Fire Blight

Erwinia amylovora

Diana Pimentel

InnovPlantProtect

10 November 2021

1 Abstract

Erwinia amylovora (Burrill) Winslow et al. is the causal agent of fire blight in approximately 200 species of the *Rosaceae* family. Fire blight is one of the most devastating diseases of pear and apple. The most common symptoms of fire blight are the blackening and wilting of blossoms and fresh shoots, which reflects the main areas of entrance and proliferation of the pathogen, and the necrosis and withering of organs or entire sections of the affected plants caused by the proliferation of the pathogen in the host tissues and vascular system. *E. amylovora* is a protected zone quarantine pest. Since Portugal is no longer a protected zone, this pathogen is considered a regulated non-quarantine pest (RNQP). *E. amylovora* is widely distributed across North and Central America and Europe, and is present in the Middle East and southern Mediterranean area. Temperature and humidity are critical factors for the development of fire blight, being temperatures from 18 to 29°C and high relative humidity (90–95%) the most favourable conditions. *E. amylovora* can be spread by insects, rain, wind, infected propagation material and non-disinfected pruning tools. Some predictive models have been developed, such as Maryblyt and Cougarblight, based on risk factors like temperature and humidity. Fire blight can be detected through visual inspection in the field and confirmed through laboratory tests. Available control methods in Portugal include chemical treatments (copper, fosetyl-Al, acibenzolar-S-methyl, laminarin and prohexadione-calcium) and biological control agents (*Bacillus amyloliquefaciens* QST 713 and *Bacillus amyloliquefaciens* subsp. plantarum, strain D747). Sanitation of pruning tools, removal of overwintering cankers, grafting on resistant rootstock and reduce nutrient application are cultural practices important to prevent fire blight infection.

2 Resumo

Erwinia amylovora (Burrill) Winslow et al. é uma bacteria Gram-negativa pertencente à família Enterobacteriaceae e responsável pela doença denominada fogo bacteriano, afectando aproximadamente 200 espécies da familia *Rosaceae*, incluindo tanto arvores de fruto (pereiras, macieiras e marmeleiros) como plantas ornamentais. *E. amylovora* é um organismo de quarentena de zonas protegidas (Regulamento de Execução (UE) 2019/2072 da Comissão, Anexo IV). Estando a doença distribuida em todo o território, Portugal perde, em 2018, o estatuto de zona protegida e *E. amylovora* passa a ser considerada uma praga regulamentada não sujeita a quarentena (RNQPs). O fogo bacteriano tem grande impacto económico na produção de pêras e maçãs. Os sintomas mais comuns incluem o desenvolvimento de necroses de cor castanha a negra dos gomos e raminhos, presença de exsudado bacteriano, cancro nos ramos e tronco e mumificação dos frutos. A principal fonte do inóculo são os cancros. Durante o inverno, as bacterias hibernam nas margens dos cancros, onde por vezes se torma visivel um exudado composto por bactérias viáveis numa matrix de polissacáridos. Na primavera com o aumento da temperatura, as bactérias tornam-se activas, multiplicam-se e propagam-se para as flores através da chuva, vento e insectos. O fogo bacteriano foi descrito pela primeira vez nos Estados Unidos da América em 1780 e actualmente está amplamente presente na America do Norte e Central, Europa e Médio Oriente. Em Portugal, o fogo bacteriano foi identificado pela primeira vez em 2005 no Fundão, e subsequentemente erradicado. Novos casos foram detectados em 2010 em várias regiões do país, nomeadamente Alcobaça, Bombarral, Torres Vedras, Caldas da Rainha, Guarda, Viseu, Ferreira do Alentejo e Alandroal, tendo sido implementadas medidas fitosanitárias que incluiam a destruição de plantas infectadas. Em 2017 foram detectados casos distribuidos ao longo de todo território continental: Mafra (*Pyrus*

communis), Penafiel (*P. communis* cv. Rocha), Viana do Castelo (*Rubus fruticosus* cv. Ouachita), Figueira Castelo do Rodrigo (*Cydonia oblonga*), Montemor-o-Novo (*Cydonia*, *Malus* and *Pyrus*), Tavira (*M. domestica*), e Monchique (*C. Oblonga*). Em 2021, observa-se grande incidência do fogo bacteriano na região do Oeste. Os principais factores de risco para o desenvolvimento do fogo bacteriano incluem temperaturas de 18 a 29°C, elevada humidade relativa (90-95%) ou chuva após períodos quentes, presença de evelados níveis de inóculo, presença de insectos polinizadores e existência de florações secundárias. *E. amylovora* pode ser disseminada por insectos, chuva, vento, material de propagação infectado e pelo uso de material agricola não desinfectado.

Existem vários modelos de previsão, como os modelos Maryblyt e Cougarblight, no entanto estes, embora eficazes, não estão totalmente adaptados a Portugal. Actualmente, o projecto Fire4Cast desenvolvido pelo COTHN, BioISI e INIAV tem como objectivo a criação de um modelo epidemiológico de previsão adaptado à zona do Oeste, Cova da Beira e Alentejo.

O fogo bacteriano pode ser detectado pela avaliação visual dos sintomas e confirmado por testes laboratoriais, que incluem teste serológicos e de PCR. Os métodos de contolo aprovados pela DGAV em Portugal incluem tratamentos químicos (cobre, fosetil, laminarina, prohexadiona-cálcio e acibenzolar-S-methyl) e agentes de controlo biológico (*Bacillus amyloliquefaciens* QST 713 e *Bacillus amyloliquefaciens* subsp. *plantarum*, estirpe D747). Para reduzir o nível de inóculo e evitar a propagação, os cancros devem ser removidos durante o outono e inverno, devem ser feitos cortes 30-50 cm abaixo do local do sintoma, o material resultante da poda deve ser queimado, o material de poda deve ser desinfectado, usar porta-enxertos resistentes, e deve reduzir-se a aplicação de abubos nutritivos.

3 Introduction

Erwinia amylovora (Burrill) Winslow et al., belonging to the Enterobacteriaceae family, is the causal agent of fire blight in most species of the subfamily *Maloideae* of the family *Rosaceae*. The name fire blight is descriptive of its major characteristic, which is a blackening of twigs, flowers, and leaves as if burned by fire (Palacio-Bielsa *et al.*, 2012). The genus *Erwinia* contains other plant pathogens of lesser economic importance such as *Erwinia pyrifoliae* and *Erwinia piriflorinigrans* (Palacio-Bielsa *et al.*, 2012). *E. amylovora* has been thoroughly studied due to its early description and significant economic impact, since it affects important fruit crops, such as pear and apple. Fire blight has been described in approximately 200 species, which are included in all four subfamilies of the *Rosaceae* family: *Maloideae* (syn. *Pomoideae*), *Rosoideae*, *Amygdaloideae* (syn. *Prunoideae),* and *Spiraeoideae* (Palacio-Bielsa *et al.*, 2012). Some plant species can act as a reservoir of the pathogen for future infections, such as observed for *Pyracantha* spp. in California (Schroth *et al.*, 1974).

Symptoms of *E. amylovora* infection have been observed in *Rosaceae* plants since 1780 in North America, from where it extended to other countries (van der Zwet and Keil, 1979). In the early XX century was firstly reported in New Zealand, and then in European and Middle East countries. Nowadays, fire blast can be found in most of North America, New Zealand, South Korea, Europe, Middle East and North Africa. Nevertheless, it has been eradicated in Australia and samples coming from Japan are considered belonging to a possibly different species (Thapa *et al.*, 2013). Long distance fire blight spread is most likely associated with the introduction of infected plant material from abroad, such as plants and branches for propagation or fruits.

The most common symptoms of *E. amylovora* infection are the blackening and wilting of blossoms and fresh shoots, which reflects the main areas of entrance and proliferation of the pathogen, and the necrosis and withering of organs or entire sections of the affected plants caused by the proliferation of the pathogen in the host tissues and vascular system (Paulin, 1996). Fire blight infection can be divided into multiple phases: canker blight (develops due to renewed activity by the bacteria at the margins of overwintering cankers from the previous season), blossom blight (develops due to infections of open flowers), shoot blight (develops from direct infections of vegetative shoot tips), and trauma blight (develops following injuries in plant tissues).

The first *E. amylovora* full genome was sequenced in 2010 (Smits *et al.*, 2010). Currently, 141 assemblies are publicly available in NCBI genome database, including 14 with complete genome sequences, three at the chromosome level, 99 at the scaffold level, and 25 at the contig level. *E. amylovora* genome is small with, in mean, 3.81 Mb.

E. amylovora was considered a quarantine organism by the European Union (Directive 2000/63/EU Annex II/A2). However, and due to the fact that it is now present in most European countries, it has been removed from the list of quarantine pathogens in 2019 (Commission Implementing Regulation (EU) 2019/2072). In Portugal, the pathogen has been reported since 2005. The first foci were quickly eradicated, nevertheless in 2010 the bacterium was identified again in several regions, particularly in the Oeste, Center and Alentejo region (DGADR *et al.*, 2011). After those outbreaks, Portugal lost its status as the last south European country not having *E. amylovora* as an endemic pathogen. In 2017, fire blight outbreaks were detected in several municipalities, from north to south (EPPO, 2018).

