Erwinia pyrifoliae

Figure 1. Fire blight (*Erwinia amylovora*) symptoms in pear, including blackened leaves and branch tips. Courtesy of Melinda Sullivan, USDA-APHIS-PPQ.

Scientific Name
Erwinia pyrifoliae Kim et al., 1999

Synonyms
None

Common Name(s)
Asian pear blight
Bacterial shoot blight of pear

Type of Pest
Bacterium

Taxonomic Position
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Erwiniaceae

Reason for Inclusion in Manual
Additional pest of concern

Background Information
Erwinia pyrifoliae was first formally described in symptomatic Asian pear trees in South Korea (Kim et al., 1999). Symptoms in these trees were similar to those shown by the closely related bacteria *Erwinia amylovora* (Fire blight), but molecular analysis determined that *E. pyrifoliae* was a distinct species (Kim et al., 1999)*.* After the initial description of *E. pyrifoliae*, researchers in Japan examined some bacterial samples which were previously thought to be *E. amylovora* (Beer et al., 1996) and determined that they were also *E. pyrifoliae* (Geider et al., 2009; Thapa et al., 2013). In 2013, *E. pyrifoliae* was unexpectedly found in the Netherlands, in greenhouses containing strawberry plants (EPPO, 2014; Wenneker and Bergsma-Vlami, 2015). The geographic origin of this pathogen is undetermined, and a comparative analysis of reference strains from the Netherlands, Japan, and South Korea is ongoing (Wenneker and Bergsma-Vlami, 2015).

Pest Description
"Cells are Gram-negative, non-spore-forming, peritrichous, straight rods. The strains are facultatively anaerobic, oxidase is not produced. Nitrates are not reduced. The species conforms to the definition of the family *Erwiniaceae.* Strains grow on YPDA medium, producing colonies that are 2 mm after 48 hr. at

Figure 2. Fire blight (*Erwinia amylovora*) symptoms on common pear, including blackened leaves and branch tips also known as 'Shepherd's crook'. Courtesy of Melinda Sullivan.

28ºC (82ºF). Colonies are circular white, well-domed and opaque. Glucose (dextrose) is fermented without gas production. Voges-Proskauer test is (weakly) positive" (Kim et al., 1999).

The genome of the Japanese strain *E. pyrifoliae* Ejp617 is 3.9 Mb, smaller than the Korean strain Ep1/96 which has a size of 4.0 Mb. Nucleotide homology between the two strains is 95% (Thapa et al., 2013). A direct comparison of genomes of Japanese and Korean strains revealed numerous insertion/deletion rearrangements and inversions in the central regions of the chromosomes (Thapa et al., 2013).

"The genome of (Japanese strain) Ejp617 encodes numerous virulence factors, including a type VI secretion system, an exopolysaccharide synthesis cluster, and another protein secretion system present in plant pathogenic *Erwinia* strains" (Thapa et al., 2013).

Biology and Ecology

The biology of *E. pyrifoliae* is not well studied, but it may be similar to *E. amylovora*, which is a better understood pathogen of pear. *Erwinia amylovora* infects host plants through wounds and migrates through the xylem and other tissues, spreading the infection. It then overwinters in annual cankers that were formed on diseased branches during the previous season. *Erwinia amylovora* also can reside as an endophyte within apparently healthy plant tissue, such as branches, limbs, and budwood (Johnson, 2000). *Erwinia amylovora*, was shown to survive for up to five weeks in untreated soil from a field (Hilderbrand et al., 2001). *Erwinia amylovora* can disperse short distances in the air (Hilderbrand et al., 2001) and is also transmitted mechanically by insects, surviving for up to 12 days after contact with aphids (Hildebrand et al., 2000).

The biology of *E. pyrifoliae* is not well understood in strawberry. Depending on the time of the strawberry growing season when *E. pyrifoliae* infection starts, economic losses can greatly vary from low (when infection occurs late in the stages of fruit development) to very high (when infection occurs at the initial stages of fruit development) (EPPO, 2014). It is currently unknown how *E. pyrifoliae* survives in the absence of a host (NPPO, 2014). Wenneker et al. (2016) demonstrated that *E. pyrifoliae* can be transported by honey bees, and the pathogen can be detected in bee colonies introduced into a greenhouse before symptoms are visible in infected strawberries in the same greenhouse.

Symptoms/Signs

In pear: "Black-to-brown stripes in the midribs of leaves, brown-to-black leaf spots and necrotic petioles were observed on Asian pear (*Pyrus pyrifolia* cv. Shingo). Symptoms spread to entire branches and also affected blossoms and fruitlets. Large areas of trees were found with these necrotic symptoms and a major portion of trees in an orchard could be affected" (Rhim et al., 1999).

