MINIREVIEW

XYLELLA FASTIDIOSA: ITS BIOLOGY, DIAGNOSIS, CONTROL AND RISKS

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SUMMARY

The bacterium *Xylella fastidiosa*, a xylem-inhabiting, vector-transmitted, Gram-negative, very slow growing bacterium, was cultured and properly described for the first time in 1987 in the USA as the cause of Pierce's disease (PD) of grapevine, *Vitis vinifera* (disease observed already in 1884) and as the cause of phony peach disease (PPD) in peach, *Prunus persica* (disease observed in 1890 in the USA) and in 1993 in Brazil as the cause of citrus variegated chlorosis (CVC) or citrus X disease. Moreover, it was found that the bacterium also causes a number of so-called leaf scorch diseases in *Prunus* spp. (including almond leaf scorch or ALS in *Prunus amygdalus* and plum leaf scald or PLS in *Prunus domestica*), *Acer* spp., *Carya illinoinensis* (pecan), *Coffea arabica* (CLC, in Brazil isolated in 1995 and also pathogenic to *Citrus*), *Hedera helix*, *Morus rubra, Nerium oleander* (OLS)*, Platanus occidentalis, Quercus* spp., and *Ulmus americana*. It infects also *Medicago sativa* (alfalfa dwarf) and *Vinca major* (wilting symptoms). Many wild plants may carry the pathogen with, but more often without showing symptoms, such as grasses, sedges and trees. A list of main hosts is presented. All these diseases are not seed-borne and occur mainly in tropical/subtropical areas, although leaf scorch diseases also occur in much colder climate, e.g. oak leaf scorch in eastern North America up to Canada. Several pathogenic varieties of the bacterium have been described, that are often host-specific (e.g., the PD strain will not cause disease if introduced to peach or plum). The following subspecies have been described: (i) *Xylella fastidiosa* subsp. *fastidiosa* (erroneously named *X. f.* subsp. *piercei*), PD and LSA, strains from cultivated grape, alfalfa, almond, and maple; (ii) *X. fastidiosa* subsp. *multiplex*, PPD, PLS, strains from peach, elm, plum, pigeon grape, sycamore and almond; (iii) *X. fastidiosa* subsp. *pauca*, CVC, strains from citrus and probably those from coffee (CLC); (iv) *X. fastidiosa* subsp. *sandyi*, strains from *Nerium oleander* (OLS); (v) *X. fastidiosa* subsp. *tashke*, strains from the ornamental tree *Chitalpa tashkentensis*. Vectors are mainly sharpshooters and froghoppers or spittlebugs (Cicadellidae) that lack a latent period, and have no transstadial or transovarial transmission of the bacterium. The pathogen shows persistence in the vector adults, and ability to multiply in the foregut. In North America main vectors (for PD unless indicated) are *Cuerna costalis* (PPD), *Draculacephala minerva* (green sharpshooter) important also in ALS in California; *Graphocephala atropunctata* (blue-green sharpshooter), most important before the introduction of the glassy winged sharpshooter; *G. versuta* (PPD); *Hordnia circellata*, most efficient; *Homalodisca vitripennis* [formerly *H. coagulata* (glassy-winged sharpshooter or GWSS)]; *H. insolita* (PPD), *Oncometopia nigricans*, *O. orbona* (PPD), *Xyphon fulgida* [formerly *Carneocephala fulgida* (red-headed sharpshooter)]. CVC vectors in Brazil are *Acrogonia terminalis*, that lays eggs externally on leaves, *Dilobopterus costalimai* and *Oncometopia fascialis*. Local possible vectors for Europe are *Cicadella viridis* and *Philaenus spumarius* (meadow spittle bug). *X. fastidiosa* is an emerging threat in the south-west USA, mainly due to recent establishment of *H. vitripennis*, providing much more efficient transmission than local vectors, and leading to very serious outbreaks of PD in grapevine, ALS and OLS. GWSS probably first entered California as eggs in plants. The eggs are deposited into plant tissues. In Central and South America *X. fastidiosa* has become very noxious due to the rapid expansion (most likely via distribution of infected planting material) of CVC in *Citrus*, leading to more than a third of all trees in the area having symptoms of CVC, and CLC in coffee. For Europe there are until now only a few unconfirmed reports of the presence of *X. fastidiosa* in grapevine from Kosovo [erroneously mentioned as Slovenia in Janse (2006)] and in France, based on disease symptoms observation. Since *X. fastidiosa* has more that 150 hosts and many of them, including *Vitis* planting material, were and are imported, risk of introduction (especially in latent form) must not be underestimated. Absence of the diseases caused by *X. fastidiosa* will mainly be due to the absence of suitable vectors. However, introduction of the pathogen and vectors with plant material can not be excluded for certain. More-

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over, also local Cicadellidae (see above) could become (potential) vectors. Therefore, *X. fastidiosa* has the A1 quarantine status in the EPPO region and *H. vitripennis*, that has a very large host range and also feeds on almond, peach and plum, was recently put on the EPPO alert list. As in the more northern parts of the USA, *Vitis* varieties in Europe are very susceptible to *X. fastidiosa* and this is really a risk should a vector that could survive the winters of southern Europe become established, also in wild hosts (e.g. wild and domestic plums and wild cherry are symptomless reservoirs in the USA) and cause spring infections that would most likely to persist over the years. The same risk holds true for *Citrus* (sweet oranges, mandarins, and tangerines) and other hosts, such as almond, plum and peach that are widely grown in south-east and south-west Europe, especially in the warmer Mediterranean basin (where a disease-favourable combination of warm nights, regular rainfall/high humidity and long growing season, is present). Possible ways to prevent introduction and to control eventual outbreaks are indicated. The conclusion is that *X. fastidiosa* is a real and emerging threat for Europe, not only for *Vitis* and *Citrus* but also for stone fruits (almond, peach and plum) and oleander (e.g. GWSS likes to feed on oleander), that is difficult to prevent from entering and difficult to control once established, deserving more attention than up till now. Resistance in European grapes is scarce or even absent. Vector control proved not to be very effective in the USA. Cultural practices to keep plants in optimum condition are of importance, but not sufficient and the use of avirulent strains for cross-protection is still in its infancy.

INTRODUCTION AND HISTORY

In the 1880's a 'mysterious' vine disease destroyed *ca.* 14,000 ha of grapes (*Vitis* spp.) and *ca.* 50 wineries had to close down in the Los Angeles area in California. This disease was described in detail in 1887 by N.B. Pierce (1856-1916) and was later named after him: Pierce's disease (PD) of grapevine. Now, 125 years later, PD is still a main concern for grape and wine producers in southern USA (especially California, Texas and Florida). For a long time the causal agent could not be cultured outside the host and was generally regarded as a virus or a non-culturable bacterium (Rickettsia-like organism or RLO). A related disease was recorded in peach (*Prunus persica*) in 1890 in the USA, with outbreaks (mainly in Georgia) in 1929, 1951 and 1976 and was named phony peach disease (PPD).

