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REVIEW

# This tree is on fire: a review on the ecology of *Erwinia amylovora*, the causal agent of fire blight disease

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## Abstract

Fire blight represents a great threat to apple and pear production worldwide. The ability of its causal agent, *Erwinia amylovora*, to spread rapidly in the host plants makes this devastating disease difficult to manage. Copper and antibiotics are still the most effective solutions to control fire blight, although their application contribute to environmental pollution and to the development of *E. amylovora* resistant populations. Thus, there is an urgent need to find new alternatives to such plant protection products. In this review, we summarized what is known on *E. amylovora* biology, as the knowledge of the plant pathogen biology is essential to develop eco-friendly management strategies. Notably, the presence of *E. amylovora* alone does not necessarily result in the disease development as it is the final outcome of multiple interactions established between *E. amylovora* cells, flower microbiota, plant host, insect vectors and environment. For instance, specific humidity and temperature create the suitable conditions for *E. amylovora* to grow and reach the specific cell density needed for plant infection. Once fire blight develops, insects act as potential vectors of *E. amylovora*, playing a role in the dispersal of the disease. The host plant represents an important factor as its susceptibility varies among the species belonging to the Rosaceae family. Recent studies showed apple flower microbiota might promote or hinder the infection progress, thus representing a possible source of new biocontrol agents effective in controlling *E. amylovora*.

**Keywords** *Erwinia amylovora* · Microbial ecology · Environment · Insects · Plant resistance · Microbiota

## Introduction

Fire blight is a devastating disease affecting a wide range of plant species belonging to the Rosaceae family, including apple (*Malus domestica* L.) and pear (*Pyrus communis* L.) that are the major hosts. Since its first description in New York State at the end of the 18th century, this destructive disease spread to many countries and it is now present in New Zealand, Europe, northern Africa, Asia, and the Middle East (Van der Zwet et al. 2012; Jock et al. 2013; Park et al. 2017; Gaganidze et al. 2018, 2021; Doolotkeldieva et al. 2021). Even though fire blight outbreaks are often sporadic, disease development can rapidly lead to the loss of entire apple and/or pear orchards, therefore representing a great threat to the apple and pear production of many regions worldwide (Doolotkeldieva et al. 2016). The causal agent of fire blight is *Erwinia amylovora*, a Gram-negative bacterium reported in the European and Mediterranean Plant Protection Organization (EPPO) A2 lists of the of pests recommended for regulation as quarantine pests (EPPO 2022). Although *E. amylovora* exploits small wounds in plant tissue caused by insects or strong winds to invade the plant, flowers are considered the main sites of infection. Colonization of flowers of Rosaceae plants by *E. amylovora* cells allows them to grow on stigma surfaces and subsequently enter the plant through the hypanthium (Cui et al. 2021c). Once *E. amylovora* enters the plant, it moves systemically through the parenchyma where its accumulation and its production of biofilms break the epidermis, leading to ooze formation that represents a secondary source of inoculum (Schouten 1989a, b; Slack et al. 2017). Ooze attracts insects that can become potential vectors and further spread the disease (Boucher et al. 2021a). Notably, the presence of *E. amylovora* within the flowers does not inevitably result in

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the disease development. Indeed, several studies report fire blight only occurs when there are specific environmental conditions that facilitate *E. amylovora* cell movement and multiplication (Dagher et al. 2020; Pusey 2000). However, weather conditions are not the only environmental factors to consider. Increasing evidence indicates that the microbial communities living in flowers may play an important role in the earliest stage of host colonization when *E. amylovora* multiplies on stigma surfaces (Cui et al. 2021b).

Since the knowledge of the ecology and biology of plant pathogens is essential to find new eco-sustainable approaches in plant protection (Morris et al. 2017), this review will focus on the interactions between *E. amylovora* and all the other factors involved in the development of fire blight, providing an insight in the ecology of this plant pathogenic bacterium (Fig. 1).