Figure 3. Fire blight (*Erwinia amylovora*) Symptoms on common pear, including undeveloped fruit. Florida Division of Plant Industry, FDACS, Bugwood.org

Symptoms of *E. pyrifoliae* on pear trees Korean orchards remotely resembled those of fire blight (*Erwinia amylovora*) (Fig. 1, 2, 3) (Kim et al., 1999). Symptoms caused by the Japanese strain of *E. pyrifoliae* in pear are similar to symptoms caused by Korean strains (Beer et al., 1996; Kim et al., 1999).

In strawberry (Fig. 4): "Strawberry plants expressed an intense brown to black discoloration of their immature fruits, their fruit calyx, and the attached stems. There were no symptoms observed on the leaves. The discoloration was also observed inside the young fruits, including an intense shining of the fruit tissue. In several cases, the release of bacterial slime on the surface of the young fruits and their attached stems has been observed. Fruits did not develop at all or were in many cases heavily malformed. Crop losses of up to 40% were found" (Wenneker and Bergsma-Vlami, 2015).

Figure 4: Symptoms in strawberry fruit infected with *Erwinia pyrifoliae.* Infected fruits tend to blacken*,* ooze bacteria slime, or become misshapen. Courtesy of M. Wenneker, Applied Plant Research, Wageningen University & Research.

In apple: Symptoms in experimentally inoculated apple cultivars were generally weak and included leaf wilting (Kim et al., 2001). Symptoms were not always observed in apple, and the extent of damage caused by *E. pyrifoliae* is unclear and may not be significant (Kim et al., 2001).

Pest Importance

Pear and strawberry, which are both affected by *E. pyrifoliae*, are important commodities in the United States. Damage caused by fire blight outbreaks in pear orchards can be significant, and severe loss of fruit production may result from the disease (Kim et al., 1999; Rhim et al., 1999). Crop losses up to 40% occurred in strawberry in infected greenhouses (Wenneker and Bergsma-Vlami, 2015). Symptomatic fruit were unmarketable (EPPO, 2014). Apple is also an experimental host of *E. pyrifoliae* and may be susceptible to damage caused by this pathogen (Kim et al., 2001).

In 2014, strawberry was planted on 59,895 acres in the United States, and the value of utilized production was over $2.8 billion (USDA-NASS, 2016). Pear was grown on 49,300 acres in 2014, and the value of utilized production was over

$457 million (USDA-NASS, 2016). Apple was grown on 327,380 acres in 2014, and the value of utilized production was over $2.9 billion (USDA-NASS, 2016).

Known Hosts

Major hosts: *Fragaria ananassa* (strawberry) (EPPO, 2014) and *Pyrus pyrifolia* (Asian pear) (Kim et al., 1999, 2001).

In the Netherlands, the most commonly infected strawberry cultivar appears to be 'Elsanta'. Other susceptible cultivars include: 'Selva, Clery, Malling Opal, and Ischia' (Wenneker et al., 2016).

Experimental hosts: *Malus domestica* (apple), and *Pyrus communis* (European pear) (Kim et al., 2001).

Experimentally susceptible European pear cultivars include: '4703/78, Bartlett, Conférence, Doyenné du Comice, Harrow Sweet, INRA hybrid, Old Home, US 65.063.13 (Kim et al., 2001; Wenneker et al., 2016). Experimentally susceptible apple cultivars include: 'Empire, Fortune, Idared, and McIntosh' (Beer et al., 1996; Kim et al., 2001).

Known Vectors (or associated insects)

Erwinia pyrifoliae is not known to be transmitted by vectors. However, it can be transmitted by phoresy on honeybees (Wenneker et al., 2016). In addition, *E. amylovora* can be transmitted by phoresy on insects including *Aphis pomi* (Aphids) and *Chrysoperla carnea* (Green lacewing) (Hildebrand et al., 2000). In addition, *Ceratitis capitata* (Mediterranean fruit fly) (Ordax et al 2015), and many other insects are also potential vectors of *E. amylovora.*

Known Distribution

Asia: Japan (Beer at al., 1996), and South Korea (Kim et al., 1999).
Europe: Netherlands (EPPO, 2014).

There is some uncertainty about the current distribution of *E. pyrifoliae*. Despite extensive surveying in South Korea, this pathogen has not been found there since 1998 after phytosanitary measures were initiated in the area of the original outbreak (Geider et al., 2001; Kim et al., 2001). In Japan, the record of *E. pyrifoliae* is reported to be, 'unreliable' by EPPO (2015). There was also a reported finding of *E. pyrifoliae* in a single strawberry field in Belgium in 2011, confirmed by polymerase chain reaction (PCR), but pathogenicity tests with the isolated bacteria were inconclusive (NPPO, 2014).

