EPPO Datasheet: *Xanthomonas fragariae*

Last updated: 2023-06-19

IDENTITY

Preferred name: *Xanthomonas fragariae*
Authority: Kennedy & King
Taxonomic position: Bacteria: Proteobacteria: Gammaproteobacteria: Lysobacterales: Lysobacteraceae
Common names: angular leaf spot of strawberry, leaf blight of strawberry, vascular collapse of strawberry
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EPPO Categorization: A2 list
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EU Categorization: RNQP (Annex IV)
EPPO Code: XANTFR

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Notes on taxonomy and nomenclature

Xanthomonas fragariae is a phenotypically and genotypically homogeneous species and clearly distinct from the other xanthomonads (Vauterin *et al*., 1995; Roberts *et al*., 1996 and 1998; Rademaker *et al*., 2000 and 2005; Parkinson *et al*., 2007; Albuquerque *et al*., 2011). Strains show a strong clonal relationship, although some variation in fatty acid, RFLP, rep-PCR and AFLP profiles, not related to geographic origin or virulence, was observed by Roberts *et al*., 1998 and Stöger *et al*., 2008. A deviating, virulent strain, causing crown infection was reported from Liaoning province in China (Li *et al*., 2021). Maas *et al*. (2000) noted some difference in virulence between strains.

X. fragariae was the first xanthomonad where the CRISPR (clustered regularly interspaced short palindromic repeats) locus spacer typing and MLVA (Multilocus variable number of tandem repeats – VNTR - analysis), assisted in determining a (micro)-evolutionary trend among isolates. Two major groups and four subgroups were distinguished and data suggest that the two main groups were potentially responsible for the spread of the disease worldwide and the relative homogeneity of the species (Gétaz *et al*., 2018).

A related bacterial pathogen, causing so-called bacterial leaf blight, in the early 1990s in strawberry cultivations in Northern Italy, was described in 2001 as *Xanthomonas arboricola* pv. *fragariae* (Janse *et al*. 2001). Subsequently, the pathogen was also found in strawberry plantlets in Türkiye (Ustun *et al*. 2007). Symptoms are necrotic, reddish-brown lesions on leaves that enlarge, often with a chlorotic halo, that are not water-soaked as in infections of *X. fragariae*. Moreover, often large brown V-shaped lesions, surrounded by a chlorotic halo develop along the leaf margin in *X. arboricola* pv. *fragariae* infections. In the final stages leaves may completely wither and die. As opposed to infections of *X. fragariae*, no small, water-soaked lesions in early stages of the infection and no bacterial exudate was observed. High humidity and lower temperatures are important for infections of *X. arboricola* pv. *fragariae* to appear. Sometimes, however, this pathogen has been co-isolated with *X. fragariae* (Scortichini and Rossi 2003; Vandroemme *et al*. 2013b). Vandroemme *et al*. (2013b) found genetic variability within a relatively small collection of *X. arboricola* pv. *fragariae* strains. In an apparent failure to obtain symptoms in their artificial inoculations with those strains (Vandroemme *et al*., 2013b), they concluded, incorrectly (see below), that *X. arboricola* pv. *fragariae* was non-pathogenic. In some studies, other authors also failed to prove pathogenicity for certain strains of *X. arboricola* pv. *fragariae* (e.g. Fischer-Le Saux *et al*., 2015; Gétaz *et al*., 2020a). However, Ferrante & Scortichini (2018), using proper conditions during inoculation, unequivocally proved and reconfirmed pathogenicity using the pathovar type strain of *X. arboricola* pv. *fragariae*.