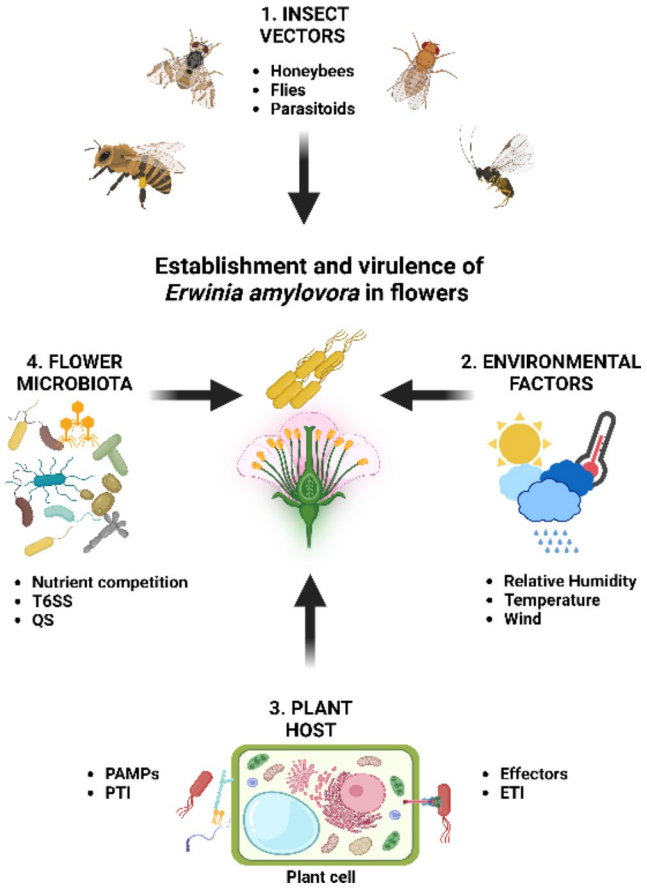


**Fig. 1** Multiple interactions occurring in the environment that may affect the establishment and virulence of *Erwinia amylovora* cells in the flowers of Rosaceous plants. **(1)** Insects may promote the dissemination of *E. amylovora* cells and allow the plant pathogen to reach the plant hosts; **(2)** Environmental factors such as temperature and relative humidity may affect *E. amylovora* ability to colonize flowers and infect the plant hosts; **(3)** Plant hosts may hinder the success of the infection by *E. amylovora* cells through the perception of Pathogen Associated Molecular Patterns (PAMPs) and effectors leading to the activation of the PAMP Triggered Immunity (PTI) and Effector Triggered Immunity (ETI), respectively; **(4)** Microbial communities residing in the flowers may compete with *E. amylovora* cells for space and nutrients. Moreover, bacterial communities may interact directly and/or indirectly through Type 6 Secretion System (T6SS) and chemical communication signals involved in the Quorum Sensing (QS), thus affecting *E. amylovora* growth and virulence. (Created with Biorender.com)

## Influence of environmental factors on the establishment and virulence of *Erwinia amylovora*

As reported by Stevens (1960), a favourable environment is one of the three main factors needed for disease development. But what is meant by 'favourable environment' in the case of fire blight?

First of all, relative humidity (RH) showed to strongly affect *E. amylovora* population size during flower colonization, whose density must exceed $10^4$ bacterial cells per flower to cause the infection in apple plants (Philion 2014). At a stigmatic level, it was seen the epiphytic growth of *E. amylovora* can reach a concentration of more than $10^6$ bacterial cells per crab apple flower only when RH is above 55%, while a RH higher than 80% was required in the floral cup (hypanthium) (Pusey 2000). Rainy weather plays therefore a key role in creating a wet environment suitable for *E. amylovora* cell multiplication, but flower humidity can be also enhanced by the dew occurring throughout the night, as recently hypothesised (Slack et al. 2022). According to the same study, this phenomenon would be influenced even by light wind (Slack et al. 2022). Besides *E. amylovora* cell population size, disease development requires the activation of the Type 3 Secretion System (T3SS), a crucial virulence factor. High RH would contribute to the expression of the genes related to T3SS, thus enabling *E. amylovora* to inject proteins into the host plant to start the infection (Cui et al. 2021a).

Together with RH, temperature is another environmental factor essential to reach the high cell density required for flower infection (Canada AeA 2006; MAAARO 2011). The optimal temperature for the growth of *E. amylovora* is 28 °C (Santander et al. 2017; Van der Zwet et al. 1979), which is consequently the most suitable temperature for fire blight development. However, it was recently shown *E. amylovora* pathogenicity is maintained even at colder temperatures (14 °C and 4 °C), proving that the low bacterial cell growth rates slowed down the infection without necessarily preventing it (Santander et al. 2017). Moreover, it is worthy to note the pH is also a parameter to consider. Indeed, the optimum pH for the growth of *E. amylovora* is around 7.5 (Shrestha et al. 2005) and it was reported this affects both growth and chemotaxis in *E. amylovora* (Raymundo et al. 1980). In addition, Pester and colleagues (2012) also

showed acidic conditions might affect *E. amylovora* pathogenicity. Indeed, the expression of T3SS genes is no longer induced in acidic conditions (pH 4), proving pH has a role in *E. amylovora* virulence (Pester et al. 2012).

All the studies reported above highlighted the environmental conditions that may favour the establishment of *E. amylovora* cells in plant flowers. However, *E. amylovora* cells has also to withstand environmental conditions that may be unfavorable (i.e., dryness). So, it comes naturally to wonder how *E. amylovora* can cope with adverse environmental conditions and what strategies this bacterium may implement to survive and persist in the environment.

The factors described so far are all environmental variables. To a certain extent, the flower itself could be seen as a confined environment in continuous evolution. By their opening, apple and pear flowers undergo several changes that could explain why flower age hinders the colonization ability of *E. amylovora* (Pusey et al. 2008b; Slack et al. 2022). One of these changes is related to the composition of stigmatic exudates which differs in correlation to flower opening stage (Pusey et al. 2008a). Moreover, visitors such as pollinators can disperse microorganisms that constitute potential competitors for the nutritional sources harboured within the flower, contributing to a shift in the bacterial community (Cui et al. 2021b, c).