Pathway

The most probable pathway of entry for *E. pyrifoliae* into the United States is through the shipment of infected plant material. Currently, there are multiple open pathways of entry from the Netherlands. The import of *Malus* spp. and *Pyrus* spp. propagative material from the Netherlands is allowed (USDA, 2017). Since 2006,

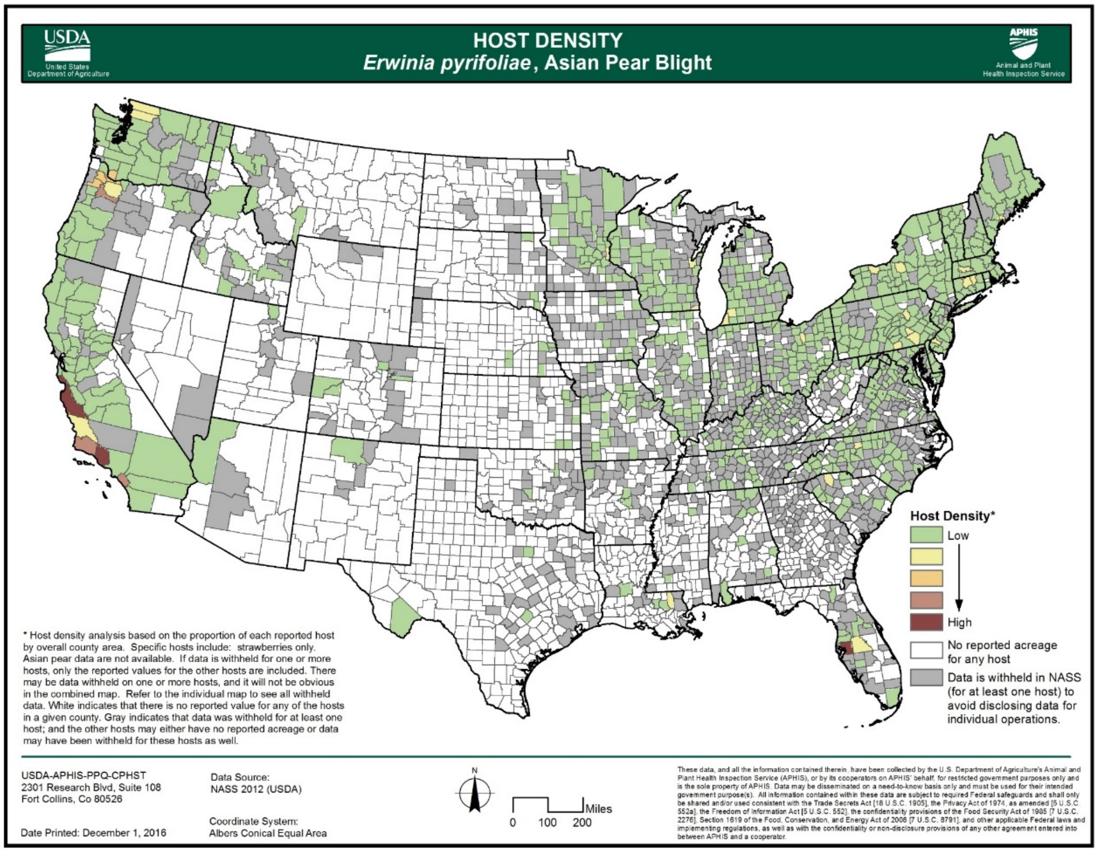

Figure 5: Host density map in the continental United States. Map courtesy of USDA-APHIS-PPQ-CPHST.

there have been 124 shipments of *Malus* spp. plant material from the Netherlands containing a total of over 10.1 million plant units (AQAS, 2017). In addition, there have been 217 shipments of *Fragaria* spp. plant material from the Netherlands since 2006, with the largest shipment containing 950,000 plant units (AQAS, 2017). There was also one shipment of *Pyrus* spp. plant material from the Netherlands (AQAS, 2017).

Since 2009, there have been interceptions of *Erwinia* spp. at U.S. ports of entry from Costa Rica (2), Tahiti (1), and Vietnam (2) (AQAS, 2017). The interceptions were likely not *E. pyrifoliae* based on the countries of origin, but these interceptions demonstrate the ability of *Erwinia* spp. to travel long distances.

Since 2006, there were 199 interceptions of *Malus* spp. plant material intended for propagation from the Netherlands (AQAS, 2017). These interceptions occurred on permit cargo, baggage, and general cargo. Since 2006, there have also been interceptions of *Fragaria* spp. plant material from Japan (16), Netherlands (9), and South Korea (8) (AQAS, 2017). Most of these interceptions were fruit intended for consumption, but two of them contained plants intended for propagation. Since 2006, there have also been interceptions of *Pyrus* spp.

plant material from South Korea (32) and Japan (9) (AQAS, 2017). One interception from Japan contained plant material intended for propagation.