4 The pest and its biology

4.1 Taxonomy

Name: *Erwinia amylovora* (Burrill, 1883) Winslow et al., 1920

Synonyms: *Micrococcus amylovorus* Burrill, 1883;
Bacillus amylovorus (Burrill, 1883) Trevisan, 1889;
Bacterium amylovorus (Burrill, 1883) Chester, 1897;
Erwinia amylovora f. sp. *rubi* Starr, Cardona & Falson, 1951.

Taxonomic tree:
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Erwinia*
Species: Erwinia amylovora

Common names: Fire blight (US), fireblight (GB), fogo bacteriano (portuguese), fuego bacteriano (spanish), feu bactérien (french).

EPPO code: ERWIAM (Erwinia amylovora).

Phytosanitary categorization: EPPO A2 list no. 52, EU Protected Zone Quarantine Pest (Annex III), EU Regulated Non-quarantine Pest (Annex IV)

Erwinia amylovora is a gram-negative bacterium, with rod shaped of about 0.3 µm × 1–3 µm in size and motile with peritrichous flagella (van der Zwet and Keil, 1979). The genus *Erwinia* includes pathogenic and epiphytic bacterial species which are associated to a wide range of botanic families, including the Rosaceae members. Besides *E. amylovora*, pathogenic species include *E. pyrifoliae*, *E. piriflorinigrans* and *E. uzenensis*, whereas non-pathogenetic species include *E. billingiae* and *E. tasmaniensis (Palacio-Bielsa et al.*, 2012). *E. pyrifoliae* is the closest related species to *E. amylovora (McGhee et al.*, 2002). *E. amylovora* can be divided into four clades, three of which mainly infect plants of the Amygdaloideae subfamily, while the fourth clade exclusively infects *Rubus* species (Parcey *et al.*, 2020). Comparative genomic analysis revealed a low level of genetic diversity between *E. amylovora* genomes (Smits *et al.*, 2010; Singh and Khan, 2019; Parcey *et al.*, 2020).

Genomic based taxonomic classification of GTDB (https://gtdb.ecogenomic.org/) has kept all genomes from NCBI classified as *Erwinia amylovora* indicating that there are no problems with the taxonomic classification of the species.

E. amylovora are very susceptible to low concentrations of surfactant agents like SDS (Chatterjee, Buss and Starr, 1977). The lipidic composition of the cell envelope has been thoroughly studied, and it is now possible to distinguish between different strains and also to find out whether the bacteria are streptomycin resistant or not using gas-liquid chromatography (van der Zwet and Wells, 1993). CCT growth medium is a good selection and growth medium to *E. amylovora*, in which colonies are smooth large, pulvinate, light blue opalescent with craters (Ishimaru and Klos, 1984).

E. amylovora is a facultative anaerobe, and is also incapable of reducing nitrate to nitrite, which is extremely uncommon in Enterobacteriaceae (Paulin, 2000). Moreover, nicotinic acid is essential to growth and proposed as a biochemical test for *E. amylovora* characterization (Holt *et al.*, 1994; Paulin, 2000).

4.2 EU and PT regulatory status

In the European Union (EU), *E. amylovora* is a protected zone quarantine pest (Commission Implementing Regulation (EU) 2019/2072, Annex III, Point a (1)), for the whole territory of Estonia, Latvia and Finland, certain regions of Spain and Italy, Corsica island in France and Isle of Man and Channel Islands in United Kingdom. Annex IX lists the plants and live pollen for pollination other than fruits and seeds that are prohibited from introduction into the protected zones if they do not originate in countries or areas free from *E. amylovora* (Commission Implementing Regulation (EU) 2019/2072).

Out of the protected zones, *E. amylovora* is considered a regulated non-quarantine pests (RNQPs) (Commission Implementing Regulation (EU) 2019/2072, Annex IV). A RNQP is defined as ‘a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party’ (IPPC, 1997).

Measures to prevent the presence of RNQPs on specific plants for planting relevant to *E. amylovora* are present in Annex V of Commission Implementing Regulation (EU) 2019/2072 in Part C, which applies to propagating material of ornamental plants and other plants for planting intended for ornamental purposes. These are directed towards ornamental plants for planting, other than seeds, of *Amelanchier*, *Chaenomeles*, *Cotoneaster*, *Crataegus*, *Cydonia*, *Eriobtrya*, *Malus*, *Mespilus*, *Photinia davidiana*, *Pyracantha*, *Pyrus* and *Sorbus*.

Since Portugal has lost the status of Integral Protected Area within the European Union due to the establishment of fire blight in the main production areas of pear and apple, E. amylovora is a regulated non-quarantine pest (RNQP). The decree-law No. 67/2020, of 15/09, ensures the execution and compliance with the phytosanitary control measures to prevent and combat plant pests and diseases (Regulation (EU) 2016/2031).

When installing new orchards:

- Acquisition of plants in officially controlled nurseries;
- Verification of the existence of a plant passport, mandatory for the host species of the disease.

In the maintenance of orchards:

- Early detection of symptoms and immediate communication to official entities (DRAP, DGAV, GNR);
- Copper-based treatments before the rainy season and, preferably, after pruning and before sprouting;
- During pruning, the cutting tools must be disinfected between each cutting and from orchard to orchard;
- Start-up and destruction by fire on site, and under the control of DRAP and GNR, from all affected vegetables or with suspicious symptoms as well as all surrounding host vegetables.

4.3 Disease distribution

Fire blight was firstly identified in the USA in 1780 and has spread throughout North America. This disease was later reported in New Zealand (1920) and in England (1957) from where it spread to most European countries and Middle East. Nowadays, fire blight is widely distributed across North and Central America and Europe, and present in the Middle East and southern Mediterranean area.

According to EPPO Global database (2021, Figure 1), in Europe, *Erwinia amylovora* is:

- present with few occurrences in: Belarus, Ireland, Italy (Sicilia), Latvia, Lithuania and Slovakia;
- present with restricted distribution in: Albania, Armenia, Austria, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Denmark, France (mainland, Corse), Georgia, Germany, Greece (Kriti), Hungary, Italy, Luxembourg, Norway, Poland, Portugal, Russia (Central Russia, Southern Russia), Serbia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom (Northern Ireland, Scotland);

- widespread in: Bulgaria, Cyprus, Greece, Montenegro, Netherlands, North Macedonia, Romania, Turkey, United Kingdom (England).

In Portugal, *Erwinia amylovora* was firstly observed in 2005 in apple and pear orchards at Fundão and subsequently eradicated. In 2010, new outbreaks were reported in the municipalities of Alcobaça, Bombarral, Torres Vedras, Caldas da Rainha, Guarda, Viseu, Ferreira do Alentejo and Alandroal (DGADR, 2012). Official phytosanitary measures were implemented (Portaria n.º287/2011, de 31 de outubro) to eradicate fire blight which included intensive surveys and destruction of infected plants. In 2017, several outbreaks were detected in Mafra (*Pyrus communis*), Penafiel (*P. communis* cv. Rocha), Viana do Castelo (*Rubus fruticosus* cv. Ouachita), Figueira Castelo do Rodrigo (*Cydonia oblonga*), Montemor-o-Novo (*Cydonia*, *Malus* and *Pyrus*), Tavira (*M. domestica*), and Monchique (*C. Oblonga*) (EPPO, 2018).

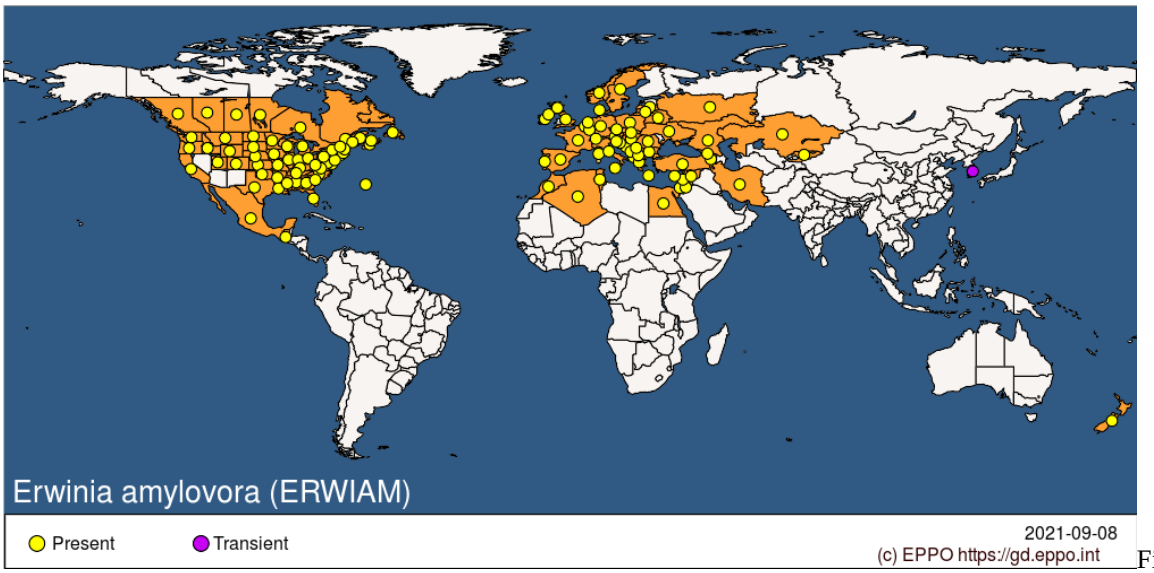

Figure 1: Distribution of fire blight disease (EPPO Global Database, 2021).