The causal agent of PD was isolated from grape in pure culture for the first time in 1978 (Davis *et al.*, 1978). However, this xylem-inhabiting, vector-transmitted, Gram-negative, very slow growing bacterium, was only properly described, classified and named *Xylella fastidiosa* in 1987 (Wells *et al.*, 1987). Already in the 1940's sharpshooter leafhoppers and spittlebugs were identified as vectors of PD and PPD (Severin, 1949). Once the bacterium was described and more easily cultured, *X. fastidiosa* was found in large number of other hosts, with or without symptoms. The most important are the so-called leaf scorch and scald diseases of *Prunus* spp. (including almond leaf scorch or ALS in *Prununs amygdalus* and plum leaf scald or PLS in *Prunus domestica*), *Acer* spp., *Carya illinoinensis* (pecan), *Coffea arabica* (coffee leaf scorch or CLS), in Brazil (isolated in 1995 and also pathogenic to *Citrus*), *Hedera helix*, *Morus rubra, Nerium oleander* (oleander leaf scorch or OLS, Grebus *et al*., 1996), *Platanus occidentalis, Quercus* spp., *Ulmus americana*. Furthermore the bacterium was found in *Medicago sativa* (alfalfa dwarf), *Vinca major* (wilting symptoms), and in avocado *Persea americana* (Montero-Astúa *et al*., 2008). Many wild plants were found to carry the pathogen (often latent only), such as grasses, sedges and trees (Freitag, 1951; Blake, 1993; Hartman, 1991, 1992, 2003; Hernandez-Martines *et al*., 2007; Raju *et al*., 1983).

In 1987 in Brazil, a rapidly spreading disorder, similar to PD, called citrus variegated chlorosis (CVC) or citrus X disease of *Citrus,* was observed and *X. fastidiosa* was also isolated from diseased trees in 1993 (Chang *et al.,* 1993). Characterization of *X. fasdidiosa* culminated in whole genome sequencing, and a citrus strain of *X. fastidiosa* actually was the first plant pathogenic bacterium from which the whole genome was sequenced. The sequences of three *Xylella* strains (from almond, oleander and citrus) are now available at Integrated Genomics. Till now, diseases caused by *X. fastidiosa* have only been reported from North and South America (especially Brazil). In Europe, there is only one unconfirmed report from Kosovo (Berisha *et al*., 1998). However, it can not be excluded that the pathogen is present on a low scale and goes still undetected because of unfamiliarity with the symptoms and (still) lack of efficient vectors. This because (wild) grape rootstocks from North America have been imported on a large scale to Europe for their resistance to phylloxera since the end of the nineteenth century. Moreover, the import of other of its many hosts (Table 1) could have lead to incidental introductions that still go unnoticed. Given this situation and the recent outbreak in California due to the introduction of *H. vitripennis* as a new vector, an overview and preliminary risk evaluation of this important pathogen appears desirable.

SYMPTOMS AND TRANSMISSION

Grapevine. First symptoms are sudden drying of large parts of a green leaf. These parts become brown

necrotic and the surrounding tissues become yellow to red. The necrosis is often present at the leaf margins (Fig. 1A). Scorched (burnt-like) leaves usually drop from the distal and not from the usual basal end of the petiole, leaving bare petioles attached to canes, often well after normal leaf fall. PD can be confused with other disorders such as salt toxicity, boron, copper or phosphorus deficiency. In later stages, more yellowing occurs and leafs shrivel and drop. Defoliation, shoot dwarfing and cane stunting, as well as dehydration of fruit clusters may occur. Irregular patches of brown and green tissue can be found on the canes. The trees may be reduced in growth, stunted and have a low and short production which may lead to plant death.

Main (persistent) vectors are *Xyphon fulgida* (= *Carneocephala. fulgida*), *Draeculacephala minerva*, *Graphocephala atropunctata, Homalodisca vitripennis* and *Oncometopia nigricans* in North America. Possible vectors in southeast Europe are *Cicadella viridis* and *Philaenus spumarius* (meadow spittle bug) (Redak *et al*., 2004).

Peach. First symptoms are stunted young shoots that have earlier, more numerous and darker green leaves than normal. Moreover they show early blooming and both leaves and flowers remain on the shoots longer than normal. Twigs on diseased trees have shortened internodes and increased lateral branching. Lateral branches grow horizontally or droop. Fruit production is severely impaired and fruits are small and early ripening. Trees that will be infected before bearing will never be productive. Symptom development is often slow (up to 18 months or more after infection) and may be present in one scaffold limb or in the entire tree. An extremely dry summer seems to delay symptom development for at least a year. Phony-infected leaves are generally broader, slightly more flat, and stay on the tree longer in the fall. Phony-infected trees when completely infected appear uniform across the top, a hedged look (Fig. 1D). Trees are generally not killed but are more susceptible to other diseases and arthropods. Main (persistent) vectors are *H. vitripennis*, *H. insolita*, *Oncometopia orbona*, *Graphocephala versuta* and *Cuerna costalis*.

Citrus. Symptoms can be observed especially on sweet orange trees from nursery up to 10-year-old (older trees show just a few diseased branches). Plants do not usually die. Small interveinal chlorotic spots (slightly raised and gummy on upper side and brown on lower side of the leaf) on leaves in parts of a tree or all over the tree, very similar to zinc deficiency symptoms. In later stages brown necrotic spots develop on the lower side of the leaf, corresponding to the chlorotic areas on the upper side. Wilting may occur. Fruits remain small, have higher sugar content and a harder rind than normal and ripen earlier (Fig. 1B) Water stress or senescence may aggravate the symptoms. A chronic form of the disease

Table 1. Natural host plants of *X. fastidiosa* in which the pathogen was surely identified (source: http://www.cnr.berkeley.edu/xylella/). For a complete list of hosts, see the same site.