It is now widely accepted that biofilm is one of the most important virulence factors required by *E. amylovora* to cause disease (Koczan et al. 2009, 2011; Kharadi and Sundin 2021; Peng et al. 2021). Biofilm consists of *E. amylovora* cells and a polymeric matrix whose main components are amylovoran, levan and cellulose (Castiblanco et al. 2018; Koczan et al. 2009, 2011) namely exopolysaccharides (EPSs). In addition, EPS capsule synthesized by *E. amylovora* enhances both dry and cold tolerance, since under these stressful conditions their production is increased, in particular at low temperature (Jock et al. 2005; Santander et al. 2017). Moreover, it was reported that levan might protect *E. amylovora* cells from plant defence mechanisms (Geier and Geider 1993), while amylovoran had a protective effect against desiccation and salinity (Geider 2000, 2009). Similarly, Ordax and colleagues (2010) showed amylovoran and levan protected *E. amylovora* cells against the toxic effect of copper ions. Moreover, both amylovoran and levan can be exploited by *E. amylovora* cells as an alternative carbon source when the nutrient availability in the environment is limited (Ordax et al. 2010).

The lack of nutrients is one of the several stresses plant pathogenic microorganisms are exposed to, especially when they are not in the host plant. It was observed *E. amylovora* undergoes numerous morphological changes under starvation, altering cell size and shape, as well as producing vesicles whose function has not been determined yet (Santander et al. 2014). Additionally, nutrient deprivation resulted in loss of motility even though flagellar biosynthesis was not reduced, as proved by gene expression analysis (Santander et al. 2014). Strikingly, *E. amylovora* cells kept under starvation condition were still able to cause symptoms comparable to cells kept under optimal conditions (Santander et al. 2014).

Similarly to other Gram-negative plant pathogenic bacteria (Grey et al. 2001; Kong et al. 2014), *E.amylovora* cells may also enter into Viable But Not Culturable (VBNC) state to withstand unsuitable environmental conditions. For instance, the entry into VBNC is a strategy used by *E. amylovora* cells to counteract the presence of chlorine and copper ions (Ordax et al. 2006, 2009; Santander et al. 2012) as well as resist starvation. VBNC in *E. amylovora*, is also triggered under starvation and is particularly influenced by temperature (Santander et al. 2014), proving once again how much the environment may influence life of *E. amylovora* cells.

Recent findings showed the expression of virulence genes in *E. amylovora* changes throughout apple flower infection (Schachterle et al. 2022). Specifically, T3SS and flagella are highly induced during the colonization of flower stigmas and at the flower base (Schachterle et al. 2022). The biosynthesis of amylovoran have a similar regulation, while genes involved in the sulfur/oxidative stress are expressed during all stages of the infection (Schachterle et al. 2022). These outcomes suggest the *E. amylovora* might modulate its virulence according to the environmental stimuli perceived. Previous studies were carried out to understand how this takes place at a molecular level (Schachterle et al. 2019a, b, 2022; Yang et al. 2020; Kharadi et al. 2022). Yang and colleagues (2020) reported the RelA/SpoT system is activated in *E. amylovora* under adverse conditions, such as starvation and oxidative stress, leading to an increase in the production of the nucleotide second messenger (p)ppGpp that positively regulates T3SS and motility (Yang et al. 2020). Cyclic di-GMP, another nucleotide second messenger, has an important role during xylem invasion. Indeed, its accumulation inside *E. amylovora* cells promoted the surface attachment mediated by the Type IV pilus and the production of EPSs, two factors involved in the biofilm formation (Kharadi et al. 2022). However, *E. amylovora* virulence traits and oxidative stress adaptation are also influenced by ArcZ, OmrAB and RmaA, small RNAs regulators acting at a transcriptional and post-transcriptional level (Schachterle et al. 2019a, b). Overall, these observations highlighted the complexity of the regulatory systems activated by *E. amylovora* in response to the environment.

## Influence of the interaction with insect on the dispersal and infection by *Erwinia amylovora*

Due to their essential role in agriculture, honeybees and other pollinators have always been seen as potential carriers of plant pathogenic microorganisms (McArt et al. 2014; Cellini et al. 2019). This is of particular interest in the case of fire blight because pollination takes place in flowers, which are regarded as the primary sites of infection. Honeybees' involvement in *E. amylovora* dispersal was demonstrated in the first half of the 1900 (Keitt et al. 1941; Pierstorff et al. 1934). To be transmitted, *E. amylovora* cells must survive inside and/or outside the body of the insects. Early results reported contaminated honeybees can carry *E. amylovora* on the body surface and in the intestine for up to 48 and 36 h, respectively. However, *E. amylovora* cell longevity strongly depended on temperatures (Alexandrova et al. 2002a, b; Sabatini et al. 2006). In contrast, Choi et al. (2022) recently revealed *E. amylovora* cells can be detected for a longer period inside the honeybees that can disseminate the plant pathogenic bacterium in a time span of 10 days after contamination. Moreover, the same authors showed honeybees may acquire *E. amylovora* from diseased apple plants and further spread the pathogen to healthy plant hosts (Choi et al. 2022). This raises questions on the way the interaction involving *E. amylovora*, pollinators and host plants is modulated. Honeybees seem to be more attracted by healthy apple flowers rather than infected ones and this phenomenon could be driven by the emission of Volatile Organic Compounds (VOCs) (Cellini et al. 2019). It was hypothesised honeybees may distinguish between diseased and non-diseased apple flowers by recognizing their VOCs profile. In particular, methyl salicylate, whose emission is associated to *E. amylovora* infection, would discourage pollinators' visits (Cellini et al. 2019). If on one hand the honeybee preference for healthy flowers would prevent the dissemination of the *E. amylovora*, on the other hand the attractiveness of healthy flowers towards honeybees infected with *E. amylovora* might facilitate disease transmission (Cellini et al. 2019). The epidemiological implications behind these observations highlighted by Cellini et al. (2019) are unclear yet and further studies are needed to disentangle the complicate interaction between honeybee-*E. amylovora*-apple flowers.