4.4 Disease cycle

E. amylovora enters through natural openings and wounds and can infect all parts of the plant including flowers, leaves, branches, stems, fruits, and roots.

Bacterial cells overwinter in the margins of cankers, which are likely sources of primary inoculum (Thomson, 2000; Agrios, 2005). Besides, resident bacteria from healthy (asymptomatic) buds can also be a source of inoculum (Tancos *et al.*, 2017). Sometimes a visible ooze, composed of viable bacteria in a hygroscopic polysaccharide matrix, can be found as droplets on the surface of cankers from previous growing seasons or on freshly-infected flowers, fruits, and shoots (Thomson, 2000; Zeng, Puławska and Schachterle, 2021). This ooze can be a sticky and viscous liquid or it may dry to a hard, shiny, amber-coloured glaze (Thomson, 2000). *E. amylovora* cells present in the ooze droplets actively expressed virulence genes such as *hrpL*, *dspE*, and *amsK*, suggesting that bacterial cells in ooze droplets are already primed for infection *(Slack et al.*, 2017). The population size of *E. amylovora* present in ooze droplets was shown to be approximately 10^8 CFU per microlitre of the ooze droplet (Slack *et al.*, 2017). Ooze exudation was observed to occur through erumpent mounds

and small (10-µm) tears in tissue, suggesting that ooze exudation occurs through wounds induced by the infection instead of through natural openings (Slack *et al.*, 2017).

In the spring, with the rise in temperature, bacteria in the cankers become active again, multiply, and spread to opening flowers by wind, rain, and insects (Agrios, 2005). Before actually invading host tissues through the natural openings, *E. amylovora* grows epiphytically on plant surfaces, mainly on flower surface (Zeng, Puławska and Schachterle, 2021). On flowers, several studies demonstrate that *E. amylovora* mostly colonizes on stigma surfaces, however, it was also observed in other parts, such as anthers and nectary (reviewed by Zeng, Puławska and Schachterle, 2021). Bacteria enter the tissues of the flower through the nectarthodes and once inside (endophytic infection), bacteria multiply quickly and advance into the intercellular spaces and cause death and collapse of nearby cells (van der Zwet and Keil, 1979; Agrios, 2005). It was also observed that *E. amylovora* can also colonize and enter hosts through petal fall junctions (Zeng, Puławska and Schachterle, 2021). From the flower, bacteria move down the pedicel into the fruit spur. Infection of the spur results in the death of all flowers, leaves, and fruit on it (Agrios, 2005).

On leaves, the process is similar to flowers, *E. amylovora* can enter through wounds or natural openings, such as stomata and hydathodes. Wounds created by wind, thunderstorms or hailstorms, as well as injuries caused by insects, like aphids, leafhoppers, pear psylla, can serve as entry points of *E. amylovora* into leaves (reviewed by Zeng, Puławska and Schachterle, 2021). From the leaf, bacteria pass into the petiole and the stem (Agrios, 2005).

Young, tender twigs may be infected by bacteria through their lenticels, wounds, and flower and leaf infections. In the twig, bacteria travel intercellularly or through the xylem. Nearby cortical or xylem parenchyma cells collapse and break down, forming large cavities (Agrios, 2005).

At the end of the growing season (summer-autumn), when environmental conditions start to be less favourable, *E. amylovora* multiplication reduces or ceases. The colonized tissues in branches or trunks develop cankers in which *E. amylovora* can overwinter until the beginning of a new cycle next spring (Thomson, 2000). *E. amylovora* can also survive for a few weeks in the soil, however it is unlikely a source of inoculum (Thomson, 2000).

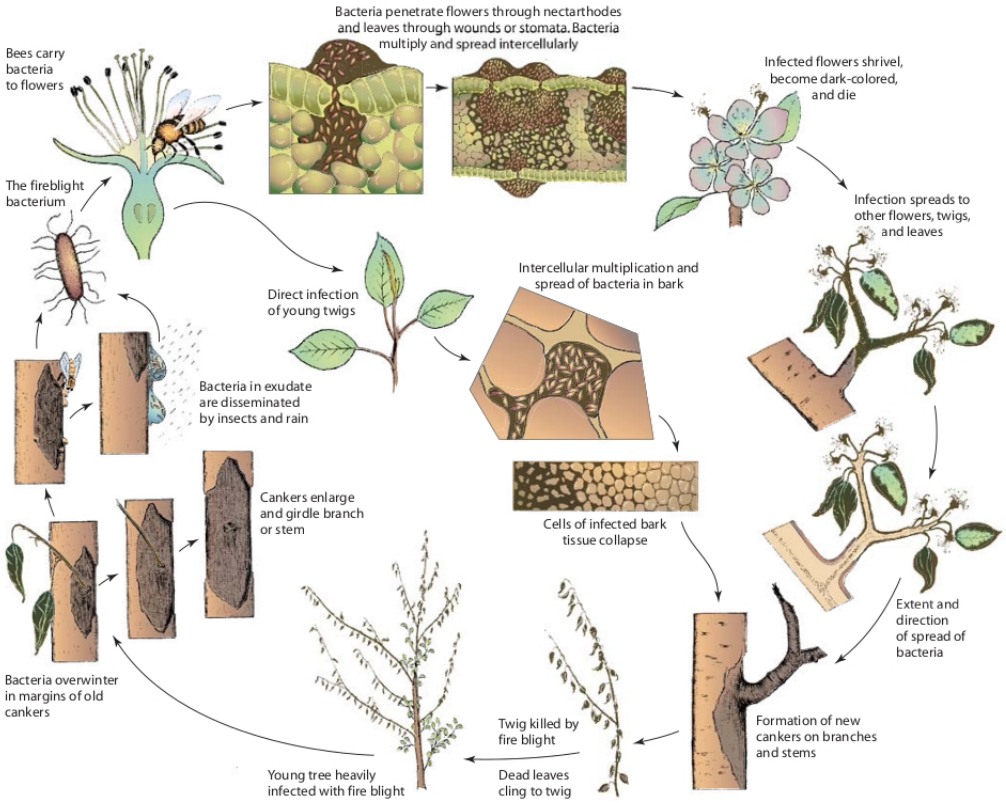

Figure 2: Disease cycle of fire blight caused by *Erwinia amylovora* (Agrios, 2005).

5 Host Plant Target population

5.1 Host range and main hosts

The fire blight disease affects plants within the Rosaceae family, particularly those in the subfamily Maloideae, such as apple (*Malus domestica*) and pear (*Pyrus communis*). *E. amylovora* affects both fruit trees, such as *Eriobotrya spp.*, *Cydonia spp.*, *Malus spp.*, *Mespilus spp.* and *Pyrus spp.*, and ornamental plants (Palacio-Bielsa and Cambra Álvarez, 2009).

Host list: *Amelanchier alnifolia*, *Amelanchier canadensis*, *Amelanchier laevis*, *Aronia melanocarpa*, *Chaenomeles japonica*, *Chaenomeles*, *Cotoneaster bullatus*, *Cotoneaster buxifolius*, *Cotoneaster dammeri*, *Cotoneaster horizontalis*, *Cotoneaster lacteus*, *Cotoneaster lucidus*, *Cotoneaster microphyllus*, *Cotoneaster moupinensis*, *Cotoneaster niger*, *Cotoneaster salicifolius*, *Cotoneaster x crispii*, *Cotoneaster x watereri*, *Cotoneaster*, *Crataegus laevigata*, *Crataegus monogyna*, *Crataegus x prunifolia*, *Crataegus*, *Cydoniaoblonga*, *Eriobotrya japonica*, *Fragaria x ananassa*, *Malus baccata*, *Malus coronaria*, *Malus domestica*, *Malus floribunda*, *Malus*, *Mespilus germanica*, *Photinia davidiana*, *Prunus armeniaca*, *Prunus cerasifera*, *Prunus domestica*, *Prunus salicina*, *Pseudocydonia sinensis*, *Pyracantha coccinea*, *Pyracantha crenatoserrata*, *Pyracantha*, *Pyrus betulifolia*, *Pyrus bourgaeana*, *Pyrus communis*, *Pyrus elaeagnifolia*, *Pyruspyraster*, *Pyrus pyrifolia*, *Pyrus spinosa*, *Pyrus ussuriensis*, *Pyrus*, *Rosa canina*, *Rosa rugosa*, *Rosa*, *Rubus fruticosus*, *Rubus idaeus*, *Sorbus aria*, *Sorbus aucuparia*, *Sorbus*, *Spiraea prunifolia* (EPPO, 2021).