Scientific name	Common name
Acacia longifolia	golden wattle
Acer macrophyllum	big leaf maple
Aesculus californica	California buckeye
Ampelopsis arborea	peppervine
Artemisia douglasiana	mugwort
Avena fatua	wild oat
Baccharis pilularis	coyote brush
Bromus rigidus	ripgut grass
Callicarpa americana	American beautyberry
Chenopodium ambrosioides	Mexican tea
Citrus sinensis	sweet orange
Cynodon dactylon	Bermuda grass
Cytisus scoparius	Scotch broom
Digitaria sanguinalis	hairy crabgrass
Duranta repens	pigeon-berry
Echinochloa crus-galli	water grass
Escallonia montevidensis	*Escallonia*
Eugenia myrtifolia	Aust. brush-cherry
Fraxinus dipetala	California ash
Fuchsia magellanica	*Fuchsia*
Genista monspessulana	French broom
Hedera helix	English ivy
Hydrangea paniculata	*Hydrangea*
Lolium multiflorum	Italian ryegrass
Majorana hortensis	sweet majoram
Medicago hispida	bur clover
Melilotus sp.	sweet clover
Melissa offcinalis	garden balm
Oenothera hookeri	evening primrose
Parthenocissus quinquefolia	Virginia creeper
Parthenocissus tricuspidata	Boston ivy
Paspalum dilatatum	dallisgrass
Platanus occidentalis	sycamore
Poa annua	annual bluegrass
Polygonum persicaria	ladys thumb
Prunus sp.	wild plum
Quercus agrifolia	coast live oak
Quercus lobata	valley oak
Rosa californica	California wild rose
Rosa californica	California wild rose
Rosmarinus offcinalis	rosemary
Rubus sp.	blackberry
Rubus ursinus	California blackberry
Rubus ursinus	California blackberry
Rumex crispus	curly dock
Sambucus canadensis	American elder
Sambucus mexicana	blue elderberry
Sambucus mexicana	blue elderberry
Symphoricarpos albus	snowberry
Toxicodendron diversilobum	poison oak
Toxicodendron diversilobum	poison oak
Trifolium repens var. latum	Ladino clover
Umbellularia californica	California bay or laurel
Uritca dioica ssp.gracilis	stinging nettle
Veronica sp.	speedwell
Vinca major	greater periwinkle
Vitis californica	Calif. wild grape
Vitis rupestris	St. George
Vitis vinifera	grape

Fig. 1. A. Typical leaf scorch symptoms, caused by *Xylella fastidiosa* subsp. *fastidiosa* on a grapevine leaf (source: http://www.pdg-wss.net/Board_Info/FAQs.htm) B. Typical symptoms on leaves (variegated spots) and fruits (dwarf growth) of *Citrus* caused by *Xylella fastidiosa* subsp. *pauca* (source: A.H. Purcell). C. Severe symptoms of leaf scorch on a *Platanus occidentalis* (sycamore) caused by *X. fastidiosa* subsp. *fastidiosa* (source: A.H. Purcell). D. Symptoms of Phony peach disease on *Prunus persica* (peach), reduced growth of the tree on the left (source: http://www.aces.edu/mt/peachipm/archives/001409.php).

shows stunting and dieback of twigs (Crop Protection Compendium, 2005; EPPO, 2004). The bacterium also occurs in the roots (Hopkins *et al.*, 1991). Medium and long distance dissemination is by infected planting material. Out of the 11 sharpshooter vectors found in Brazil, *Acrogonia citrina, Bucephalogonia xanthophis*, *Dilobopterus costalimai*, *Macugonalia leucomelas*, and *Oncometopia fascialis* are the most important. Since they are the most common on citrus, these hosts are the most important source of inoculum (Almeida *et al.*, 2005). Although the pathogen is considered not to be seed borne, transmission from seeds to seedlings of sweet orange has been reported (Li *et al.*, 2003).

Coffee. First symptoms appear on young shoots as large scorched areas on the top or at the margins of mature leaves. Dwarf growth of new shoots, small, pale green to yellow leaves, shoot dieback, and overall plant stunting occur. Fruit size and yield are impaired. Symptoms are severe under conditions of water stress, but trees generally do not die or only after some years (Lima *et al.*, 1998).

Other hosts. The symptoms caused by *X. fastidiosa* in other hosts resemble by and large those described above, namely: early symptoms are a slight chlorosis or bronzing along the leaf margin or tip that intensifies and that may become water-soaked before browning and drying.

These symptoms are first found on a few branches, later on almost all foliage. The affected area is delineated by a narrow chlorotic band that becomes especially clear in autumn. A premature defoliation takes place with new malformed leaves formed. Abnormally shaped fruit may also be formed and stems may show internal and external discoloration, dieback and abnormal growth, leading to eventual death of the host. Transmission to other hosts is largely unknown. Based on epidemiological information available from intensive studies of diseases incited by *X. fastidiosa* in economically important crops, it is assumed that the main type of transmission of this bacterium in minor crops or host plants in spontaneous flora is by insect vectors feeding on more than one plant species. Giving the fact that there are thousands of insect species potential vectors worldwide, studying of their potential role and efficiency in the pathogen transmission is an important part of pest risk assessment within a particular geographical region.

BIOLOGY

X. fastidiosa is a Gram-negative, strictly aerobic, xylem-inhabiting, non-flagellated pathogen with a growth optimum of 26-28°C. It moves downstream, but also upstream in plants. The upstream movement is possible with long type IV pili (twitching motility). In advanced stages of infection, sap blocking biofilms are formed both in the host plant and in the foregut of the vectors. Type I pili play the most important role in biofilm formation and aggregation of cells. The biofilms in plant and vector differ in composition and they are actively (signal-based) produced by the pathogen. (Li *et al*., 2007; Meng *et al*., 2005). In the vector biofilm the bacterial cells are pearly attached (Newman *et al*., 2004). The pathogenicity of *Xylella fastidiosa* shows similarities to that of *Xanthomonas campestris* pv. *campestris*: it produces a wide variety of pathogenicity factors for host-specific colonization such as a large