Even though bees and hoverflies represent the most important pollinators of apple flowers (Delaplane and Mayer 2000; Klein et al. 2007; Pardo and Borges 2020), orchards are visited by a wide population of non-pollinators throughout the seasons. Among them, fruit flies such as *Ceratitis capitata* (the Mediterranean fruit fly, or medfly) and the common *Drosophila melanogaster* attracted the attention of fire blight research. Medfly is classified as one of the most dangerous threats to fruit production worldwide due to its invasiveness and ability to easily settle in new areas, that make it difficult to control this pest (Aluja and Norrbom 2000). It was seen this pest can acquire *E. amylovora* by feeding on inoculum drops of infected apples and harbour the plant pathogenic bacterium both in inner and outer parts of insect's body for 8 and 28 days, respectively, similarly to the previously observations conducted on honeybees (Ordax et al. 2015).

Trees infected by fire blight present small bacterial ooze droplets on fruits or other plant tissues depending on how the stage of disease is advanced (Johnson 2000). In contrast to honeybees, flies such as *D. melanogaster* can be contaminated by *E. amylovora* directly from ooze and later transfer the pathogen, even though this transmission was only tested on selective medium (Boucher et al. 2019). *E. amylovora* cell acquisition, but not its abundance, was positively correlated to the amount of time *D. melanogaster* individuals were exposed to bacterial ooze (Boucher et al. 2019) tested 3, 6, 12 and 24 h of exposure, but it is questionable these times represent what really happens in the field, where flies can be attracted by several ooze sources and could probably keep flying from one source to another. So, it is difficult to know whether the time they are exposed to ooze is sufficient to acquire enough *E. amylovora* cells to further spread, also because the *E. amylovora* population harboured in ooze could differ.

A recent study carried out on *Delia* (*De.*) *platura*, another fly species, underlined EPSs contained in ooze would also enhance the attachment of the *E. amylovora* cells to the surface of the insects (Boucher et al. 2020). Surprisingly, insects showed no preference for infected apples with or without ooze droplets (Boucher et al. 2021a), although it is known the attractive effect exerted by ooze on insects (Agrios 2008). Investigating the role of odours emitted by infected and uninfected apples emerged that *De. platura* seems to prefer healthy fruits to those diseased when the infection was in an advanced stage (Boucher et al. 2021a). To a certain extent, this is consistent with what reported by Cellini et al. (2019) on the role of VOCs in the honeybee transmission. These observations were conducted on controlled experimental conditions, but what happens in open field? According to recent findings, the role of pollinators in the *E. amylovora* dispersal may be overestimated (Boucher et al. 2021b). However, it is conceivable that data collected in open field are strongly affected by several environmental factors, such as temperature and humidity. In addition, the outcomes reported by Boucher et al. (2021b) are just preliminary and limited to a specific area. Further studies on other orchards are needed to provide other data to confirm what observed.

To summarise, both pollinators and non-pollinators showed the ability of acquiring *E. amylovora*, which can be maintained in internal parts of the insect body, particularly in the digestive tract. *E. amylovora* internalisation might result in its transmission to the progeny. Research carried out on the egg parasitoid *Anaphes nitens* focussed on this aspect. By studying the endosymbionts of this insect, Ribeiro et al. (2022) found *E. amylovora* was inherited by the F1 and F2 generations, but it was absent on the eggs of *Gonipterus platensis*, the beetle species parasitised by *A. nitens* (Ribeiro et al. 2022). These outcomes represent the first evidence *E. amylovora* can be vertically transmitted in a parasitoid, raising question on the possibility this phenomenon taking place also in other insect species able to disseminate *E. amylovora*. So far, *E. amylovora* was not detected in the eggs of medfly (Ordax et al. 2015), but no data are available on honeybees.

## Impact of host plants on the pathogenicity of *Erwinia amylovora*

Plant disease could be seen as a never-ending war in which the invader, namely the plant pathogenic microorganism, fights to conquer a new niche, represented by the host plant, where it can acquire substances needed for its survival (Agrios 2005). It is an arms race in which each side tries to attack and protect itself from the other (Anderson et al. 2010). Due to their short generation time, plant pathogenic bacteria evolve more rapidly than their host plants (Frantzeskakis et al. 2020), giving them an advantage on the host and keeping the interaction with the plant constantly evolving.