HOSTS

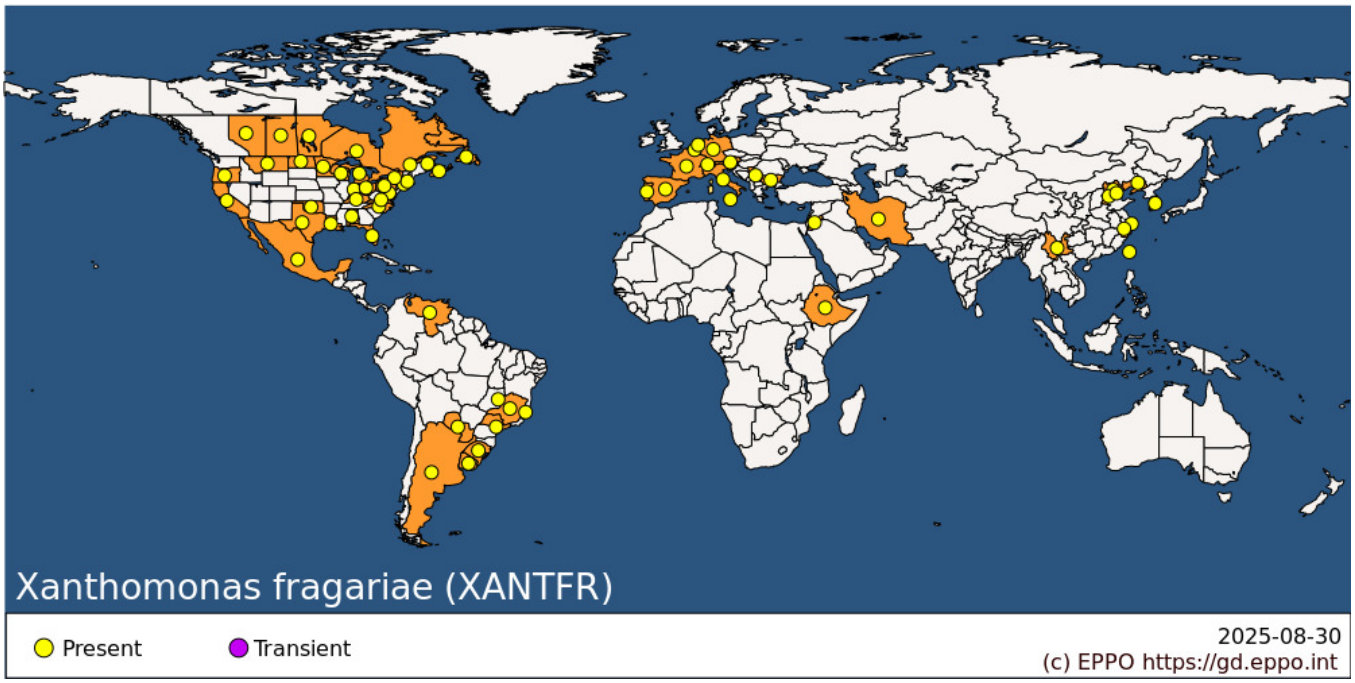
Fragaria x ananassa (the predominant cultivated strawberry, whose progenitors derive from hybridization between *F. chiloensis* and *F. virginiana*) is the main host, but its numerous cultivars vary a great deal in susceptibility (Desmet *et al*., 2009).

Stöger *et al.* (2008) isolated *X. fragariae* from *F. vesca* (wild or European strawberry) and *F. chiloensis* in the field. *F. virginiana*, *Dasiphora fruticosa* and *Potentilla glandulosa* became infected only following experimental inoculation. Among *Fragaria* spp. only *F. moschata* (musk strawberry) was found to be resistant (Kennedy & King, 1962a; Kennedy, 1965; Maas, 1998). Cultivated strawberries (*Fragaria* x *ananassa*) are the host of concern throughout the EPPO region.

Host list: *Fragaria x ananassa*

GEOGRAPHICAL DISTRIBUTION

X. fragariae, was first observed in 1960 in Minnesota, USA (Kennedy & King, 1962a). The pathogen is easily transmitted to healthy material from asymptomatic plants. It probably spread within North America and from there to many other countries in different continents, with (latently) infected planting material (e.g., Mazzucchi *et al.*, 1973; Dye & Wilkie, 1973; McGechan & Fahy, 1976; López *et al.*, 1985; Bultreys *et al.*, 2000). In Australia angular leaf spot, after several outbreaks since 1976 (McGechan & Fahy, 1976; Gillings *et al.*, 1998), has been reported as eradicated (Young *et al.*, 2011). Eradication after some outbreaks was also reported from the United Kingdom (Matthews-Berry & Reed, 2009) as well as from Réunion island (FR) in the Indian Ocean (Pruvost *et al.*, 1988). The disease is widespread in North America and the EPPO region, less so in other continents such as South America, Asia and Africa, probably related to the spread and intensity of strawberry cultivation.