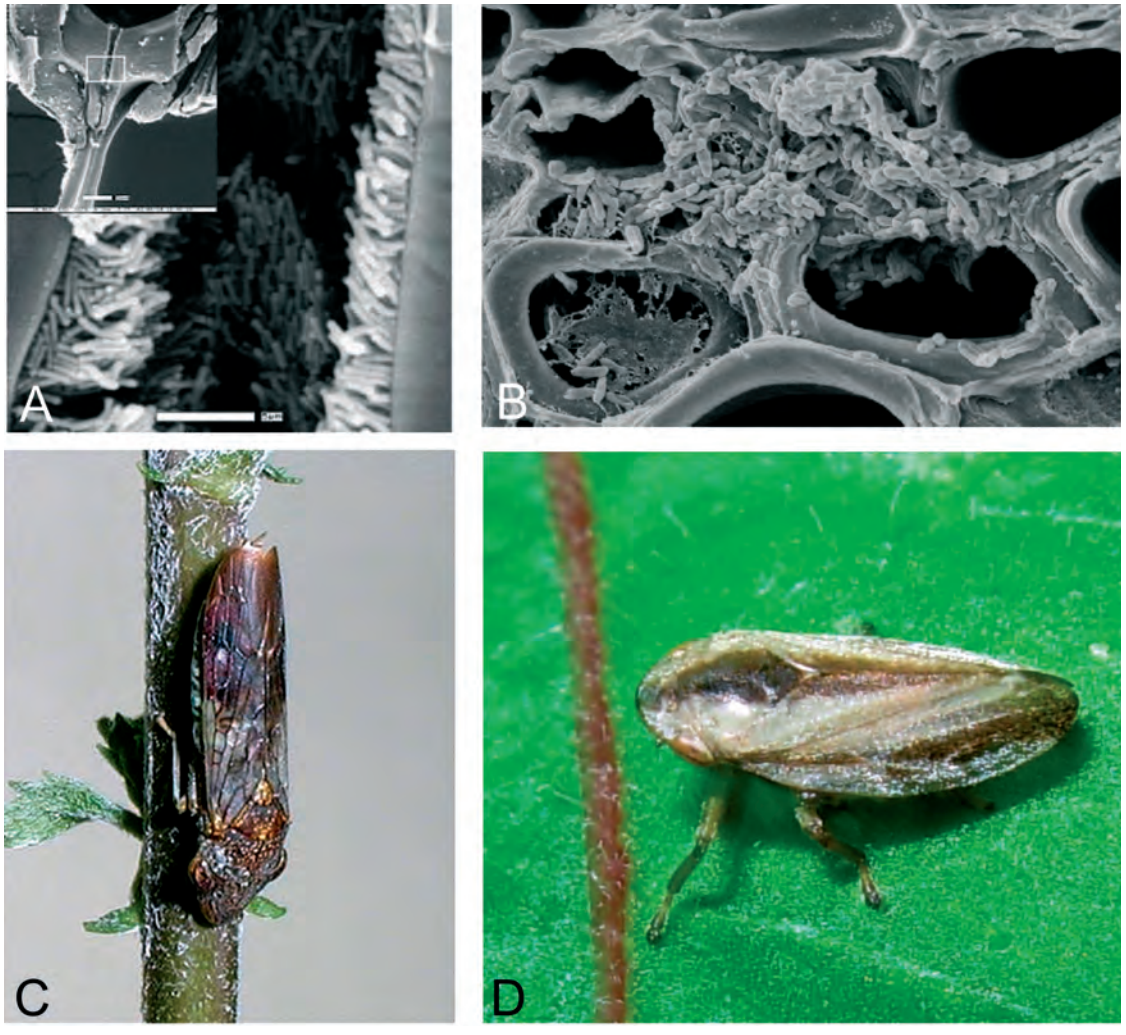

Fig. 2. A. Cells of *X. fastidiosa* in biofilms polarly attached on the cuticle of foregut (source: A. Almeira) B. Cells of *X. fastidiosa* in xylem vessels (source: A. Almeira). C. The glassy-winged sharpshooter *Homalodisca vitripennis* (formerly *H. coagulata*), a vector of *Xylella fastidiosa* (source: http://www.apsnet.org/bookstoretitles/EPPCD/Images/3-14.htm). D. *Philaenus spumarius* (meadow spittle bug) a potential vector of *X. fastidiosa*, occurring in Europe (source: EPPO).

number of fimbrial and afimbrial adhesins for attachment. The exopolysaccharidal slime (EPS) is similar to the xanthan gum produced by *Xanthomonas campestris* pv. *campestris*. It does not have a type III secretion system; however, genes for a type II secretion system for export of exoenzymes that degrade the plant cell wall and allow the bacterium to colonize the plant xylem were determined. In some aspects (biofilm formation) *X. fastidiosa* is more similar to animal pathogens (Machado *et al*., 2001; Chatterjee *et al*., 2008). Biofilm formation and attachment (Fig. 2A) are under control of the *GacA* gene, which plays a similar role in other phytopathogenic bacteria. It is also involved in physiological processes that may enhance the adaptation and tolerance of *X. fastidiosa* to environmental stresses and the competition within the host xylem (Shi *et al*., 2009), The bacterium can enter neighbouring vessels through pits, after degradation of the pith membranes, which is apparently also triggered by a diffusible signal from the bacterium (Newman *et al*., 2004). The bacterium is also present in roots and can therefore be transmitted by root grafting. Vessels can become occluded by dense colonization (Fig. 2B) and high frequencies of blocked vessels are associated with disease symptom development. EPS and polygalacturonase also play a role in the break down of pit membranes and xylem occlusion (Roper *et al*., 2007a). EPS also entraps hydrolytic products that can be utilised by the bacteria as carbon source (Roper *et al*., 2007b).

X. fastidiosa has been found in a latent state in many symptomless hosts, i.e. mugwort (*Artemisia douglasiana*) and watergrass (*Echinochloa cruz-galli*), that serve as a source of inoculum for vectors (Hopkins and Purcell, 2002), although they did not move systemically in most of the symptomless hosts. Systemic movement was found in symptomless blackberry (*Rubus procerus*). *X. fastidiosa* is irregularly distributed in infected tissues (Hopkins and Adlerz, 1988), thus longer plant access time may increase chances of the vector probing infected vessels.

BACTERIUM -VECTOR RELATIONSHIP

X. fastidiosa is transmitted persistently by xylem-sap sucking insect vectors as follows: (i) acquisition from a source plant; (ii) attachment and retention to vector's foregut cuticle; (iii) detachment and inoculation into a new host. Vectors are mainly sharpshooters and spittlebugs (Cicadellidae), have no transstadial or transovarial transmission (nymphs shed cuticle) and the bacterium does not need a latent period. Once infected with *X. fastidiosa*, insects remain infective with the pathogen, which multiplies in the foregut and the bacterium becomes persistent in adult insects. Only a few bacterial cells are required for transmission (Hill and Purcell, 1995).

It appears that most of xylem-feeding Cicadellidae species can be or are vectors in nature (Purcell, 1989), where probing behaviour (e.g. preference for young shoots or, as in *H. vitripennis*, preference of woody tissues, even transmitting the bacterium to dormant vines, leading to winter-persisting populations of the pathogen) and foregut morphological characteristics determine the efficiency of bacterial transmission (Almeida *et al*., 2005; Hopkins and Purcell, 2002). It has been determined that for PD, alfalfa dwarf and CVC 27, 22 and 11 species of Cicadellinae were vectors of *X. fastidiosa* (Redak *et al*., 2004). Some 39 species of 19 genera of Cicadellinae (sharpshooters) and 5 species of Cercopidae (spittlebugs) have been reported as vectors. Vectors have been mainly identified in North America. The most important are (for PD, unless indicated) in North America: *Cuerna costalis* (PPD); *Draeculacephala minerva* (green sharpshooter) important also for ALS in California; *Graphocephala atropunctata* (blue-green sharpshooter), most important, before GWSS, see further; *G. versuta* (PPD); *Hordnia circellata*, most efficient; *Homalodisca vitripennis* (Fig. 2C); *H. insolita* (PPD); *Oncometopia nigricans*, *O. orbona* (PPD); *Xyphon* (formerly *Carneocephala*) *fulgida* (red-headed sharpshooter). For CVC in Brazil: *Acrogonia terminalis*, lays eggs externally on leaves; *Bucephalogonia xanthophis; Dilobopterus costalimai* and *Oncometopia fascialis*. Local possible vectors for Europe are *Cicadella viridis* and *Philaenus spumarius* (meadow spittle bug) (Fig. 2D).