Plant immunity is based on two main response mechanisms, namely Pattern-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI) (Anderson et al. 2010). In PTI, plant defence reactions are activated after widely conserved microbial molecules (Pathogen-Associated Molecular Patterns, PAMPs) are recognised by specific receptors namely Pattern Recognition Receptors (PRRs) (Chisholm et al. 2006; Zhang and Zhou 2010). Transcriptional analysis revealed apple plants activate this basal defence in the first two hours after *E. amylovora* inoculation (Norelli et al. 2009), thus representing the first barrier *E. amylovora* cells must overcome to establish disease. For this reason, improving this earliest immune response of the plant would be a promising strategy to withstand fire blight. As seen for several species belonging to the Solanaceae and Poaceae families, transgenic plants expressing the *Arabidopsis thaliana* EF-TU RECEPTOR (EFR) have an increased resistance to bacterial plant pathogens (Schoonbeek et al. 2015; Schwessinger et al. 2015; Boschi et al. 2017). Once this PRR was expressed also in the susceptible apple rootstock M.26, the formation of necrotic tissue in the leaves was significantly reduced upon the infection by *E. amylovora* (Piazza et al. 2021). The case reported shows how finding candidate genes in the genome of plant species belonging to families other than Rosaceae can be a possible strategy to increase resistance in apple trees. However, the same can be done focussing on the *Malus* genus. The receptor-like kinase protein (FB_*Mfu10*) found in *M. fusca* might be involved in the resistance of this wild apple species (Emeriewen et al. 2018). Even though further studies are needed to confirm this hypothesis, this receptor harbours a domain that might bind the exopolysaccharide amylovoran, thus recognizing *E. amylovora* cells and contributing to activate the plant defence (Emeriewen et al. 2018).

To silence PTI, plant pathogens evolved a strategy based on the secretion of specific proteins called effectors. In Gram-negative plant pathogenic bacteria, such as *E. amylovora*, effectors are delivered into host cells through the T3SS encoded by the *hrp* box (Lindgren et al. 1986; Büttner et al. 2009). On the other side, plant genome can harbour genes able to specifically suppress the effector's activity, leading to resistance. This gene-for-gene resistance mechanism is called ETI (Effector Triggered Immunity). By acquiring additional effectors or modifying the recognised ones, pathogens can avoid ETI until natural selection leads plants to have new resistance genes and this never-ending evolution in attack-defence response was named 'The zig-zag model' (Jones et al. 2006).

It is difficult to apply the zigzag model to *Malus-E. amylovora* pathosystem because several studies showed how complicated it is to understand the gene-for-gene interaction between *E. amylovora* and *Malus* species. However, the possible molecular mechanisms involved in the interaction between *E. amylovora* and *Malus* spp. are summarized below.

In *E. amylovora*, T3SS gene expression is activated in the first 48 h after flower inoculation (Pester et al. 2012; Schachterle et al. 2022). Particularly, its activation would be strongly induced during the epiphytic colonization of stigmas and reduced when *E. amylovora* cells reach the hypanthium (Cui et al. 2021a). Several effector genes, namely *hrpN* and *hrpW* (Wei et al. 1992; Kim and Beer 1998); *dspE*, involved in plant cell apoptosis (Bogdanove et al. 1998); $hopPtoC_{Ea}$, whose function is not known yet (Zhao et al. 2005); $avrRpt2_{EA}$, homologous to *avrRpt* effector of *Pseudomonas syringae* pv. *tomato* (Zhao et al. 2006); *eop3*, similar to effectors belonging to HopX family (Nissinen et al. 2007); $xopX1_{Ea}$ (Bocsanczy et al. 2012); *eop1* (Wöhner et al. 2018) were identified in *E. amylovora* sequenced genomes. One of the most widely studied effectors is $avrRpt2_{EA}$, which has a single nucleotide polymorphism at

position 156 of its amino acidic sequence, where a cysteine residue can be replaced with a serine (Vogt et al. 2013). *E. amylovora* strains carrying cysteine, such as *E. amylovora* Ea273, belong to the "C-allele" group, while those presenting serine, like the Canadian *E. amylovora* strains, belong to the "S-allele" group (Vogt et al. 2013). Recently, Schröpfer and colleagues (2018) observed the effect of $avrRpt2_{EA}$ on the susceptible *M. domestica* cultivar Pinova (Schröpfer et al. 2018). Transgenic lines expressing $avrRpt2_{EA}$ activated the salicylic acid-mediated defence response and developed typical fire blight symptoms, suggesting this effector alone is sufficient to cause disease (Schröpfer et al. 2018). Moreover, the interaction between the effector HrpN and the *Malus* spp. specific protein named HIMP resulted in the disease development (Wei et al. 1992; Oh and Beer 2007). As a consequence, transgenic apple plants with a reduced expression level of *HIMP* were more resistant to *E. amylovora* infection (Campa et al. 2019).