Potential Distribution within the United States

Strawberry is primarily grown in California, which accounts for about 90% of total production in the United States (USDA-NASS, 2016). Florida accounts for roughly 6.5% of U.S. strawberry production. Other states with 1,000 or more acres of strawberry cultivation in 2015 were: Oregon, Washington, North Carolina, and New York (USDA-NASS, 2016).

European pear, an experimental host of *E. pyrifoliae*, is primarily grown in western states, with 95% of commercial production taking place in Washington, Oregon, and California (USDA-NASS, 2016). Washington alone accounts for about 42% of U.S. pear acreage (USDA-NASS, 2016). Other states with commercial pear production include New York, Pennsylvania, and Michigan (USDA-NASS, 2016).

Apple is a widely grown experimental host of *E. pyrifoliae*, with commercial cultivation reported in at least 29 states in 2014 (USDA-NASS, 2016). Washington is the leading apple producing state and accounts for about 47% of U.S. apple acreage (NASS, 2016). The top ten apple growing states by acreage in 2014 were: Washington, New York, Michigan, Pennsylvania, California, Virginia, North Carolina, Oregon, West Virginia, and Ohio (USDA-NASS, 2016).

Survey

Approved Method for Pest Surveillance*: The CAPS-approved survey method is visual survey. Collect symptomatic plant material from symptomatic plant material or plant material with signs (ooze) of the bacterium.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Visual symptoms of *E. pyrifoliae* infection in pear have been described in numerous published articles (Beer et al., 1996; Kim et al., 1999; Rhim et al., 1999). Symptoms of *E. pyrifoliae* in strawberry are also described in multiple articles (EPPO, 2014; Wenneker and Bergsma-Vlami 2015).

Key Diagnostics

Approved Method for Pest Surveillance*:

The CAPS-approved identification method is molecular diagnostics coupled with an ELISA pre-screen.

No field-based screening method for *E. pyrifoliae* is currently available. There is, however, potential for an ImmunoStrip kit to be developed but a specific one is not available. A double antibody switch indirect enzyme-linked immunosorbent assay (DASI-ELISA) kit has been developed for the *Erwinia amylovora* (http://plantprint.net/kits-and-products/bacteria-2/?lang=en) and was developed based on the work of Gorris et al. (1996) and validated and included in the EPPO official protocol (EPPO, 2013). The two species generate similar symptoms, and this method could be used to pre-screen out fire blight.

There are several published molecular diagnostic methods that are not currently validated for regulatory purposes but could be used for screening purposes (see literature based methods).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
There are several published molecular diagnostic methods for *E. pyrifoliae*. Kim et al. (1999) originally identified the species using nucleotide sequencing of the16s rRNA genes, real time PCR assay, and biochemical and physiological tests. Kim et al. (2001) developed a PCR which can distinguish *E. pyrifoliae* from *E. amylovora* and also identify *E. pyrifoliae* in necrotic plant tissue. Wensing et al. (2012) described two methods for identifying *Erwinia pyrifoliae*, including Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI–TOF MS). Additionally, novel PCR primers were designed for four *Erwinia* species, including *Erwinia pyrifoliae.* These PCR primers were used by Wenneker and Bergsma-Vlami (2015) to identify *E. pyrifoliae* in strawberry.

The genome sequences of several strains of *E. pyrifoliae* were recently published (Thapa et al., 2013). This will lead to possible new molecular identification methods in the future.

Easily Confused Species
Symptoms of *E. pyrifoliae* may be confused with other known bacterial pathogens of pear, including *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae*, which are both found in North America (Rhim et al., 1999). '*Candidatus* Phytoplasma pyri' may also cause similar symptoms in pear and is also present in the United States (Seemüller and Schneider, 2004).

There are other pathogens which infect strawberry plants in North America, but *Erwinia pyrifoliae* can be distinguished because it exhibits symptoms in strawberry fruit and not the leaves (Wenneker and Bergsma-Vlami, 2015). Viruses typically exhibit symptoms in strawberry leaves (Tzanetakis and Martin, 2013). Dozens of viruses are also known to infect strawberry in North America, including the predominant *Strawberry necrotic shock virus* (Tzanetakis and

Martin, 2013). Another bacterial pathogen, *Xanthomonas fragariae*, is the causal agent of bacterial angular leaf spot in strawberry in the United States (Roberts et al., 1997). An ELISA is available for Xanthomonas at the genus level (https://orders.agdia.com/agdia-conjugate-xan-alkphos-xanthomonas-genus-specific-eca-14600).

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