Although not all *X. fastidiosa* transmitting vectors play an important role in transmission from wild hosts to crops, it was found in California that inoculum present in weed hosts (herbs and shrubs) in adjacent riparian woods facilitated spread of PD into vineyards in early spring, when the blue-green sharpshooter, *G. atropunctata* (that overwinters as adult) apparently played an important role (Hopkins and Purcell, 2002). Adlerz and Hopkins (1979) found that *Oncometopia nigricans* (Walker) was a more important vector in central Florida that *H. vitripennis* because of larger population formation in early spring. Vectors that overwinter as eggs or nymphs are thought to be less important in the dissemination of the disease. It was therefore theorized that *X. fastidiosa* does not yet occur in Europe due to absence of vectors that survive winter as adults and, even if infected, could not cause extensive spread due to absence during the critical early spring period. (Purcell, 1997).

Transmission efficiency by vectors may vary. *H. vitripennis* for example transmits much more efficiently from grape to grape than from almond to almond (Hopkins and Purcell, 2002). Efficiency of CVC vectors is low (less than 10%), further information in Redak *et al.* (2004). *H. vitripennis* is considered now one of the most important vectors responsible for spreading of *X. fastidiosa*-caused diseases in south-east United States, espe-

cially California, such as PD and oleander leaf scorch, but also ALS, PP and PLS, furthermore inhibiting the successful culture of *V. vinifera* L. and *V. labrusca* L. In the mild winters of California, *H. vitripennis* transmits the pathogen also to dormant vines. *H. vitripennis* has a very broad host range. It has been found on more than 70 plant species in 35 families including: avocado, citrus, macadamia, and many woody ornamentals, e.g. *Fraxinus, Lagerstroemia* and *Rhus*. (Almeida *et al.,* 2005; Redak *et al*., 2004).

BACTERIUM-HOST PLANT RELATIONSHIP AND SPECIALISATION

Several pathogenic varieties of *X. fastidiosa* have been described, that are often host-specific [e.g. the PD strain will not cause disease if introduced to peach or plum; PD, ALS and alfalfa dwarf are caused by the same strains. PPD and PLS strains can be reciprocally graft-transmitted. Peach strains, however, do not cause disease in grape and grape strains do not infect peach, furthermore OLS strain does not infect grape and vice versa. But both PD and OLS strains infect almond. Reciprocal transmission between elm and sycamore leaf scorch strains was negative. A CVC strain of *X. fastidiosa* produced leaf scorch disease in coffee, and CVC and coffee strains can cause disease in grape under greenhouse conditions (Hopkins and Purcell, 2002). *X. fastidiosa* shows a certain host-specialization and other variations that warranted the following subspecific division (Schaad *et al*., 2004; Hernandez-Martinez *et al*., 2007): (i) *X. fastidiosa* subsp. *fastidiosa* (erroneously named *X. f.* subsp. *piercei*, see Schaad *et al*., 2004), PD and ALS, strains from cultivated grape, alfalfa, almond, maple (*Acer*), cherry, Spanish broom (*Genista*), one strain from western redbud (*Cercis occidentalis*); (ii) *X. f*. subsp. *multiplex*, PPD, PLS, strains from peach, elm, plum, pigeon grape (*Vitis aestivalis*), sycamore (*Platanus*), almond (one strain), olive, sweetgum (*Liquidambar*), *Ginkgo*, crape myrtle (*Lagerstroemia indica*), one strain from western redbud; (iii) *X. f*. subsp. *pauca*, CVC, strains from *Citrus* and probably those from coffee, *Coffea* (CLC); (iv) *X. f*. subsp. *sandyi*, strains from *Nerium oleander* (OLS), daylily (*Hemerocallis* spp.), *Jacaranda* and *Magnolia* (Hernandez-Martinez *et al.*, 2007); (v) *X. f*. subsp. *tashke*, strains from the ornamental tree *Chitalpa tashkentensis*; (vi) mulberry (*Morus* spp.) and heavenly bamboo *(Nandina domestica)* strains, not yet allocated to subspecies (Hernandez-Martinez *et al.*, 2007).

Host range. Apart from the already mentioned diseases PD, PPD and CVC, it was found that *X. fastidiosa* also causes a number of so-called leaf scorch diseases in *Prunus* spp. (including almond leaf scorch or ALS in *P. armeniaca* and plum leaf scald or PLS in *P. domestica*), *Acer* spp., *Carya illinoinensis* (pecan), *Coffea arabica* (CLC, in Brazil isolated in 1995 and pathogenic also to *Citrus*), *Hedera helix*, *Morus rubra* (American mulberry), *Nerium oleander* (OLS), *Platanus occidentalis* (sycamore, Fig. 1C), *Quercus* spp. (oak), *Ulmus americana* (elm tree). Furthermore it induces diseases of *Medicago sativa* (alfalfa dwarf), *Catharanthus roseus* (periwinkle) and *Vinca major* (both wilting symptoms), stunting of *Ambrosia artemisifolia* (ragweed). Many wild plants may carry the pathogen with, but more often without, showing symptoms, such as grasses, sedges and trees. A list of hosts from which *X. fastidiosa* was isolated is presented in Table 1.

Some tree species found to be infected based on PCR asays are *Acer negundo, Aesculus* x *hybrid, Celastrus orbiculata* and *Cornus florida* (McElrone *et al*., 1999). Hartman *et al.* (2002) detected the pathogen in oak species, including *Quercus coccinea* (scarlet oak), *Celtis occidentalis* (hackberry), *M. rubra* and *M. alba* (white mulberry) in Kentucky (USA) that has a temperate climate. In the USA native wild plums, especially *Prunus angustifolia* (chickasaw plum) were found to be inoculum reservoirs for *X. fastidiosa* facilitating spread of PPD. A leaf scorch described in 1990 in Taiwan on *Pyrus pyrifolia* (Japanese pear) cv. Hengshan in particular, and *P. serotina* (Asian pear), was found to be caused by a bacterium very similar (but deviating from North and South American strains serologically and in housekeeping gene sequences) to *X. fastidiosa* (Leu and Su, 1993; Chen *et al*., 2006). Asian pears have recently been introduced in central Europe (Romania) and Japanese pears, or nashis, were planted as a novelty crop in southern Europe in the 1980's.

Other natural hosts are *Ambrosia artemisiifolia, Ampelopsis arborea, Baccharis halimifolia, Callicarpa americana, Citrus jambhiri, Fragaria vesca* var. *californica, Montia linearis, Parthenocissus quinquefolia, Quercus falcata, Q. laurifolia, Q. nigra, Rhus* sp., *Rubus procerus, Sambucus canadensis, Solidago fistulosa, Vinca minor* and *Vitis rotundifolia* (Hopkins and Purcell, 2002).

For a full lists of the different types of hosts, see http://www.cnr.berkeley.edu/xylella/.