EPPO Region: Austria, Belgium, Bulgaria, France (mainland), Germany, Italy (mainland, Sicilia), Jordan, Netherlands, Portugal (mainland), Serbia, Spain (mainland), Switzerland
Africa: Ethiopia
Asia: China (Beijing, Hebei, Liaoning, Shanghai, Tianjin, Yunnan, Zhejiang), Iran, Islamic Republic of, Jordan, Korea, Republic of, Taiwan
North America: Canada (Alberta, Manitoba, New Brunswick, Newfoundland, Nova Scotia, Ontario, Québec, Saskatchewan), Mexico, United States of America (Alabama, California, Connecticut, Florida, Indiana, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Montana, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Texas, Virginia, Wisconsin)
South America: Argentina, Brazil (Distrito Federal, Espirito Santo, Minas Gerais, Rio Grande do Sul, Sao Paulo), Paraguay, Uruguay, Venezuela

BIOLOGY

Residues of infected leaves and crown infections on runners used for planting are sources of inoculum for primary infections (Maas, 1998). In the residues of infected leaves, in or on soil, the bacterium survives from one crop to the next. Survival on the leaves is much shorter, a number of weeks only, even under high humidity conditions (Kastelein *et al*., 2014) The bacterium exudes from primary lesions, and bacterial cells are spread in the form of aerosols, caused by rain and sprinkler irrigation (Hildebrand *et al.*, 2005; Kim *et al*., 2016; van der Wolf *et al*., 2018), that are transported by wind to healthy leaves. Penetration occurs through the stomata. Infections of the crowns occur through local wounds or downwards from the affected leaves. During the growing season several cycles of secondary infections may occur. The bacterium may attack flowers, and occasionally fruits. The early stages of infection and pathogenesis were studied using electron microscopy by Allan-Wojtas *et al*. (2010). From crown infection pockets, the bacterium causes lesions along the veins at the base of the youngest leaves, which develop in the apical crown region (Kennedy & King, 1962b; Hildebrand *et al.*, 1967; Maas, 1998 and 2004). In latent infections *X. fragariae* can move endophytically and systemically into the roots, crowns and runners. Occasionally this latent infection may lead to water-soaked areas at the base of newly emerged leaves with subsequent sudden plant collapse and death (Stefani *et al.*, 1989; Milholland *et al.*, 1996; Mahuku & Goodwin, 1997). This second type of symptoms, called 'vascular collapse of strawberry', lead Hildebrand *et al.* (1967) to the conclusion that the disease would better be called bacterial blight of strawberry than just angular leaf spot.

Milholland *et al.* (1996) were able to isolate the pathogen from 100% of petioles 2 weeks after artificial leaf inoculation and from up to 83% of crown-tissue samples, 12 weeks after inoculation. They also detected the bacteria in xylem vessels using IF. Petiole tissues harboring bacteria were determined to be the main source of initial inoculum in strawberry fields (Wang *et al*., 2018). Survival in soil under European conditions, in cases where crop residues were incorporated into the soil with or without haulm killing, appeared to be very limited (Kastelein *et al*., 2009). Remarkably, Kong (2010) found that *X. fragariae* could still be isolated after almost 21 years of storage from air-dried leaf spots stored in tape-sealed Petri dishes at 5°C, and that these isolates were still virulent, causing typical symptoms after inoculation in strawberry plants.

During epidemics, when environmental conditions favor exudation and spread, the bacterium may cause systemic infections associated with crown pockets. These infections may also arise under damp nursery conditions. The conditions favoring infection are moderate daytime temperatures (about 20-23°C), low night-time temperatures and high humidities, up to 100% (Maas, 1998; Kastelein *et al*., 2014). During the nursery stage in open fields in South Korea (from May to August), the pathogen was detected by PCR in mother plants, but not in soil or irrigation water. During the cultivation period, from September to March, the pathogen was detected in mother plants and their progeny, and also in soil, but not in water (Kim *et al*., 2016). Infection of propagation material mainly occurs when strawberry plants are grown outdoors, when grown in glasshouses, screenhouses or polytunnels, disease incidence is much lower (Van der Gaag *et al*., 2013).