In Portugal, fire blight disease was mainly identified in pear (*Pryus spp.*), being cv. 'Rocha' a highly susceptible variety, apple (*Malus spp.*), quince (*Cydonia spp.*) and blackberry (*R. fruticosus) (DGADR et al.*, 2011; EPPO, 2018).

In general, strains of *E. amylovora* are not host species-specific (Momol and Aldwinckle, 2000). Nevertheless, strains isolated from *Rubus* species are not pathogenic on pear or apple (Momol and Aldwinckle, 2000). Since *E. amylovora* has a wide host range, it may overwinter on plants which might not be the most economically important host and are called reservoir hosts. Any reservoir host in proximity to apple or pear orchards and nurseries should be eliminated where possible (Momol and Aldwinckle, 2000).

5.2 Environmental suitability

Temperature is a critical environmental factor affecting the development of fire blight, directly influencing the bacterial growth, the development of the host and the presence of vectors responsible for the spread. The relation between temperature and growth of the bacteria in liquid media was shown to be linear between 9°C and 18°C, with a sharp change in the growth rate at 18°C (Billing, 1974). *E. amylovora* is capable of growing in a wide range of temperatures, from 4°C to 37°C, being 25-27°C the optimal growth temperature (Paulin, 2000). *E. amylovora* can be considered a psychrotrophic microorganism, i.e., able to grow at low temperatures but with optimal and maximal growth temperatures above 15°C and 20°C, respectively (Santander and Biosca, 2017). It was shown that a temperature of 45°C for 70 min or 50°C for 50 min could destroy pure cultures of *E. amylovora*, with some variation between strains (Keck *et al.*, 1995).

Table 1: Temperature and pH needed for growth of Erwinia amylovora (van der Zwet and Keil, 1979)

Year	Temperature (°C)[1]			Thermal death point (°C)[1]	pH			Reference
	Minimum	Optimum	Maximum		Minimum	Optimum	Maximum	
1911---	----------	23–25 (73.5–77)	37 (99)	45–50 (113–122)	6.6	7.0	7.6	Jones (*484*).
1913---	----------	22–25 (71.5–77)	37 (99)	47 (117)	5.9	6.6–7.5	7.6	Stewart (*901*).
1929---	----------	25 (77)	----------	----------	4.6	----------	8.7	Howard (*438*).
1931---	----------	----------	----------	49 (120)	4.6	----------	----------	Pierstorff (*742*).
1937---	3–8 (37.5–46.5)	28 (82.5)	37 (99)	45.1–48.3 (113–118.5) 48.3–49.5 (118.5–121)	4.0–4.4	6.8	8.8	Ark (*30*).
1960---	12 (54)	21–27 (70–81)	35 (95)	----------	----------	----------	----------	Luepschen (*1115*).
1961---	3–5 (37.5–41)	25–27.5 (77–81.5)	37 (99)	47.5–50.0 (117.5–122)	5.2	6.0	8.1	Billing et al. (*87*).
1964---	10 (50)	30 (86)	35 (95)	----------	4.3	6.6–7.5	8.5	El-Helaly et al. (*264*).
1964---	3–8 (37.5–46.5)	30 (86)	37 (99)	----------	4.0–4.4	6.8	8.8	Martinec and Kocur (*630*).
1972---	----------	21–30 (70–86)	----------	----------	----------	----------	----------	Eden-Green (*1097*).
1974---	----------	18–28 (64.5–82.5)	----------	----------	----------	----------	----------	Billing (*85*).

[1]Approximate °F in parentheses.

Humidity is also an important factor, a mean percentage relative humidity (%RH) of 70 or more promotes the infection, being the optimal conditions between %RH 90-95 (Palacio-Bielsa and Cambra Álvarez, 2009). Moreover, rain above 2,5 mm in a day could initiate the infection (Palacio-Bielsa and Cambra Álvarez, 2009). Moreover, rain favours the spread of *E. amylovora*. It was also observed that *E. amylovora* can grow on the stigma at a lower RH than in the hypanthium (Pusey, 2000).

In summary, temperatures from 18 to 29°C and a high relative humidity (90–95%) are the most favourable environmental conditions for fire blight infection, when exudates are frequently observed (van der Zwet and Beer, 1999). During the flowering period, temperatures as low as 12°C still allow for infections (van der Zwet and Beer, 1999).

5.3 Spread capacity

E. amylovora is considered to spread easily both within and between orchards. After an initial outbreak in a single focus or multiple foci, fire blight generally spreads rapidly within the nearby area or towards areas with orchards and may advance up to 300 km from the original outbreak in five to six years (EFSA PLH Panel, 2014).

E. amylovora can be spread by insects that are attracted by the exudate produced on infected flowers (ooze). Particularly, pollinating insects, such as bumblebees and honeybees, are important in the secondary spread of the pathogen from infected flowers to newly opened flowers (Thomson, 2000). During pollination, these insects approach flowers from the top and have full contact with stigmas and anthers, that are the major colonization and infection sites of *E. amylovora*. Moreover, pollinators also collect pollens and nectar that are often contaminated with *E. amylovora*. Flies (Dipterans), that visit and feed on ooze, may also play a more important role in the spread of fire blight, particularly during the shoot blight stage (Zeng, Puławska and Schachterle, 2021). It was observed that *E. amylovora* can be transmitted by Mediterranean fruit flies (*Ceratitis capitata*) and survive both inside the digestive tract (for at least 8 days) and on external surface (up to 28 days) (Ordax *et al.*, 2015).

In geographical areas where rain is common during the blossom period, bacteria can also be spread from oozing cankers to flowers by rain (Thomson, 2000; Agrios, 2005). Dried ooze often forms aerial strands that can be spread by wind and act as inoculum (Agrios, 2005).

The role of birds in disease spread has been suggested and is empirically accepted (Sousa, 2021)nevertheless it was never demonstrated. It is suggested that birds carry the infected ooze from one tree to another.

Insects, rain and wind are mainly responsible for local infections. Long distance spread of fire blight happens mainly through the transportation of latently infected or undetectable contaminated propagation material (Thomson, 2000; Palacio-Bielsa and Cambra Álvarez, 2009). Another important source of primary and secondary inoculum are ornamental hosts either intentionally planted or growing wild in the vicinity of orchards, and this constitutes another frequently ignored and major source of inoculum (Thomson, 2000). Moreover, *E. amylovora* is also spread through the use of non-disinfected pruning tools (Teviotdale, Wiley and Harper, 1991). Crop management practices performed by the same workers in different orchards may also contribute to the medium-distance spread via hands, clothing, pruning and spraying tools contaminated after manipulating

infected plant material, as well as, contaminated containers (van der Zwet and Keil, 1979; Ceroni *et al.*, 2004).

5.4 Risk factor identification

E. amylovora is a major concern for farms, not only for pear and apple producers but also nursery trade, due to its fast spread, large host range and not-so effective control strategies. Identification and estimation of risk factors are essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infection by a disease in the area of interest.

Main risk factors are the presence of high levels of inoculum, presence of pollinating insects, presence of secondary buds, temperatures above 15°C during blossom, high relative humidity and rain upon warm periods, high relative humidity at night in semi-arid regions, rain in warm night regions. Mediterranean regions are highly prone to fire blight due to the favourable climatic conditions for disease development and the existence of self-rooted wild hosts.

Nurseries and garden centres where susceptible cultivated and ornamental plants are traded are potential entry points for infected plant material.

The susceptibility of plant host is also a risk factor. In general, pears are more susceptible than apples as well as cultivars with secondary flowering, as flowers produced in late spring or summer are more prone to infections than the flowers produced during the main bloom.

Moreover, agricultural practices can also impact the development of the epidemic, therefore well-maintained orchards have lower risk of infection than neglected orchards. High-density plants with weak rootstocks, predisposed to secondary flowering, favour the disease developing (Pel *et al.*, 2021). Proper fertilisation and irrigation, namely avoid bioregulators that promote floral induction and favour drip irrigation instead of sprinkler irrigation, and proper pruning to short the duration of flowering and prevent off-season flowering may reduce the risk of infections (van der Zwet and Beer, 1999). Based on the distance that bacterium could be naturally spread, the host plants within 1000 m of the risk locations could be considered to have a higher probability of infection (Pel *et al.*, 2021).

In apple, *E. amylovora* infection significantly affected the microbial communities in endosphere and episphere of fruits, twigs and leaves, reducing the species richness and diversity (Kim *et al.*, 2021). However, no significant differences were observed in the rhizosphere (Kim *et al.*, 2021). On the other hand, *E. amylovora* infection increased the microbiome diversity of apple flowers, where besides the pathogen there was an increase in the abundance of *E. tasmaniensis*, E*nterobacter tabaci*, and *Pseudomonas rhizosphaerae* (Kong *et al.*, 2021). *E. amylovora* was the dominant species in infected flowers and *Pantoea agglomerans* and *P. allii* in healthy flowers (Kong *et al.*, 2021). Nevertheless, *E. amylovora* abundance on the stigma was observed to not be enough to predict the disease, since only 42% of inoculated flowers developed symptoms (Cui *et al.*, 2021).