Geographical distribution. Diseases incited by *X. fastidiosa* occur mainly in tropical/subtropical areas, although leaf scorch diseases also occur in much colder climate, e.g. oak leaf scorch up to Canada (Table 2). The geographical distribution according to the latest findings (see http://www.eppo.org/) is as follows:

EPPO and EU region: absent. Unconfirmed reports on imported grapevine material from USA in France and from Kosovo (EPPO Reporting Service 500/02, 505/13 and 1998/9; Berisha *et al*., 1998);

Asia: India [on almonds (Jindal and Sharma, 1987); not confirmed recently] and Taiwan [pear leaf scorch, a

bacterium similar to *X. fastidiosa,* questionable serological relatedness (Leu *et al.*, 1993)];

North America: Mexico, USA (Alabama, Arizona, California, Florida, Georgia, Louisiana, Mississippi, Missouri, Montana, North Carolina, Oklahoma, 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, also for CLS (Aguilar *et al.*, 2005) and OLS (Monter-Astua *et al.*, 2008);

South America: CVC has been reported from Argentina (Brlansky *et al.*, 1991), and Brazil (São Paulo, Minas Gerais, Rio de Janeiro; rapidly spreading), PD reported from Venezuela (Jimenez, 1985) and plum leaf scald in most South American areas where *Prunus salicina* is grown.

DETECTION AND IDENTIFICATION

Cells of *X. fastidiosa* are small and narrow (0.2-0.4 x 1.0-4.0 µm) and therefore only visible using at least dark field or phase contrast microscopy. Bacteria can be found in infected tissues most easily in sap/ooze of leaf veins or vessels of petioles of scorched leaves or trunk/branch vascular tissue of non-leaf scorched symptomatic trees. With PPD, roots should be investigated. For preliminary confirmation of pathogen presence in fresh plant tissue, one ml of KOH 0.1 M can be vacuum drawn trough vessels and a resulting drop placed under the microscope. When the symptoms are not very definite the disease can be further recognised by cutting root sections and immersing them in acidified methanol (1 ml concentrated HCI in 100 ml absolute methanol). Infected roots show purplish spots within a minute or two where vessels contain bacteria.

X. fastidiosa is a slow-growing (fastidious) bacterium that does not grow on many common culture media, but some good selective media are available, such as PD2, PW, CS20 or BCYE (Schaad *et al.*, 2001). Colonies on the most frequently used Periwinkle medium (PW) are circular with entire margins, convex, opalescent-white, reaching 0.7-1.0 mm diameter after 2-3 weeks. Isolation can be performed by blotting expressed sap (after surface sterilisation) on media, by placing pieces of infected vascular tissue in PW broth, shaken and subsequently plated on agar medium or vacuum extracted. Isolation from insects is by surface sterilisation, dissection of the head which is homogenised in sterile PBS and suspension plated onto media. Plates should be kept up-side-down and sealed with parafilm, and media should be checked for up to a month for typical colonies, which should be subcultured on nutrient agar and selective media, only colonies growing on the latter should be subjected to further identification tests. PD and PPD (and other) type of strains can be differentiated by growth on PD2 (PD strains positive, others negative) and PW BCYE/CS-20 agar (PPD strains positive, PD strains negative), ELISA using different antisera and PCR (Schaad *et al.*, 2001). A full account on isolation and detection by tissue extract PCR is given in http://www.padil.gov.au/pbt/index.php?q=node/28&pbtID=109.

DNA extraction from insects can be performed with immuno-magnetic separation, or using a DNA extrac-

Table 2. Geographical distribution according to *X. fastidiosa* host/strain type (EPPO website, http://www.eppo.org/), and data sheet *Xyllela fastidiosa* (EPPO, 1992).

Strain type of *Xyella fastidiosa**	Area
PD strains	North and Central Americas, Peru, unconfirmed report from Kosovo
Alfalfa dwarf	USA, California
Almond leaf scorch	Argentina, USA, California,
Peach-plum strains Phony peach	South-eastern USA
Plum leaf scald	South-eastern USA
Citrus variegated chlorosis ('pecosita' in Argentina, 'amarelinho' in Brazil)	Argentina, Brazil
Coffee leaf scorch	Brazil
Oak leaf scorch (related to peach strains)	Eastern USA
Maple leaf scald	Eastern USA
Elm leaf scorch	Eastern USA
Sycamore leaf scorch	Eastern USA
Mulberry leaf scorch	Eastern USA
Plum leaf scald	Widespread in South America, Paraguay, Brazil
Periwinkle wilt	USA, Florida
Pear leaf scorch	Taiwan
Pecan leaf scorch	USA, Louisiana
Oleander leaf scorch	USA, California and Florida

* For differentiation into subspecies, also related to hosts, see under "Biology"

Table 3. PCR Primers useful for detection of *Xylella fastidiosa.*

Primer Name	Sequence (5'-3')	Target Gene	Reference
RST31	GCGTTAATTTTCGAAGTGATTCGA	Unique *E.coli* R 1 fragment	Minesavage *et al*., 1994
RST33	CACCATTCGTATCCCGGTG		Minesavage *et al*., 1994
XF1-F	CAGCACATTGGTAGTAATAC	16S rDNA	Firrao and Bazzi, 1994
XF6-R	ACTAGGTATTAACCAATTGC		Firrao and Bazzi, 1994

tion kit such as the DNeasy Tissue kit (Qiagen, USA) or the Genomic DNA Purification kit (Fermentas, USA). The latter performed well in the study of Bextine *et al*. (2005).

To detect *X. fastidiosa*, three specific primers sets can be used. For the PD strain the RST primers can be used (Minesavage *et al.*, 1994) (Table 3). Non-grapevine strains can be detected by XF primers (Firrao and Bazzi, 1994). A multiplex PCR for detection of all *X. fastidiosa* strains both in plant tissue and insects, using primers against *X. fastidiosa* gyrase b gene and 16S rRNA genes was developed by Rodrigues *et al.* (2003) (Table 4). Another multiplex PCR was developed by Hernandez-Martinez (2006). Primers ALM1 and ALM2 yielding a 521 bp fragment from almond strains that belong to *X. f.* subsp. *multiplex*, and XF2542-L and XF2542-R, resulting in a 412-bp fragment from PD and certain almond strains, were combined. Real-time PCR was developed by Oleivera *et al.* (2002) using the primers and probe reported in Table 5. A highly efficient (as compared to classical PCR) combined agar absorbent and bio-PCR for grape and citrus strains was developed by Fatmi *et al.* (2005).

A pathogenicity test with a pure culture of *X. fastidiosa* can be performed by hypodermic syringe/needle injection of a sterile PBS suspension of bacteria into the vascular system. For PPD strains a root inoculation is advisable (Schaad *et al*., 2005). An improved biotest on tobacco was described by Francis *et al.* (2008), using *Nicotiana tabacum* cv. SR1 (Petite Havana), yielding symptom expression already after 15 days with a final evaluation 4 to 6 weeks after inoculation. The tobacco leaves show typical scorch symptoms (see also Lopes *et al.* (2000). A complete diagnostic protocol including flow scheme of tests necessary for diagnosis, including confirmatory host test is given by EPPO (EPPO, 2004).

Table 4. A multiplex PCR for detection of all *X. fastidiosa* strains both in plant tissue and insects, using primers against *X. fastidiosa* gyrase b gene and 16 S rRNA genes.