In strawberry cultivation the (latently) infected planting material is the primary infection source, but contamination is also possible via contaminated machinery, tools, field workers and animals. Natural distribution of the bacterium in the field or glasshouse is (usually) limited to a few metres, due to splashing rain, wind or irrigation water, where (dried) exudates on leaves are also involved (Van der Gaag *et al*., 2013; Kastelein *et al*., 2009 and 2018; van der Wolf *et al*., 2017). The pathogen can survive for up to 2 weeks on metal and wood (Vermunt & Van Beuningen, 2008). Survival of *X. fragariae* was determined by Turechek *et al*. (2023) on different materials used in nurseries (corrugated cardboard, cotton balls, cotton cloth, strawberry leaf, sheet metal, plastic, rubber, wood, glass, and latex glove) stored at c. 20°C or -4°C (the latter temperature being in use for dormant plants in nurseries) for up to 365 days after inoculation (DAI), using viability real-time PCR and a bioassay. *X. fragariae* could survive on all materials at -4°C up to 7 DAI, the longest survival was on cardboard (270 DAI). At 20 °C the bacterium survived in small numbers up to 14 DAI on cardboard, cotton and strawberry leaf tissue.

Cooper (2007) found a non-pathogenic *Pseudomonas* species often associated with *X. fragariae* and aggravating the symptoms to a certain extent. When *X. fragariae* is detected in rhizomes (in which bacterial exudates are sometimes found when cut transversely), this is often accompanied by other infections by *Verticillium* and/or *Phytophthora* spp. (Van der Gaag *et al*., 2013).

Metabolic changes due to infections with *X. fragariae* and their underlying genetic basis, using ultra-performance liquid chromatography (UPLC)- quadrupole-time-of-flight (QTOF) mass-spectrometry and transcriptome analysis and gene expression of both pathogen and host, with high-throughput mRNA sequencing, were determined by Kim *et al*

. (2016) and Gétaz *et al*. (2020b).

The whole genome sequences of two strains of *X. fragariae* (*Fa*P21 and *Fa*P29) isolated in 2011 from symptomatic strawberry leaf tissue in Siskiyou County, California, USA are available (Henry & Leveau, 2016). Other genome sequences were determined by Gétaz *et al*., 2017b.

Pathogenicity of *X. fragariae* is mainly based a *hrp* gene cluster coding for structural elements of the type III secretion system (T3SS), T3SS effector s(T3E) and an essential part of the *gum* cluster coding for xanthan extracellular polysaccharide synthesis (Vandroemme *et al*., 2013b). Furthermore, pathogenicity is also linked to a type IV (T4SS) and an *xps*-coded type II secretion system (T2SS) and the production of various toxins, including hemolytic and cytolytic RTX-toxins (Gétaz *et al*., 2020b; Pu?awska *et al*., 2020).

DETECTION AND IDENTIFICATION

Symptoms

On leaves, 1-4 mm, angular, shiny, water-soaked spots appear surrounded by the smallest veins. In the early stage, leafspots are only visible on the lower surface and appear translucent against the light. They enlarge, coalesce and after about 2 weeks are also visible on the upper leaf surface as water-soaked, angular spots, which subsequently become reddish-brown. They have a shiny appearance and are usually covered by bacterial exudate, which when dry, turns brown and appears as gum-like scales. Spots may coalesce along the primary and secondary veins. The dead tissues tear and break off, and the diseased leaf may show a ragged appearance.

In the most severe cases crown infection pockets may be seen inside after dissection. They appear as localized, water-soaked zones, frequently confined to one side of the crown, where bacterial exudate may also be formed.

For more information, see Kennedy & King (1962b), Hildebrand *et al.* (1967), Mazzucchi *et al.* (1973), Panagopoulos *et al.* (1978), Maas (1998) & Van der Gaag *et al.* (2013).

A blossom blight of strawberry caused by *X. fragariae* with blighting of entire flowers, or in less severe cases, water-soaked lesions on the lower surface of the calyx and pedicel of seemingly healthy green and ripe fruits was described by Gubler *et al.* (2007) from California, USA. Symptoms on calyxes and pedicels may be confused with those caused by *Erwinia pyrifoliae*, although in the latter case there is a more general blackening of the tissues, in some cases with bacterial slime formation. Fruits are usually infected, blackened and often malformed (Wenneker & Bergsma-Vlami, 2015).