Since most of commercial apple tree varieties are susceptible to fire blight, genome mining studies aimed at identifying resistance genes mainly in wild apple genotypes. Resistance quantitative trait loci (QTLs) were found in *M.floribunda* 821 (Durel et al. 2009), *Malus* × *robusta* 5 (Peil et al. 2007), *M.fusca* (Emeriewen et al. 2018), *Malus* × *arnoldiana* (Emeriewen et al. 2021), and in the ornamental crab apple *M. evereste* (Durel et al. 2009). So far, only the *FB_MR5* gene from *Malus* × *robusta* 5 showed a gene-for-gene interaction with effector $avrRpt2_{EA}$, but this was strongly influenced by the *E. amylovora* strain inoculated. Indeed, *E. amylovora* strains belonging to the S-allele group were able to overcome plant defence, unlike strains having the cysteine allele (Vogt et al. 2013). These results were confirmed by transforming *M. domestica* cv. 'Gala' with *FB_MR5* (Broggini et al. 2014). Strain-dependent susceptibility was also observed in *M. floribunda* 821 and Evereste, whose resistance mechanism to *E. amylovora* is thought to be related to effector *eop1*, even though it was not proved yet (Wöhner et al. 2018).

In addition to the immunity response triggered by *FB_MR5* gene, other mechanisms presumably occur during the resistance response. A recent study compared the response of *Malus* ×*robusta* 5 inoculated with either the wild type strain *E. amylovora* Ea1189, triggering the ETI and therefore avirulent, and the mutant in the $avrRpt2_{EA}$, which is virulent since it is not anymore recognized by the apple plants. Several differentially expressed genes were reported in plants inoculated with either the *E. amylovora* Ea1189 virulent or avirulent strain. For instance, *Malus* ×*robusta* 5 plants induced a higher expression of genes involved in the flavonoids pathway and in the biosynthesis of (E)-ß-caryophyllene (Schröpfer et al. 2021), a VOC compound known for its antimicrobial activity (Cellini et al. 2019). These outcomes add information to the resistance reaction in *Malus* ×*robusta* 5, but further studies are needed to have a more detailed view.

Plant defence may also be triggered indirectly, without any physical contact between the plant pathogen and the host. In this regard, the VOCs profile emitted by apple (cv. Golden Delicious) plantlets infected with *E. amylovora* Ea ICMP 1540 was characterized (Cellini et al. 2018). Interestingly, Cellini and colleagues (2018) reported the exposure of apple plants to these volatiles enhanced the activation of the signalling pathways related to salicylic acid (SA), a phenolic compound known for its role in plant immunity (Vlot et al. 2009), thus highlighting a possible innovative application of VOCs in the disease management.

Looking at *E. amylovora* during the interaction with apple plants, Puławska and colleagues (2017) inoculated *E. amylovora* 650 either in a resistant or susceptible apple cultivar. The main difference was seen for *E. amylovora* genes related to stress response indicating that *E. amylovora* 650 implemented molecular mechanisms involved in the protection of secondary metabolites and cell from toxic compounds. Indeed, genes encoding heat shock proteins, as well as those related to multidrug efflux pumps, were highly expressed when *E. amylovora* 650 was inoculated in plants of the resistant apple cultivar Free Redstar (Puławska et al. 2017).

## Influence of bacterial communities on *Erwinia amylovora* establishment in flowers

An increasing body of knowledge is highlighting the importance of the plant host microbiota in the establishment of a pathogenic interaction between a plant pathogenic microorganism and its plant host.

For instance, *Ralstonia solanacearum* caused a drastic shift in the composition of tomato root-associated bacterial taxa that strongly influenced the *R. solanacearum* abundance, as well as the biochemical soil properties (Wei et al. 2018). Similar changes in the rhizosphere microbial communities have been reported also for *Verticillium dahliae*. This plant pathogenic fungus might select collaborative microorganisms within the plant host microbiota through the secretion of a specific effector, attributing these proteins a new role in plant disease (Snelders et al. 2021). These are just two examples that show manipulation of plant host microbiota might be a strategy exploited by several plant pathogenic microorganisms during plant host colonization. Is this ability shared by *E. amylovora* also?

Recently, Cui and colleagues (2021a) tried to understand what happens within the stigmatic bacterial communities upon *E. amylovora* inoculation. Metagenomic analysis

revealed *Pseudomonadaceae* and *Enterobacteriaceae* are the most represented bacterial families on stigma of healthy flowers (Cui et al. 2021a), consistently with previous observations underlining the conservation of microbial community structure among different apple cultivars (Steven et al. 2018). Moreover, it was seen *Pseudomonadaceae* family slowly take over the *Enterobacteriaceae* family in the time span of five days, which is the opposite of what observed in flowers inoculated with *E. amylovora*, where members of the *Enterobacteriaceae* family rapidly become predominant (Cui et al. 2021a). As already mentioned for other plant pathogens, it is conceivable the changes observed are the results of a selection promoted by *E. amylovora* aimed at recruiting microbial communities that can support the plant host colonization and infection. Understanding how *E. amylovora* induces a shift in the microbial communities of apple flowers and how this phenomenon takes place might shed the light to the early step of the infection.