Primer Name	Sequence (5'-3')
UP2RS	AGCAGGGTACGGATGTGCGAGCC
FXYgyr499	CAGTTAGGGGTGTCAGCG
RXYgyr907	CTCAATGTAATTACCCAAGGT
S-S-X.fas-0067-a-S-19	CGGCAGCACATTGGTAGTA
S-S-X.fas-0838-a-S-21	GCAAATTGGCACTCAGTATCG
S-S-X.fas-0838-a-A-21	CGATACTGAGTGCCAATT TGC
S-S-X.fas-1439-a-A-19	CTCCTCGCGGTTAAGCTA C

CONTROL

Since *X. fastidiosa* is "localized" on the American continent, the rest of the world is focused on implementation of quarantine and phytosanitary procedures in order to prevent "escape" of the pathogen from its place of origin. Considering the wide host range, numerous insect vectors, latent nature, global movement of plant material, these preventive administrative measures should be fortified by other prophylactic actions based on the experience from countries suffering from this bacterium. As chemical curative control of the bacterium is not possible, control of diseases caused by *X. fastidiosa* in the countries of origin concentrate on prevention, by use of resistant varieties, cultural and hygienic measures and chemical and biological vector control. These other methods, however, are often only partly successful. Reasons are e.g. that *X. fastidiosa* has many symptomless hosts, including weeds, ornamentals and other crops and possibly also still unknown vectors. Removal of diseased trees is only partly successful because of introduction of the pathogen from neighbouring ar-

Table 5. Primers used in the Real-time PCR protocol developed by Oliveira *et al.* (2002).

Name	Sequence (5'-3')	Size (bp)
CVC-1	AGATGAAAACAATCATGC AAA	424-404
CCSM-1	5'GCG CAT GCC AAG TCC ATA TTT	306-286
Probe TAQCVC	(6FAM)AACCGCAGCAGAAGCCGCTCA TC(TAMRA)P	335–313

eas. Purcell (1980) showed that PD infected plants could be cured from *X. fastidiosa* in cold winters. Furthermore bacterial populations in shoots were negatively correlated with cumulative hours below -5°C (Henneberger *et al.*, 2004). Cross-protection with weakly or avirulent strains of *X. fastidiosa* has been successful in different grapevine cultivars, including Cabernet sauvignon and Vidal blanc, to some extent, especially with strain EB92-1. The bacteria are inoculated by pin-pricking or drilling and syringe injection (Hopkins and Purcell, 2002; Hopkins, 2005).

Host resistance. Unfortunately most cultivars of European (*V. vinifera*), American (*V. labrusca*) and hybrid grapes are susceptible to Pierce's Disease. However, resistance to PD was found in *Vitis* species native to south-eastern US. The grape industry in this area of the country is based on these resistant *Vitis* species. Muscadine grapes (*V. rotundifolia*) are often highly resistant or tolerant and much used in the south-eastern US (Hopkins and Purcell, 2002). Resistance was found in different grapevine genotypes such as *Muscadinia rotundifolia*, *Vitis arizonica/candicans, V. arizonica/girdiana, V. candicans, V. girdiana*, *V. nesbittiana*, and *V. shuttleworthii* following artifical inoculation. However, *V. vinifera*, *V. aestivalis* and *V. champinii* developed very high *X. fastidiosa* concentrations in their vascular tissues (Fritschi *et al*., 2007).

Vector control. When there are recent introductions of vectors, vector control by biological agents and insecticides is an important way to slow fast spread of the insect, as is was performed in California after the introduction of *H. vitripennis*. Egg parasitoids like *Gonatocerus* sp. may be used, but their populations decrease strongly during winter when egg production of vectors is low and therefore the first generation vectors (which is very important in disease transmission) usually is only slightly parasitized. Moreover the industrial production of parasitoids is not easy. Systemic insecticides, especially neonicotinoids (imidacloprid), natural defence system enhancers (systemic acquired resistance or SAR), such as harpin, a protein from *Erwinia amylovora* and repellents, such as kaolin, a formulation of aluminum silicate, are used in vineyards but are only partly successful (Almeida *et al*., 2005; Tubajika *et al.*, 2007). The use has been tried of genetically manipulated bacteria found in the foregut of vectors and in xylem tissue of grape (as an endophyte), viz. *Alcaligenes xylosoxidans* subsp. *denitrificans* as an agent that blocks transmission of *X. fastidiosa* (Bextine *et al*., 2005), but its usefulness is questionable because the bacterium would mainly block secondary transmission (grape to grape), whereas primary infection would originate from nongrape hosts that are usually not reacting with symptoms or are non treatable.

Cultural practices. Stress is often a determining factor in the development of symptoms once a plant has become infected with *X. fastidiosa*. Cultural practices should therefore be directed towards healthy, well growing plants and adequate nutrition. Iron deprivation possibly provides a way to reduce disease severity by preventing biofilm formation in the xylem vessels (Toney and Koh, 2006). The following cultural practices have proved to be effective (source: http://edis.ifas.ufl.edu/in174): (i) cultivar selection (mainly for grape); (ii) removal of diseased trees in two to five-year old peach orchards, this extends productive orchard life; (iii) survey for the disease in June and July, pruning after diseased tree removal, with avoidance of heavy summer pruning; (iv) rouging wild plums and cherries or other hosts, within *ca.* 400 m of an orchard; (v) establishing new plantings, which should not include both peaches and plums, not closer than 400 m to existing orchards (never planting near infected orchards); (vi) weed control in and around orchards; (vii) elimination of woods, especially oaks, near orchards when possible; (viii) no routine spraying with insecticides for leafhopper populations will not substantially decrease.

RISKS AND CONCLUSIONS

X. fastidiosa is an emerging threat in the south-west US, due to recent establishment of the glassy-winged sharpshooter vector (*H. vitripennis*), leading to very serious outbreaks of PD in grapevine and also ALS and OLS, due to a much more efficient transmission of this sharpshooter than local vectors. GWSS probably first entered California as eggs in plants. The eggs are deposited into plant tissues. *H. vitripennis* is native to the southeast USA and northeast Mexico; it recent invaded of California (USA) and Tahiti (Hoddle, 2004). It was first detected in southern California in 1989 (Sorensen and Gill, 1996), and caused outbreaks of Pierce's disease in this state since 1997 with high incidences (25-97% of the plants infected, although often initially symptomless). The risk of introduction of *H. vitripennis* for Europe can be formulated as follows:

(i) very broad host range, more than 70 plant species in 35 families including: avocado (*Persea americana*), citrus, *Macadamia*, and many woody ornamentals (e.g. *Fraxinus, Lagerstroemia, Rhus*). For a full list of hosts see http://edis.ifas.ufl.edu/in174. The adult stage is persistently infected by the bacterium, transmitting it throughout the whole life and disperses widely with short hopping flights that enhance the spread of *X. fastidiosa* (Blua and Morgan, 2003);

(ii) risk of introduction with imported hosts (recent introductions reported from California, Arizona, French Polynesia, and Hawaii);

(iii) efficient vector of *X. fastidiosa* to grapevine, al-

mond, and oleander in California and of PPD and PLS in south-east USA;

(iv) highly mobile and widely distributed in various crops (Redak *et al*., 2004), although there is a relatively inefficient transmission of *X. fastidiosa* (Almeida and Purcell, 2003);

(v) precludes the culture of *V. vinifera* L. and *V. labrusca* L. in south-east USA and can inoculate dormant grapevines in winter (Almeida *et al*., 2005).