Symptoms of angular leaf spot caused by *X. fragariae*may be confused with those caused by fungi, such as *Ramularia grevillea (*formerly *Mycosphaerella fragariae)* causing common spot of strawberry and *Diplocarpon fragariae*, causing leaf scorch, as well as with the symptoms caused by *X. arboricola* pv. *fragariae* (see Notes on taxonomy and nomenclature and Janse *et al*., 2001). Definitive diagnosis should always be obtained through laboratory analysis (see below and EPPO, 2023).

Morphology

X. fragariae is an aerobic, Gram-negative, non-spore-forming, non-capsulated rod; size averaging 0.4 x 1.3 µm. Most cells are non-motile, but some have a single polar flagellum. On a suitable medium such as Wilbrink's medium colonies are circular, entire, convex, glistening, translucent to pale-yellow after 3-5 days incubation at 20 to 24°C (Bradbury, 1977; Roberts *et al.*, 1997; EPPO, 2023).

Detection and inspection methods

Rapid screening tests such as ELISA or IF and PCR/Nested PCR or a detached leaf assay (Civerolo *et al.*, 1997a and b; Randhawa & Civerolo, 2017) can be used for presumptive diagnosis of *X. fragariae*, as the bacterium is quite difficult to isolate, and its colonies are easily overgrown by those of secondary organisms. Use of purified agar (Difco) is recommended in all media because impurities from other commercial agars can inhibit the growth of *X. fragariae*

(Rowhani *et al*., 1994; EPPO, 2023). The pure culture is distinguishable on agar media from other phytopathogenic xanthomonads, including *X. a.* pv. *fragariae* (EPPO, 2023).

Stöger & Ruppitsch (2004) developed a sensitive, PCR kit called REDExtract-N-Ampk Plant PCR-Kit for the detection of *X. fragariae* in (a)symptomatic plant material. Several sensitive (nested/multiplexed) PCR detection tests, also in combination with immune-capture, targeting different loci in the *X. fragariae* genome, have been developed (Roberts *et al*., 1996; Pooler *et al*., 1996; Hartung & Pooler, 1997; Zimmermann *et al.*, 2004; Weller *et al.*, 2007; Vandroemme *et al.*, 2008; Turechek *et al.*, 2008; Vermunt and van Beuningen, 2008). These tests can be used to confirm the presence of *X. fragariae* in symptomatic plant material, and can be used for detection of (latent) infections in (symptomless) plants (Mahuku and Goodwin, 1997; Zimmerman *et al.*, 2004, EPPO, 2023; IPPC 2016). A real-time PCR was developed by Cubero *et al*. (2009), and another one, specifically tested for detection in crown tissue, by Turechek *et al*. (2008). A loop-mediated isothermal amplification assay (LAMP) and sample preparation procedure for detection was developed by Wang & Turechek (2016) and Gétaz *et al*. (2017a).

Rep-PCR has been used for identification of field isolates of *X. fragariae* (Opgenorth *et al*., 1996) as well as MALDI-TOF mass spectrometry (Vandroemme *et al.*, 2013b).

Both PEMAX-PCR (a mix of nucleic acid intercalating dyes propidium monazide - PMA and ethidium-monazide - EMA) and PMA-real-time PCR have been recently developed for the detection of viable cells of *X. fragariae* in strawberry. This so-called viability PCR (vPCR) could be useful in testing of planting material entering a country (Wang & Turechek, 2020; Immanuel *et al*., 2020).

An efficient spray-infiltration method of inoculation was published by Hazel *et al*. (1980), but also see EPPO (2023).

Details about presumptive diagnosis with rapid tests, detection and identification methods (including methods for extraction of bacterial cells and DNA), biochemical, serological and molecular and pathogenicity tests (using inoculation of bean plantlets or hilum injury/seed inoculation) for latent and symptomatic infected material, flow chart, culture media, chemicals and reference material) are provided in the EPPO Standard PM 7/65 (2) (EPPO, 2023) on *X. fragariae* and IPPC Diagnostic protocol DP 14 (IPPC, 2016).

PATHWAYS FOR MOVEMENT

Locally, *X. fragariae* can be spread by splashing water or via aerosols generated by precipitation, irrigation, or mowing (Van der Wolf *et al*., 2017; 2018). The bacterium can also be spread via contact with contaminated machinery, clothes and by animals (Maas, 2004). For example, the spread of *X. fragariae* in a strawberry field by mowing and runner cutting machinery was studied in the Netherlands. The blades of a rotary mower became heavily contaminated after trimming leaves of symptomatic plants and could spread the bacterium to healthy plants up to a distance of 4 m (Kastelein *et al*., 2018).