There are several direct and undirect mechanisms *E. amylovora* may exploit to manipulate plant host microbiota.

Type Six Secretion System (T6SS) is one of the secretion systems owned by Gram-negative bacteria (Coulthurst 2019). Unlike T3SS, whose function is limited to plant invasion, T6SS can influence the interaction among bacteria, as reported for *Agrobacterium tumefaciens* (Ma et al. 2014). *E. amylovora* harbours three T6SS gene clusters in its genome (Kamber et al. 2017; Tian et al. 2017) and they seem to have a role in antibacterial competition, as reported for *E. amylovora* NCPPB1665 (Tian et al. 2017). Moreover, they can affect both levan and amylovoran production, thus reducing *E. amylovora* NCPPB1665 virulence on immature pears (Tian et al. 2017). To see whether what reported by Tian and colleagues (2017) could also apply to other *E. amylovora* strains, single or double mutants defective in T6SS were created for *E. amylovora* CFBP 1430 by deleting either cluster one, three or both. A slightly difference in virulence was reported on apple flowers and shoots, probably due to an alteration in the motility showed by the *E. amylovora* mutants (Kamber et al. 2017). Also in this case a possible involvement of T6SSs in antibacterial competition was hypothesised. However, results of the competition assay performed with *E. coli* revealed *E. coli* survival was not so significantly higher compared with what observed for NCPPB1665 strain (Kamber et al. 2017; Tian et al. 2017).

Quorum Sensing (QS) is a regulatory system that controls gene expression according to the bacterial cell population size (Rutherford and Bassler 2012). QS relies on three main components consisting in a signal molecule released by bacteria cells, namely the autoinducer (AI), an enzyme that synthesises the AI and a transcriptional factor able to perceive the AI (Papenfort and Bassler 2016). In Gram-negative bacteria, the most common AIs are N-acyl homoserine lactones (AHLs) produced by N-acyl homoserine lactone synthases encoded by *luxI* homologs (Papenfort and Bassler 2016). However, Gram-negative bacteria may produce another AI called Autoinducer-2 deriving from cyclization of 4,5-dihydroxy-2,3-pentanedione synthesized by ribosyl-homocysteine-cleavage enzyme encoded by *luxS* homologs (Schauder et al. 2001; Pereira et al. 2013). It is now widely accepted that AIs might be at the basis of interspecies communication within plant pathogenic bacteria and bacterial communities residing on plant host organs (Dulla and Lindow 2009; Cellini et al. 2020). Homologs of the genes mentioned above were found in *E. amylovora* genomes and their functionality was confirmed by the detection of AHLs and Autoinduer-2 in several strains (Molina et al. 2005; Rezzonico et al. 2007; Venturi et al. 2004). It was reported AHL release would contribute to EPS production, virulence, and tolerance to hydrogen peroxide (Molina et al. 2005). Interestingly, AI production was not observed for some *E. amylovora* strains isolated in Germany and Switzerland (Mohammadi et al. 2007), probably because of the low titre of signal molecules that would not allow their detection. Thus, it is still questionable whether QS is strain-dependent and further research is needed to characterise its role in the interaction with the plant host microbiota and the establishment of the disease.

Both T6SS and QS can be considered as direct mechanisms possibly exploited by *E. amylovora* for microbiota manipulation. However, influence on microbial communities can be exerted indirectly through a competition for available nutrient sources (Dubinkina et al. 2019). The major components of stigmatic extracts of pomaceous plants are free sugars and amino acids (Pusey et al. 2008a). Glucose and fructose strongly prevail on sugars such as sorbitol and sucrose, and this pattern is preserved among different apple cultivars (Fuji, Gala, Golden), with an increase in sugar content related to the stage of anther dehiscence (Pusey et al. 2008a). Since their amount was very low, amino acids were detected in the order of femtograms per pomaceous flowers, with proline, asparagine, glutamine, and glutamic acid being the most abundant ones (Pusey et al. 2008a). Stockwell and collaborators (2010a) reported *E. amylovora* Ea153 might grow well in a minimal medium amended with one of the principal stigmatic compounds, giving the first evidence these components support the growth of the plant pathogen. Understanding *E. amylovora* nutrient requirements and metabolism is important because they strongly influence its cell multiplication ability and virulence. For instance, apple fruitlets and shoots inoculated with *E. amylovora* HKN06P1 mutants impaired in the biosynthetic pathways of isoleucine/valine, leucine, methionine, adenine, and tryptophan showed a reduction in the severity of symptoms (Klee et al. 2019). In addition to these amino acids, another study

