very high.

In citrus, variegated chlorosis has been described by Roistacher as "the world's most destructive disease of sweet orange". It has spread rapidly through large areas of southern Brazil and appears to be unstoppable. In a June survey made in 1990, it was reported that 13 sites out of 920 examined in São Paulo State had infected trees. In a follow-up survey in August 1991, 72 out of the 920 sites were infected (a 5-fold increase in a period of just 14 months). A total of 1.8 million trees are now infected, and some growers in São Paulo state are now planting mangoes instead of citrus.

Phytosanitary risk

EPPO considers *X. fastidiosa* as an A1 quarantine pest (OEPP/EPPO, 1989) and it is also a quarantine pest for COSAVE. In the EPPO region, it is clear that the grapevine strain of *X. fastidiosa* has the potential to kill large numbers of grapevines and to make areas unfit for growing *V. vinifera*. Its vectors in North America do not occur in the EPPO region, but vector capacity is so non-specific that one could certainly expect European Cicadellinae (e.g., *Cicadella viridis*) or Cercopidae to transmit the bacterium if introduced. The main danger in the long term is that *X. fastidiosa* could become established in natural vegetation which would then act as a reservoir for infection of vineyards. It is less likely that Pierce's disease would become a problem in the production of planting material, for it is easily detected and rapidly self-eliminating. Nevertheless, infected planting material could introduce the disease to new areas. *X. fastidiosa* is only likely to establish in the warmer parts of the EPPO region such as the southern Iberian and Italian peninsulas and the lowlands of Greece, which have winter temperatures approaching those of the southern USA. However, its potential natural range in Europe may depend on the biology of potential vectors and is accordingly rather difficult to assess.

The South American strain on citrus also presents a major risk, for the climatic conditions of Mediterranean countries seem favourable to its development. The reported damage in Brazil suggests potential damage even greater than for the grapevine disease. No critical evaluation of possible European vectors has yet been made, but the arguments developed above for grapevine should certainly also apply to citrus.

The peach strain is relatively less important, but *X. fastidiosa* still presents a definite danger for peaches, almonds, oaks and, *sensu lato*, for other fruit and amenity trees (see Hosts) (Dunez, 1981).

PHYTOSANITARY MEASURES

Grapevine-growing countries should prohibit or severely restrict importation of grapevine planting material from countries where *X. fastidiosa* occurs. As recommended by EPPO (OEPP/EPPO, 1990), if planting material is imported under licence, it should be maintained in post-entry quarantine for 2 years and shown to be free from the pest. Imported plants and fruits should be free from vectors, possibly by use of an appropriate treatment. A temperature treatment has been shown to eliminate the bacterium (45°C for at least 3 h) (Goheen *et al.*, 1973), and may have potential as a phytosanitary measure.

Citrus-growing countries should similarly prohibit or severely restrict importation of citrus planting material from South America. Peach and other *Prunus* material from a country where the peach strain occurs should come from a reliable certification scheme, with particular emphasis on preventing reinfection of healthy material via the vectors.

While the hazard presented by *X. fastidiosa* in other hosts (oak, plane, maple and others) still has to be evaluated, inspection services should be aware that these hosts also present a certain risk.

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