Within a field crop, *X. fragariae* is not free-living in the soil, but it can overwinter in the soil in association with previously infected plant material (Maas, 1998).

Over short and long distances, the movement of infected plants for planting is the main pathway. Commercial strawberry runners used for planting may spread the bacterium as they may still bear old, whole or torn, infected leaves or have crown infection pockets. Moreover, almost invisible fragments of infected leaves may be hidden in the apical crown region or between the roots (Kennedy & King, 1962a). Viable cells of *X. fragariae* could be detected in fruits produced for the fresh market (Immanuel *et al*., 2020), but fruits are considered as a minor pathway.

PEST SIGNIFICANCE

Economic impact

Like in other strawberry leaf spot and blight diseases caused by e.g., *Phomopsis obscurans* and *X. arboricola* pv. *arboricola* and leaf blotch caused by *Zythia fragariae*, bacterial blight causes a certain reduction in yield, but generally, the disease is not destructive. However, heavy losses may occur under very wet weather conditions or

frequent overhead sprinkler irrigation. The highest losses (up to 75% fruit loss) were reported by Epstein (1966) in the USA, but in most cases losses are much lower, since only leaves and calices are infected, leading to a moderate reduction of photosynthesis and disfigured calices which very occasionally rendered fruits unmarketable (Legard *et al*., 2003). Earlier substantial losses reported by Mazzucchi *et al*. (1973), López *et al*. (1985) and Bosshard & Schwind (1997) were not observed in later years, although the disease is widespread in Western Europe. In the USA, yield losses up to 25% were reported during some years in the 1990s, but the biggest impact was due to import restrictions from Mexico and the European Union for planting material (Roberts *et al*., 1997; Van der Gaag *et al*., 2013). In South Korea, the implementation of a strict control programme for several years reduced the disease incidence from 45% to 5% (Yoon *et al*., 2016).

Control

The use of healthy planting material and avoidance of conditions favouring the disease (e.g. high humidity, high nitrogen fertilizing) are the main control methods. Crop operations, using machinery proved important for disease and pathogen spread in the field (Gigot *et al*., 2017; Kastelein *et al*., 2018) and should be taken into account when considering control measures. Other cultural measures include the use of drip irrigation and irrigation early in the morning to obtain faster drying of crop, as well as monitoring and removal of diseased plants. Treatments with copper-containing products have shown some effectiveness, but have to be applied very intensively, with a risk of phytotoxicity (Kennedy-Fisher, 1997).

Mixtures of copper compounds and the fungicide mancozeb were found to be effective against *X. fragariae*, but they may lead to phytotoxicity (Roberts *et al*., 1997). Alternatives to copper such as acibenzolar-S-methyl, and kasugamycin showed lower disease severity than untreated controls (Cooper, 2007). An organic acid-based biopesticide (OAB, containing 10.73 g L^{-1} of citric acid and 21.37 g L^{-1} of lactic acid) could reduce disease incidence by up to 50% when 7 sprays were applied, in trials under natural field infection conditions, in Canada, where the product is registered (Dubois *et al*., 2017). Oxidate (hydrogen dioxide 27%) was registered in the USA for control of angular leaf spot and has been used with varying success (Anco & Ellis, 2011). Braun & Hildebrand (2013) found that foliar applications of various antioxidants, such as ?-tocopherol and mannitol, along with the plant activators acetylsalicylic acid and acibenzolar-S-methyl, and the fungicide fosetyl-Al, substantially reduced disease incidence. Kim *et al*. (2016) used oxolinic acid, with an 87% control during the nursery period, and the antibiotic validamycin-A, with a 95% control during the cultivation stage (control effect 95%). A post-harvest propylene oxide fumigation also had a control effect. Amino-thiazidol and zinc thiazidol alone or in low-rate combination with kasugamycin or copper compounds reduced disease incidence when applied pre-harvest to more than 90% (Haack *et al*., 2019).

Henry *et al*. (2016) observed a strong reduction in symptom formation when *X. fragariae* was co-inoculated with tannic acid (a chelating agent of iron) onto strawberry plants, suggesting a kind of nutritional immunity and possibilities for control by restriction to iron access on or in the plant.