Soils with high clay content, poorly drained, with high acidity and over fertilized are conducive to fire blight development (van der Zwet and Beer, 1999). Moreover, orchard soils should be maintained at pH 5. 5- 6. 5 (van der Zwet and Beer, 1999). The major nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), and the minor elements boron (B), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe), should be applied at rates necessary to

maintain a good balance, since imbalance might increase disease severity (van der Zwet and Beer, 1999).

5.4.1 Predictive models

Predictive computational models have been developed based on climatic factors (i.e. temperature, humidity, precipitation, frost, hail and strong wind) and on fruit phenological data. These models were developed to forecast for blossom blight occurrence and to ensure optimal timing of applications in relation to risks of infection.

Most of these predictive models were created with data collected in USA and UK, causing a loss of their predictive power when tested in other regions with different climatic conditions (Billing, 2000; Dewdney, Biggs and Turechek, 2007).

One of the most used model is the **Maryblyt**. Maryblyt is a predictive program for forecasting fire blight disease in both apples and pears that was developed in blight-prone apple orchards in Maryland, USA, where weather is often wet and warm (Steiner, 1990a, 1990b), and was continually updated until 2018 (http://grapepathology.org/maryblyt). This model was built on the assumption that there is an abundance of inoculum, so for a blossom infection event to occur, four strict conditions must occur in a sequence:

- Flowers must be open with stigmas exposed for colonization and petals intact;
- Accumulation of at least 110 Degree Hour1 (DH) > 18.3 °C within the last 44.4 Degree Day1 (DD) > 4.4 °C for apples or within the last 66.7 DD>4.4°C for pears (defines the epiphytic infection potential for the oldest open and, hence, most colonized flower in the orchard).
- A wetting event occurring as dew or more than 0.25 mm of rain, or more than 2.5 mm of rain the previous day (allows movement of bacteria from colonized stigmas to the nectarthodes).
- An average daily temperature of more than 15.6 °C (this may influence the rate at which the bacteria migrate into the nectarthodes as well as the multiplication of bacteria needed to establish infections).

Based on these events, the infection risk is:

- **None** if the blossoms are not open;
- **Low** if there is open blossoms and no other positive condition;
- **Moderate** if there is open blossoms and 1 positive condition;
- **High** if there is open blossoms and 2 positive conditions;
- **Occurs** if there is open blossoms and all 3 positive conditions.

Another well known predictive model is the **Cougarblight** model, which estimates the risk of blossom blight infections using a Temperature Risk Value and predicted Blossom Wetting event (Smith, 1993; Smith and Pusey, 2011). Thresholds were created based on observations of more than 30 years in Washington and Oregon (USA). Hourly Temperature Risk Values are calculated using

the *E. amylovora* growth with a lower threshold of 10°C and upper threshold of 35°C. Blossom Wetting is estimated based on 15-min weather data. A tenth of an inch or more is considered rain and relative humidity over 80% is considered dew. The basic components and assumptions of the CougarBlight fire blight infection risk model are (Smith and Pusey, 2011):

- Orchard fire blight history: there are important differences among orchards relating to the potential of blossom contamination and initial population size of the pathogen on the stigma. The grower is asked to first select an orchard fire blight history that most closely describes the orchard.
- Flower life/colony growth: in the range of temperatures that are likely to lead to infection, a flower is assumed to be open and receptive to contamination and infection for a total of four days.
- Bacterial growth: the relationship of hourly temperature during any given day to growth rate of the pathogen on stigmas is quantified in this model. They were based on the average pathogen growth rate per 24 hours, which was divided by 24 to derive an hourly growth rate at any given temperature. Then once again arbitrarily divided by 1000 to make the numbers easier to use. These hourly-by-temperature values are assigned to each hour of the day and totaled for the four days leading up to the wetting event.
- Wetting as a trigger to infection: wetting of the flowers by rain, dew of two hours or more, mist from sprinklers, or any other situation that results in flowers being wet for two hours or more is considered a potential infection event.

Recently, COTHN, BioISI and INIAV are working on the Fire4Cast project to develop and implement a predictive model for forecast of fire blight outbreaks in Portugal, mainly in Oeste, Cova da Beira and Alentejo region.

6 Detection and identification

6.1 Detection and identification in the field

To detect the fire blight in the field, it is necessary to make inspections during the growing season, when the symptoms are visible. Therefore, inspection should be performed from after flowering until late summer, when the symptoms are more easily detected. Nevertheless, asymptomatic material should be sampled and tested when the climatic conditions are favourable for *E. amylovora*, i.e. temperatures above 20°C and high humidity, and preferably in the second half of the growing season. During the winter, on dormant plants, disease detection is difficult because cankers are not always visible (EPPO, 2021).

The symptoms can be observed in all the above-ground parts of the plant and the most common are (EPPO, 2021):

- Wilt and death of **flowers or whole clusters**, in the spring. The infected flowers become dry and turn into a dark-brown to black colour and usually remain attached to the plant.

- Wilt and death of young **shoots and twigs**, which are the most susceptible parts to rapidly develop symptoms. Infected shoots and twigs wither, turn brown or black and in most cases the tip of the shoot bends in a characteristic way called ‘shepherd's crook’.
- **Leaves** on heavily affected shoots turn brown or black together with the twigs, which appear to be scorched by fire.
- **Leaf blight:** infected leaves display either necrotic patches starting from the margin of the leaf blade or blackening of the midrib and the petiole.
- **Fruit blight:** infected fruits initially turn grey/green and look water-soaked. Then, they turn brown or black and remain attached to the spur, with a mummified appearance.
- **Limb and trunk blight:** in infected trunks a yellow to orange/brown ooze appears on the outside of the bark. *E. amylovora* can also cause cankers, which cause quick death of branches or the whole tree by girdling. The cankers are recognized, externally, through their slightly sunken surface surrounded by irregular cracks in the bark.
- In general, a whitish/yellowish mucoid bacterial ooze may exude from infected shoots, petioles, cankered bark and infected fruit and blossoms.

Moreover, early detection of *E. amylovora* is also possible by monitoring the presence of the bacteria in pollen from beehives. A study showed that *E. amylovora* could be detected through PCR-ELISA in pollen samples from hives even before any symptoms appear in the orchard (Ghini *et al.*, 2002).

6.2 Detection and identification in the lab

Fireblight symptoms may be confused with those caused by other diseases that have similar symptoms, like *Pseudomonas* (necrosis of young tissues), *Phytophtora* (cankers), *Janus compressus* (shepherd crook), besides the possibility of latent infection, so detection should be confirmed by isolation and laboratory tests. These include immunofluorescence (Paulin, 1981), dot-ELISA (Zutra, Shabi and Lazarovits, 1986), serological kits, nested PCR (Llop *et al.*, 2000), real-time PCR (Hinze *et al.*, 2016), LAMP (Moradi *et al.*, 2012; Bühlmann *et al.*, 2013). Serological kits, such as Ea Agristrip kit (Braun-Kiewnick *et al.*, 2009) and Pocket Diagnostics (Forsite Diagnostics, UK), are very easy to use and a useful tool for non-laboratory personnel, such as phytosanitary inspectors and orchard growers, nevertheless, real time PCR assays provide higher sensitivity, specificity and reliability of pathogen detection in combination with several DNA extraction methods based on isopropanol extraction, silica columns or magnetic beads (EUPHRESCO, 2009). Within real time PCR assays, Ams assay was shown to be more accurate in detection of *E. amylovora* than ITS and plasmid assays. Real time PCR (Ams assay) was also shown to be more sensitive than nested PCR (EUPHRESCO, 2009). Detection methods are described in EUPHRESCO Final Report (EUPHRESCO, 2009) and EPPO Standard PM 7/20 (2) (EPPO, 2013).

7 Economic and social impact

E. amylovora causes severe damages in susceptible hosts, particularly in pear and apple. It can destroy all trees affecting fruit productivity, and in extreme conditions lead to the destruction of the

complete orchard. Besides, next year's productivity is also significantly reduced due to the destruction of fruiting spurs. Moreover, fire blight can also affect the production and commercialization of vegetative propagation material, since it also affects ornamental plants that can act as inoculum.

Fire blight has been causing several losses in multiple countries. In the 1990s, more than a half million trees were destroyed in Italy (Vanneste, 2000). In 1998, losses of more than 68 million dollars (US) were estimated in the northwest United States and more than 10 million dollars (NZ) in the Hawke's Bay region of New Zealand (Vanneste, 2000). In Switzerland, the financial burden of control measures and the compensation payments for destroyed plants were estimated to be about 35 million EUR from 1989 to 2003 (Duffy *et al.*, 2005).