In Central and South America *X. fastidiosa* has become very noxious due to the rapid expansion (most likely via distribution of infected planting material) of CVC in Citrus, leading to more than a third of all trees in the area having symptoms of CVC and CLC in coffee.

For Europe there are until now only a few unconfirmed records of *X. fastidiosa* on grapevine from Kosovo [erroneously mentioned as Slovenia in my book Phytobacteriology, Principles and Practice (Janse, 2006)] and France. The finding of *X. fastidiosa* in grape material originating from Kosovo (Berisha *et al*., 1998) prompted Serbian authorities to do pest risk assessment. Although the location of origin of tested material was not accessible, in 2005/06 the Serbian Ministry of Agriculture carried out surveys of vineyards in the region neighbouring Kosovo. The purpose of this survey was to collect plants showing symptoms resembling PD and to detect *X. fastidiosa* in suspicious material. Sampling was performed in late summer-early autumn and material was subjected to laboratory analysis according to the detection procedure recommended by EPPO (EPPO, 2004). Results of this analysis showed no indication of *X. fastidiosa* presence in the tested samples (A. Obradovic, unpublished information).

Since *X. fastidiosa* has more that 150 hosts and for many of them, including *Vitis,* planting material is imported, the risk of introduction (especially in latent form) must not be underestimated. Absence of the diseases caused by *X. fastidiosa* will mainly be due to the absence of suitable vectors. However, introduction of the pathogen and vectors with plant material can not be excluded. Moreover also local Cicadellidae (see above) could become potential vectors. Due to its potential risks and absence in the region *X. fastidiosa* is on the A1 Quarantine list of EPPO and *H. vitripennis*, which has a very large host range and feeds on almond, peach and plum, was recently put on the EPPO alert list. *H. vitripennis* could easily be introduced or perhaps has already been introduced via its many host plants (nursery productions, cut flowers, propagating material, fruits) into the EPPO region. Of course, it should be investigated how much these and local vectors are adapted not only to transmission, but also to their new hosts and environments. Many known vectors do not play a substantial role in disease outbreaks in certain crops (Redak *et al*., 2004). Further risks of *X. fastidiosa* for Europe can be formulated as follows:

(i) bacterium irregularly distributed in host tissues and sometimes difficult to detect, often occurring in a latent form;

(ii) detection techniques are often not sensitive. As an example: direct PCR assays of grape and citrus 13% and 33% positive, Agar absorbent-PCR, 97% and 100% positive for grape and citrus, respectively (Fatmi *et al*., 2005);

(iii) massive importations of wild grape rootstocks from America to Europe (*Phylloxera*-resistant) provide opportunity to introduce *X. fastidiosa*;

(iv) wide range of (symptomless) hosts of *X. fastidiosa* should have allowed periodic introductions of the bacterium into Europe;

(v) many Cicadellidae transmit *X. fastidiosa,* including some European species such as *Cicadella viridis* and *P. spumarius* (meadow spittle bug);

(vi) it is unknown if in Europe there are vectors surviving winter as adults, able to spread the disease once it is present;

(vii) vectors may overwinter unnoticed as adults in woods and weeds adjacent to vineyards. For instance, *G. atropunctata* from riparian woods causes *X. fastidiosa* infections early in the spring in California (Purcell and Saunders, 1999). Some spittlebugs vectors, not found on grapevines, occur on herbs and shrubs nearby vineyards and alfalfa fields (DeLong and Severin, 1950). They may maintain inoculum in weed hosts;

(viii) the apparent absence of *X. fastidiosa* in Europe till now may be due to lack of vectors that overwinter as adults that could establish early season infections (Purcell, 1997), but due to recent climatic changes, the Mediterranean basin climatic conditions may be more congenial to certain vectors than initially thought (Hoddle, 2004);

(ix) cold winters may cure PD (demonstrated in potted grapevines) (Purcell, 1980). Nevertheless, inoculum sources important for epidemics to develop from primary spread may build up unnoticed and will cause epidemics when hosts, vectors and pathogen find the ideal conditions. In California adjacent riparian woods, alfalfa fields, and pastures serve as major reservoirs of *X. fastidiosa* (Hopkins and Purcell, 2002);

(x) in Central and South America *X. fastidiosa* has become very noxious due to the rapid expansion (most likely via distribution of infected planting material) of CVC in citrus.

As in the more northern parts of the USA, *Vitis* varieties in Europe are very susceptible to *X. fastidiosa* and this is really a risk when a vector would become established that could survive the winters in South Europe and would also establish in wild hosts (e.g. wild and domestic plums and wild cherry are symptomless reservoirs in the USA) and cause spring infections that are most likely to persist over the years. The same risk holds

true for citrus (sweet oranges, mandarins, and tangerines) and other hosts, such as almond, plum and peach that are widely grown in southeast and southwest Europe, especially in the warmer Mediterranean basin, where a disease-favourable combination of warm nights, regular rainfall/high humidity and long growing season is present.

In a computer simulation program (CLIMEX) study concerning climatic conditions and possibilities of spread of *X. fastidiosa*, Hoddle (2004) concluded: 'CLIMEX predicted that cold stress accumulation would exclude Pierce's disease-causing strains of *X. fastidiosa* from France and northern and central grape producing areas of Spain and Italy. This result is incongruous with Pierce's disease reports from Kosovo in the Balkans and may suggest that cold-tolerant strains of *X. fastidiosa* that cause Pierce's disease exist which could exhibit invasion potential and establish in areas of Europe contrary to results reported here. When observing the reports from eastern USA (up to Canada) and comparing climatic conditions of those areas and California, especially with those of the Mediterranean basis, we are pretty sure that even without a possible change towards cold-tolerant strains (for which there is no evidence yet), *X. fastidiosa* has too many chances for establishing itself in Europe and the Mediterranean basin.

The conclusion is that *X. fastidiosa* is a real and emerging threat for Europe, not only for *Vitis* and *Citrus* but also for stone fruits (almond, peach and plum) and oleander (e.g. GWSS likes to feed on oleander), that is difficult to prevent from entering and difficult to control once established, deserving more attention than up till now. Resistance in European grapes is scarce or even absent and vector control proved not to be very effective in the USA. Cultural practices to keep plants in optimum condition are of importance, but not sufficient and the use of avirulent strains for cross-protection is still in its infancy.

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