Dipping plants in a solution of 10% chlorine bleach and use of UV-C radiation and a concomitant removal or trimming remnant leaf and petiole tissue from nursery-trimmed plants, reduced disease significantly but not completely in planting stock (Turechek *et al*., 2013).

Heat treatment was successfully applied to nursery stock where bacterial populations were exposed to 44°C for 4 h or 48°C for 2 h. These treatments minimally affected vegetative growth of plants bagged dry or wet (Turechek & Peres, 2009).

Hildebrand *et al*. (2005) found the cultivar Tristar as well as two clones, US 4808 and US 4809, that were derived from *F. virginiana*, to be much less susceptible than most of the cultivars tested. The latter two were released for resistance breeding. An important locus determining the resistance of the two wild genotypes mentioned above was found (designated *FaRXf1*) which could play a role in marker-assisted selection in order to develop resistant cultivars (Roach *et al*., 2016). Maas *et al*. (2002) used the above mentioned octoploid highly resistant clones US 4808 and US 4809, see also Jamieson *et al*. (2013). Recent breeding experiments with these genotypes showed that their resistance is based on three or four unlinked loci (Lewers *et al*., 2003), leading to only limited inheritance.

A high level of plant host resistance to angular leafspot disease has been found in certain *Fragaria* species, such as *F. moschata* (2n=6x) and *F. vesca* (2n=2x) and occasionally in clones of *F. virginiana* (2n=8x) but not in octoploid

cultivated strawberries and in *F. x ananassa* cv. Tristar (Roberts *et al*., 1997; Maas *et al*., 2000, 2002; Hartung *et al*., 2003; Xue *et al*., 2005; Hildebrand *et al*., 2005; Cooper, 2007; Pérez-Jiménez *et al*., 2012; Jamieson *et al*., 2013). In addition to *F. moschata* some accessions of *F. pentaphylla*, a tetraploid species of wild strawberry native to China showed resistance towards *X. fragariae* (Xue *et al*., 2005).

The old German strawberry variety Sieger was found to be resistant against two strains of *X. fragariae* in Spain and could also be useful in breeding programs (Pérez-Jiménez *et al*., 2012). To obtain more understanding and potential contributors to breeding for resistance Bestfleisch *et al*. (2015) screened 145 *Fragaria* genotypes of which 6, with variable polyploidy showed moderate resistance, belonging to *Fragaria vesca* f. *alba*, *F. nilgerrensis* 'Yunnan', *F. vesca* 'Illa Martin' and *F. moschata* 'Bauwens'.

Phytosanitary risk

The main pathway for (international) spread of *X. fragariae* is via asymptomatic plants for planting with latent infections. Infected planting material is therefore the main risk for uncontaminated areas. However, over the years it has been observed that actual losses due to bacterial angular leaf spot remain generally low to very low and that current cultural methods, in combination with some preventive sprays of bactericidal compounds, can sufficiently limit its importance (Van der Gaag *et al*., 2013). Based on this information the European Union changed the status of *X. fragariae* from a quarantine pest (Annex IIA2) to a regulated non-quarantine pest (RNQP) (EU, 2019).

PHYTOSANITARY MEASURES

It can be recommended that strawberry planting material from infested countries should be derived from mother plants kept free from *X. fragariae* as part of a certification scheme (EPPO, 2008a), and that the place of production should have been found free from the disease during the last five growing seasons. In addition, visual inspections during the dormant period can be useful. Inspectors should look for typical angular spots on old leaves or on their remains still attached to the runners. Samples from lots kept in cold storage must be inspected immediately after the runners are taken out and thawed. The spots can no longer be seen after only 1 day at room temperature.

Visual inspection and sampling methods for strawberry planting material (at import) are described in the commodity specific EPPO phytosanitary procedure PM 3/73 (1) (EPPO 2008b) and for places of production in PM 3/83(1) (EPPO, 2017). Certification for pathogen-tested material, including tests for *X. fragariae* are laid down in EPPO PM 4/11 (2) (EPPO, 2008a). Detailed risk management measures against *X. fragariae* have also been proposed on the use of non-certified and certified strawberry planting material during the RNQP project (EPPO, 2018). Detection of viable cells of *X. fragariae* using viability PCR (vPCR) has been suggested as a method that could be useful in post-entry quarantine and for detection in fresh fruits in New Zealand (Immanuel *et al*., 2020).

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