According to APAS (*Associação dos Produtores Agrícolas da Sobrena*), the average incidence of fire blight in Portugal is 10%, with an annual loss of 11 550 000€ to the producers and 33 000 tons in production. In 2021, removal of around 300 ha of new orchards (< 15 years) is expected due to high disease incidence (APAS, personal communication, November 2021).

Table 2: Economical impact of fire blight in Portugal

	APAS (2021) [1]
Average incidence	10%
Annual economical losses for the producers (€)	11550000
Annual economical losses for the value chain (€)	44550000
Annual production losses (tonnes)	33000
Annual cost of control measures (pruning, phytochemicals, etc.; €)	8800000

8 Available control/prevention methods

8.1 Chemical

Chemical treatments can be divided in four subclasses: copper compounds, antibiotics, carbamates and miscellaneous compounds (van der Zwet and Keil, 1979).

Copper compounds have been used since the first years of the XX century, but most of these compounds can be phytotoxic when applied in the concentrations needed to control *E. amylovora*, and even though resistance to copper has not yet been reported in this species it was observed in other phytopathogenic bacteria (Psallidas and Tsiantos, 2000).

Several antibiotics have been shown to reduce the growth of the pathogen *in vitro*, but only few of them are effective in planta. The most used and effective one has been streptomycin, nevertheless resistance has been observed for some strains (Loper, 1991). An alternative antibiotic is

[1] Calculated by João Azevedo (from Associação dos Produtores Agrícolas da Sobrena) based on the assumptions:
- Total orchard area in Portugal – 11 000 ha;
- Average productivity – 30 ton/ha
- Average value paid to producer – 0,35€/kg
- Average value paid by retailers – 0,85€/kg
- Retail selling margin – 100%
- Average value for the remaining value chain – 1,35€/kg

oxytetracycline, which has been approved to be used in fields with pathogens resistant to streptomycin even though it is less effective and more toxic (McManus and Jones, 1994). No resistance has been reported to oxytetracycline (Loper, 1991). More recently, the antibiotic kasugamycin was shown to be very effective against *E. amylovora.* Nevertheless, antibiotics are not approved as chemical control methods in EU.

Another compound that has been proven to be effective in controlling *E. amylovora* infections is acibenzolar-S-methyl (ASM), which works by eliciting the SAR immune response in plants.

In Portugal, the approved chemical treatments to fire blight include copper (Cuprital, Cuprital SC, Cuproxi Flo, Cuproxat), fosetyl-Al (Aliette Flash, Etylit Premier, Protect Garden Fungicida Sistémico WG, Alfil, Fosletis 80 WG, Maestro 80 WG), Acibenzolar-S-methyl (biostimulant, BION 50 WG), laminarin (biostimulant, Vacciplant) and prohexadione-calcium (growth regulator, Regalis Plus) (https://sifito.dgav.pt/divulgacao/usos).

8.2 Biological

The use of **biological control agents (BCAs)** to control the spread of *E. amylovora* infection is a promising approach to overcome the issue caused by the presence of antibiotic-resistant strains of the pathogen, besides being more ecologically sustainable. The only downside is the relatively low efficacy, which might delay its widespread use (Ngugi, Lehman and Madden, 2011). Several BCA-based products against fire blight in pears and apples are already commercially available in USA, such as

- BlightBan A506 (*Pseudomonas fluorescens* A506, isolated from leaves of pear trees) (Wilson and Lindow, 1993);
- BlightBan C9-1 (*Pantoea vagans* C9-1, isolated from apple stem tissue) (Ishimaru, 1988);
- Serenade Optimum (*Bacillus subtilis* QST713, isolated from soil) (Edgecomb and Manker, 2006);
- Double Nickel (*Bacillus amyloliquefaciens* D747, isolated from soil);
- BioproTM (*Bacillus subtilis* BD170);
- Bloomtime Biological (*Pantoea agglomerans* E325) (Pusey, 1999);
- Blossom Protect (*Aureobasidium pullulans* strains DSM 14940 and DSM 14941).

These products were originally isolated from many different sources like soil, leaves, stem tissues and blossoms, and have different ways to help decrease the incidence of *E. amylovora*, including antibiosis and growth inhibition by resource depletion and size decrease of growth space. Nonetheless, some of these products are not available in the EU due to concerns on possible health issues.

In **Portugal**, *Bacillus amyloliquefaciens* QST 713 (Serenade Max and Serenade ASO) and *Bacillus amyloliquefaciens* subsp. plantarum, strain D747 (AMYLO-X WG) are BCAs approved by DGAV to fight *E. amylovora.* In addition, *Aureobasidium pullulans* strains DSM 14940 + DSM 14941 (BOTECTOR) are also approved for commercialization but against *Botrytis cinerea* (https://sifito.dgav.pt/divulgacao/usos).

Recently, the company Kimitec Agro (Almería, Spain) is developing a phage-based solution to prevent and treat *E. amylovora* infections (PhageFire project, https://www.phagefire.eu/). Bacteriophages are viruses that require bacteria to survive and have been observed to positively control *E. amylovora* infection (Schnabel and Jones, 2001; Gill *et al.*, 2003; Besarab *et al.*, 2020).

8.3 Cultural

Cultural practices are an important part in controlling fire blight infection. The first method is a strict sanitation of pruning tools. Also the removal of overwintering cankers from the plants before the spring was proven to be of utmost importance (Schroth *et al.*, 1974), as well as grafting on resistant rootstock and reducing plant vitality by reducing nutrient application (van der Zwet and Keil, 1979).

After disease establishment, eradication of the most affected trees is mandatory. Moreover, sanitary pruning should be carried out on less affected trees, allowing the orchard a longer producing life (DGADR *et al.*, 2011)

8.4 Breeding

Breeding for resistance to fire blight is considered as one of the best ways to fight the disease, since it reduces the amount of pesticides needed to limit the spread of the pathogen (Lespinasse and Aldwinckle, 2000). Resistance to the disease appears to this day to be of polygenic nature, and only a few single genes have been associated with a very low level of resistance; most known QTLs are also strain-specific (Brisset *et al.*, 2002; Emeriewen *et al.*, 2019). Apples, pears, peaches and ornamental Rosaceae all have breeding programmes designed to increase resistance to *E. amylovora*. These programmes use several strategies: some explore genetic resources coming from regions where the pathogen and the host co-evolved, they may need large progeny sizes and parent generations with traits of interest that have less than the higher level of resistance available, while retaining good horticultural characteristics (Flachowsky *et al.*, 2009).

In recent years there has also been an increasing interest in the development of transgenic lines of apples and pears. There are well-established methods of *A. tumefaciens* transformation of apples and pears (Matsuda *et al.*, 2005; Szankowski *et al.*, 2009; Vanblaere *et al.*, 2011; Nakajima *et al.*, 2013), but to this day only apples have been transformed to obtain *E. amylovora* resistance. Transformed genes may include insect bactericidal proteins like attacin E (Borejsza-Wysocka *et al.*, 2010), or cisgenic resistance genes such as *MR5 (Kost et al.*, 2015).

8.5 AgroEcological

As previously stated, pollinators are one of the main transmission vehicles for *E. amylovora*. Thus, an important source of inoculum may be epiphytic growth in other plants present in orchards. Johnson *et al.* (2006) studied the ability of E. amylovora to grow in pants often present in pear orchards in the US. Their results showed that *E. amylovora* growth on non-Rosaceous plants was generally lower than on Rosaceous plants, with *Cytisus scoparius* and *Acer macrophylum* being particularly good at restricting pathogen growth.

9 References

Agrios, G.N. (2005) *Plant pathology*. 5th ed. Amsterdam ; Boston: Elsevier Academic Press.

Besarab, N.V. *et al.* (2020) 'Isolation and characterization of Hena1 - a novel Erwinia amylovora bacteriophage', *FEMS microbiology letters*, 367(9), p. fnaa070. doi:10.1093/femsle/fnaa070.

Billing, E. (1974) 'The effect of temperature on the growth of the fireblight pathogen, Erwinia amylovora', *The Journal of Applied Bacteriology*, 37(4), pp. 643–648. doi:10.1111/j.1365-2672.1974.tb00488.x.

Billing, E. (2000) 'Fire blight risk assessment systems and models.', in Vanneste, J.L. (ed.) *Fire blight: the disease and its causative agent, Erwinia amylovora.* Wallingford: CABI, pp. 293–318. doi:10.1079/9780851992945.0293.

Borejsza-Wysocka, E. *et al.* (2010) 'Stable expression and phenotypic impact of attacin Etransgene in orchard grown apple trees over a 12 year period', *BMC Biotechnology*, 10(1), p. 41. doi:10.1186/1472-6750-10-41.

Braun-Kiewnick, A. *et al.* (2009) 'Ea AgriStrip - a new rapid test for detection of fire blight.', *Obst- und Weinbau*, 145(14), pp. 7–10.

Brisset, M.N. *et al.* (2002) 'Induced resistance to Erwinia amylovora in apple and pear', *Acta Horticulturae*, (590), pp. 335–338. doi:10.17660/ActaHortic.2002.590.49.

Bühlmann, A. *et al.* (2013) 'Erwinia amylovora loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site diagnosis of fire blight', *Journal of Microbiological Methods*, 92(3), pp. 332–339. doi:10.1016/j.mimet.2012.12.017.

Ceroni, P. *et al.* (2004) 'Survival of Erwinia amylovora on pears and on fruit containers in cold storage and outdoors', *EPPO Bulletin*, 34(1), pp. 109–115. doi:10.1111/j.1365-2338.2004.00705.x.

Chatterjee, A.K., Buss, R.F. and Starr, M.P. (1977) 'Unusual Susceptibility of *Erwinia amylovora* to Antibacterial Agents in Relation to the Barrier Function of its Cell Envelope', *Antimicrobial Agents and Chemotherapy*, 11(5), pp. 897–905. doi:10.1128/AAC.11.5.897.

Cui, Z. *et al.* (2021) 'Temporal and spatial dynamics in the apple flower microbiome in the presence of the phytopathogen Erwinia amylovora', *The ISME Journal*, 15(1), pp. 318–329. doi:10.1038/s41396-020-00784-y.

Dewdney, M.M., Biggs, A.R. and Turechek, W.W. (2007) 'A Statistical Comparison of the Blossom Blight Forecasts of MARYBLYT and Cougarblight with Receiver Operating Characteristic Curve Analysis', *Phytopathology*, 97(9), pp. 1164–1176. doi:10.1094/PHYTO-97-9-1164.

DGADR *et al.* (2011) *Manual de boas práticas para o controlo do Fogo Bacteriano (*Ewinia amylovora*)*. Lisboa: Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território.

Duffy, B. *et al.* (2005) 'Regulatory measures against Erwinia amylovora in Switzerland', *EPPO Bulletin*, 35(2), pp. 239–244. doi:10.1111/j.1365-2338.2005.00820.x.

Edgecomb, D.W. and Manker, D. (2006) 'Bacillus subtilis strain QST 713, bacterial disease control in fruit, vegetable and ornamental production.', *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft*, (No.408), pp. 167–169.

EFSA PLH Panel (2014) ‘Scientific Opinion on the pest categorisation of Erwinia amylovora (Burr.) Winsl. et al.’, *EFSA Journal*, 12(12), p. 3922. doi:10.2903/j.efsa.2014.3922.

Emeriewen, O.F. *et al.* (2019) ‘Malus Hosts–Erwinia amylovora Interactions: Strain Pathogenicity and Resistance Mechanisms’, *Frontiers in Plant Science*, 10, p. 551. doi:10.3389/fpls.2019.00551.

EPPO (2013) ‘PM 7/20 (2) *Erwinia amylovora*’, *EPPO Bulletin*, 43(1), pp. 21–45. doi:10.1111/epp.12019.

EPPO (2018) *EPPO Reporting Service no. 05–2018. PM 1/2(28) English.* Article 2018/103. Available at: https://gd.eppo.int/reporting/article-6297.

EPPO (2021) *EPPO Global Database (available online)*. Available at: https://gd.eppo.int (Accessed: 14 September 2021).

EUPHRESCO (2009) *Development and validation of innovative diagnostic tools for the detection of fire blight (Erwinia amylovora) (ERWINDECT)*.

Flachowsky, H. *et al.* (2009) ‘A review on transgenic approaches to accelerate breeding of woody plants’, *Plant Breeding*, 128(3), pp. 217–226. doi:10.1111/j.1439-0523.2008.01591.x.

Ghini, S. *et al.* (2002) ‘Environmental monitoring of the phytopathogen *Erwinia amylovora* - the causative agent of fire blight - with the use of honeybee [*Pyrus communis* L.]’, *Informatore Fitopatologico (Italy)* [Preprint].

Gill, J.J. *et al.* (2003) ‘Bacteriophages of Erwinia amylovora’, *Applied and Environmental Microbiology*, 69(4), pp. 2133–2138. doi:10.1128/AEM.69.4.2133-2138.2003.

Hinze, M. *et al.* (2016) ‘Real-time PCR detection of *Erwinia amylovora* on blossoms correlates with subsequent fire blight incidence’, *Plant Pathology*, 65(3), pp. 462–469. doi:10.1111/ppa.12429.

Holt, J.G. *et al.* (1994) *Bergey’s Manual of Determinative Bacteriology*. 9th edn. Baltimore, Maryland, USA: Williams and Wilkins.

IPPC (1997) *International Plant Protection Convention*. Available at: https://www.ippc.int/static/media/files/publications/en/2013/06/06/1329129099_ippc_2011-12-01_reformatted.pdf (Accessed: 28 September 2021).

Ishimaru, C. and Klos, E.J. (1984) ‘New Medium for Detecting *Erwinia amylovora* and Its Use in Epidemiological Studies’, *Phytopathology*, 74(11), pp. 1342–1345. doi:10.1094/Phyto-74-1342.

Ishimaru, C.A. (1988) ‘Multiple Antibiotic Production by *Erwinia herbicola*’, *Phytopathology*, 78(6), p. 746. doi:10.1094/Phyto-78-746.

Keck, M. *et al.* (1995) ‘Heat treatment of plant propagation material for the control of fire blight’, *Plant Pathology*, 44(1), pp. 124–129. doi:10.1111/j.1365-3059.1995.tb02724.x.

Kim, S.-H. *et al.* (2021) ‘Comparison of Bacterial Community of Healthy and Erwinia amylovora Infected Apples’, *The Plant Pathology Journal*, 37(4), pp. 396–403. doi:10.5423/PPJ.NT.04.2021.0062.

Kong, H.G. *et al.* (2021) ‘Microbial Community Dysbiosis and Functional Gene Content Changes in Apple Flowers due to Fire Blight’, *The Plant Pathology Journal*, 37(4), pp. 404–412. doi:10.5423/PPJ.NT.05.2021.0072.

Kost, T.D. *et al.* (2015) ‘Development of the First Cisgenic Apple with Increased Resistance to Fire Blight’, *PLOS ONE*. Edited by B.A. Vinatzer, 10(12), p. e0143980. doi:10.1371/journal.pone.0143980.

Lespinasse, Y. and Aldwinckle, H.S. (2000) ‘Breeding for resistance to fire blight.’, in Vanneste, J.L. (ed.) *Fire blight: the disease and its causative agent, Erwinia amylovora.* Wallingford: CABI, pp. 253–273. doi:10.1079/9780851992945.0253.

Llop, P. *et al.* (2000) ‘Development of a highly sensitive nested-PCR procedure using a single closed tube for detection of Erwinia amylovora in asymptomatic plant material’, *Applied and Environmental Microbiology*, 66(5), pp. 2071–2078. doi:10.1128/AEM.66.5.2071-2078.2000.

Loper, J.E. (1991) ‘Evaluation of Streptomycin, Oxytetracycline, and Copper Resistance of *Erwinia amylovora* Isolated from Pear Orchards in Washington State’, *Plant Disease*, 75(3), p. 287. doi:10.1094/PD-75-0287.

Matsuda, N. *et al.* (2005) ‘Development of an Agrobacterium-mediated transformation method for pear (Pyrus communis L.) with leaf-section and axillary shoot-meristem explants’, *Plant Cell Reports*, 24(1), pp. 45–51. doi:10.1007/s00299-005-0924-1.

McGhee, G.C. *et al.* (2002) ‘Relatedness of Chromosomal and Plasmid DNAs of Erwinia pyrifoliae and Erwinia amylovora’, *Applied and Environmental Microbiology*, 68(12), pp. 6182–6192. doi:10.1128/AEM.68.12.6182-6192.2002.

McManus, P.S. and Jones, A.L. (1994) ‘Role of wind-driven rain, aerosols, and contaminated budwood in incidence and spatial pattern of fire blight in an apple nursery.’, *Plant Disease*, 78(11), pp. 1059–1066.

Momol, M.T. and Aldwinckle, H.S. (2000) ‘Genetic diversity and host range of Erwinia amylovora.’, in Vanneste, J.L. (ed.) *Fire blight: the disease and its causative agent, Erwinia amylovora.* Wallingford: CABI, pp. 55–72. doi:10.1079/9780851992945.0055.

Moradi, A. *et al.* (2012) ‘Development and evaluation of a loop-mediated isothermal amplification assay for detection of Erwinia amylovora based on chromosomal DNA’, *European Journal of Plant Pathology*, 133(3), pp. 609–620. doi:10.1007/s10658-012-9939-y.

Nakajima, I. *et al.* (2013) ‘*Agrobacterium*-mediated genetic transformation using cotyledons in Japanese pear (*Pyrus pyrifolia*)’, *Breeding Science*, 63(3), pp. 275–283. doi:10.1270/jsbbs.63.275.

Ngugi, H.K., Lehman, B.L. and Madden, L.V. (2011) ‘Multiple treatment meta-analysis of products evaluated for control of fire blight in the eastern United States’, *Phytopathology*, 101(5), pp. 512–522. doi:10.1094/PHYTO-08-10-0221.

Ordax, M. *et al.* (2015) ‘Medfly Ceratitis capitata as Potential Vector for Fire Blight Pathogen Erwinia amylovora: Survival and Transmission’, *Plos One*, 10(5), pp. e0127560–e0127560. doi:10.1371/journal.pone.0127560.

Palacio-Bielsa, A. *et al.* (2012) ‘Erwinia spp. from pome fruit trees: similarities and differences among pathogenic and non-pathogenic species’, *Trees*, 26(1), pp. 13–29. doi:10.1007/s00468-011-0644-9.

Palacio-Bielsa, A. and Cambra Álvarez, M.A. (2009) *El fuego bacteriano de las rosáceas (Erwinia amylovora)*. Madrid: Ministerio de Medio Ambiente y Medio Rural y Marino, Centro de Publicaciones.

Parcey, M. *et al.* (2020) ‘Comparative genomic analysis of Erwinia amylovora reveals novel insights in phylogenetic arrangement, plasmid diversity, and streptomycin resistance’, *Genomics*, 112(5), pp. 3762–3772. doi:10.1016/j.ygeno.2020.04.001.

Paulin, J.P. (1981) ‘Overwintering of *Erwinia amylovora*: sources of inoculum in spring.’, *Acta Horticulturae*, (117), pp. 49–54. doi:10.17660/ActaHortic.1981.117.7.

Paulin, J.P. (2000) ‘Erwinia amylovora: general characteristics, biochemistry and serology.’, in Vanneste, J.L. (ed.) *Fire blight: the disease and its causative agent, Erwinia amylovora.* Wallingford: CABI, pp. 87–115. doi:10.1079/9780851992945.0087.

Pel, C. *et al.* (2021) ‘Pest survey card on Erwinia amylovora’, *EFSA Supporting Publications*, 18(7), p. 6767E. doi:10.2903/sp.efsa.2021.EN-6767.

Psallidas, P.G. and Tsiantos, J. (2000) ‘Chemical control of fire blight.’, in Vanneste, J.L. (ed.) *Fire blight: the disease and its causative agent, Erwinia amylovora.* Wallingford: CABI, pp. 199–234. doi:10.1079/9780851992945.0199.

Pusey, P.L. (1999) ‘Laboratory and field trials with selected microorganisms as biocontrol agents for fire blight’, *Acta Horticulturae*, (489), pp. 655–662. doi:10.17660/ActaHortic.1999.489.117.

Pusey, P.L. (2000) ‘The Role of Water in Epiphytic Colonization and Infection of Pomaceous Flowers by Erwinia amylovora’, *Phytopathology*, 90(12), pp. 1352–1357. doi:10.1094/PHYTO.2000.90.12.1352.

Santander, R.D. and Biosca, E.G. (2017) ‘Erwinia amylovora psychrotrophic adaptations: evidence of pathogenic potential and survival at temperate and low environmental temperatures’, *PeerJ*, 5, p. e3931. doi:10.7717/peerj.3931.

Schnabel, E.L. and Jones, A.L. (2001) ‘Isolation and Characterization of FiveErwinia amylovora Bacteriophages and Assessment of Phage Resistance in Strains of Erwinia amylovora’, *Applied and Environmental Microbiology*, 67(1), pp. 59–64. doi:10.1128/AEM.67.1.59-64.2001.

Schroth, M.N. *et al.* (1974) ‘Epidemiology and Control of Fire Blight’, *Annual Review of Phytopathology*, 12(1), pp. 389–412. doi:10.1146/annurev.py.12.090174.002133.

Singh, J. and Khan, A. (2019) ‘Distinct patterns of natural selection determine sub-population structure in the fire blight pathogen, Erwinia amylovora’, *Scientific Reports*, 9(1), p. 14017. doi:10.1038/s41598-019-50589-z.

Slack, S.M. *et al.* (2017) ‘Microbiological Examination of Erwinia amylovora Exopolysaccharide Ooze’, *Phytopathology*, 107(4), pp. 403–411. doi:10.1094/PHYTO-09-16-0352-R.

Smith, T.J. (1993) ‘A PREDICTIVE MODEL FOR FORECASTING FIRE BLIGHT OF PEAR AND APPLE IN WASHINGTON STATE.’, *Acta Horticulturae*, (338), pp. 153–160. doi:10.17660/ActaHortic.1993.338.21.

Smith, T.J. and Pusey, P.L. (2011) ‘CougarBlight 2010, a Significant Update of the CougarBlight Fire Blight Infection Risk Model’, *Acta Horticulturae*, (896), pp. 331–336. doi:10.17660/ActaHortic.2011.896.45.

Smits, T.H.M. *et al.* (2010) ‘Complete genome sequence of the fire blight pathogen Erwinia amylovora CFBP 1430 and comparison to other Erwinia spp’, *Molecular plant-microbe interactions: MPMI*, 23(4), pp. 384–393. doi:10.1094/MPMI-23-4-0384.

Sousa, R.M. de (2021) ‘Aspetos práticos e operativos de campo para o controlo do Fogo Bacteriano’. *Palestra técnico cientifica sobre Fogo Bacteriano*, Cadaval, Portugal, 19 July. Available at: https://www.youtube.com/watch?v=CgAEop4LF9c&t=559s.

Steiner, P.W. (1990a) ‘PREDICTING APPLE BLOSSOM INFECTIONS BY ERWINIA AMYLOVORA USING THE MARYBLYT MODEL’, *Acta Horticulturae*, (273), pp. 139–148. doi:10.17660/ActaHortic.1990.273.18.

Steiner, P.W. (1990b) ‘PREDICTING CANKER, SHOOT AND TRAUMA BLIGHT PHASES OF APPLE FIRE BLIGHT EPIDEMICS USING THE MARYBLYT MODEL’, *Acta Horticulturae*, (273), pp. 149–158. doi:10.17660/ActaHortic.1990.273.19.

Szankowski, I. *et al.* (2009) ‘Highly scab-resistant transgenic apple lines achieved by introgression of HcrVf2 controlled by different native promoter lengths’, *Tree Genetics & Genomes*, 5(2), pp. 349–358. doi:10.1007/s11295-008-0191-8.

Tancos, K.A. *et al.* (2017) ‘Fire Blight Symptomatic Shoots and the Presence of Erwinia amylovora in Asymptomatic Apple Budwood’, *Plant Disease*, 101(1), pp. 186–191. doi:10.1094/PDIS-06-16-0892-RE.

Teviotdale, B., Wiley, M. and Harper, D. (1991) ‘How disinfectants compare in preventing transmission of fire blight’, *California Agriculture*, 45(4), pp. 21–23.

Thapa, S.P. *et al.* (2013) ‘Comparative genomics of Japanese Erwinia pyrifoliae strain Ejp617 with closely related erwinias’, *Genome*, 56(2), pp. 83–90. doi:10.1139/gen-2012-0094.

Thomson, S.V. (2000) ‘Epidemiology of fire blight.’, in Vanneste, J.L. (ed.) *Fire blight: the disease and its causative agent, Erwinia amylovora*. Wallingford: CABI, pp. 9–36. doi:10.1079/9780851992945.0009.

Vanblaere, T. *et al.* (2011) ‘The development of a cisgenic apple plant’, *Journal of Biotechnology*, 154(4), pp. 304–311. doi:10.1016/j.jbiotec.2011.05.013.

Vanneste, J.L. (ed.) (2000) *Fire blight: the disease and its causative agent, Erwinia amylovora*. Wallingford, Oxon, UK ; New York, NY, USA: CABI Pub.

Wilson, M. and Lindow, S.E. (1993) ‘Interactions Between the Biological Control Agent *Pseudomonas fluorescens* A506 and *Erwinia amylovora* in Pear Blossoms’, *Phytopathology*, 83(1), pp. 117–123. doi:10.1094/Phyto-83-117.

Zeng, Q., Puławska, J. and Schachterle, J. (2021) ‘Early events in fire blight infection and pathogenesis of Erwinia amylovora’, *Journal of Plant Pathology*, 103(1), pp. 13–24. doi:10.1007/s42161-020-00675-3.

Zutra, D., Shabi, E. and Lazarovits, G. (1986) ‘Fire Blight on Pear, a New Disease in Israel’, *Plant Disease*, 70(11), p. 1071. doi:10.1094/PD-70-1071.

van der Zwet, T. and Keil, H.L. (1979) *Fire blight, a bacterial disease of Rosaceous plants.* Washington, D. C. (Agriculture Handbook, Science and Education Administration).

van der Zwet, T. and Wells, J.M. (1993) ‘Application of fatty acid class analyses for the detection and identification of *Erwinia amylovora*’, *Acta Horticulturae*, (338), pp. 233–234. doi:10.17660/ActaHortic.1993.338.34.

van der Zwet, Tom. and Beer, S.V. (1999) *Fire blight-its nature, prevention, and control : a practical guide to integrated disease management*. Washington, DC: U.S. Dept. of Agriculture. doi:10.5962/bhl.title.134796.