highlighted *E. amylovora* HKN06P1 mutated in the arginine biosynthetic pathway lost its pathogenicity on immature apples (Ramos et al. 2014). This auxotrophic mutant, but not its dead cells, inhibited the colonization of the *E. amylovora* HKN06P1 wild type strain when applied on flowers (Klee et al. 2019). It is not known yet whether this effect is due to nutrient competition alone or other mechanisms (i.e., plant immunity stimulation) are involved. However, Klee and collaborators (2019) proved specific amino acids are necessary for full virulence of *E. amylovora* and they are not sufficiently available in the host, forcing the plant pathogen to synthesise them. Iron is another important element for bacterial cell growth because DNA replication, as well as tolerance to oxidative stress, would not be possible without this cofactor (Krewulak et al. 2008; Cornelis et al. 2011). Thus, iron might represent a limiting factor for bacterial cell survival, particularly when several microorganisms are present in the same environmental niche. Latest findings confirmed this hypothesis by comparing the growth of *E. amylovora* CFBP1430 deficient in the iron-uptake receptor gene *foxR* on greenhouse and orchard apple flowers. Greenhouse flowers, that can be considered as semi sterile, well supported the colonization of the *E. amylovora* CFBP1430 mutant in contrast to those collected from the orchards, whose established microbial communities might be potential iron-competitors (Müller et al. 2022).

## Application of microorganisms for the sustainable control of *Erwinia amylovora*

For many years antibiotics and copper-based compounds have been the most effective solutions to limit *E. amylovora* spread and damages. However, these plant protection products cause environmental pollution and contribute to the development of resistance that make their application useless, as reported in the USA where streptomycin- and copper-resistant *E. amylovora* strains were isolated already in 1991 (Loper et al. 1991). For these two main reasons, research has focused on finding new strategies that can be both effective and eco-friendly.

Besides increasing the knowledge on the fire blight, unveiling how interaction between flower microbiota and *E. amylovora* occurs may also help to improve the sustainable management of the disease. Indeed, microorganisms, such as yeasts and bacteria, able to reduce the ability of plant pathogens to cause disease might be used as biocontrol agents (BCAs) and developed as the main active ingredient of commercial biopesticides (Gupta et al. 2021).

Over the years, microorganisms tested against *E. amylovora* were isolated from different environmental niches (Aktepe et al. 2022; Barbé et al. 2022; Mikiciński et al. 2016; Pourjafari et al. 2022; Pusey et al. 2009). Nevertheless, it is conceivable those one isolated from apple flowers can represent the best antagonistic candidates against *E. amylovora* because their adaptation to live in such an environment. Recent findings showed apple stigma-colonizing bacteria can reduce disease severity when applied on flowers, even though efficacy strongly depends on the bacterial mixtures and is not comparable to streptomycin treatment (Cui et al. 2021b). Among the bacterial isolates tested, treatments including *Pantoea* sp. CT-1039 gave the best reduction of disease incidence, indicating this strain may be responsible for the effect observed (Cui et al. 2021b). Strains belonging to *Pantoea* spp. are indeed the active ingredients of commercial biopesticides used in the fire blight management. At the moment, commercial biopesticides developed to control *E. amylovora* are based on Gram-negative bacteria isolated from apple trees, such as *Pantoea* (*Pa.*) *agglomerans* E325 (Bloomtime), *Pa. vagans* C9-1 (BlightBan C9-1) and *Pseudomonas* (*Ps.*) *fluorescens* A506 (BlightBan A506) (Sundin et al. 2009). All these strains can produce antibiotics, such as herbicolin O and pantocin A (Kamber et al. 2017; Stockwell et al. 2010b). Interestingly, *Ps. fluorescens* A506 can release the antibacterial compound only in an iron-rich environment, which is not the case of apple and pear floral surface (Temple et al. 2004). The efficacy of *Ps. fluorescens* A506 may be mainly attributed to the nutrient/niche competition (Stockwell et al. 2010a), even though the number of carbon sources utilized by *Ps. fluorescens* A506 was lower than the one of *Pa. vagans* C9-1 and *E. amylovora* Ea135 (Stockwell et al. 2010a). On the opposite, the two BCAs share almost all the nitrogen requirements with *E. amylovora* Ea135 (Stockwell et al. 2010a). In comparison to *E. amylovora* Ea135, both *Pa. vagans* C9-1 and *Ps. fluorescens* A506 had a faster growth when cultivated in the sugar compounds contained in the apple stigmatic exudates (Pusey et al. 2008a; Stockwell et al. 2010a). Unexpectedly, the application of both the BCAs did not enhance the reduction of fire blight symptoms because *Ps. fluorescens* A506 produces a protease degrading the antibiotic produced by *Pa. vagans* C9-1, thus erasing its effect (Stockwell et al. 2010b). This study highlighted the incompatibility of BCAs is an important aspect to consider when developing new biopesticides. Considering Gram-positive bacteria, the biopesticide Serenade is based on *Bacillus subtilis* QST713 whose antimicrobial activity is related to the production of lipopeptides (Sundin et al. 2009). The application of Serenade on apple blossoms reduced fire blight symptoms similarly to BlightBan A506, BlightBan C9-1 and Bloomtime (Sundin et al. 2009). Another product used to control fire blight is Blossom Protect, whose active ingredients are the yeast-like fungal strains *Aureobasidium pullulans* CF10 and CF40. Regardless of the incubation temperature, these

strains isolated from apple fruits were effective in reducing the fire blight severity both on detached apple flower and in open field (Kunz 2004). In a recent study, Temple et al. (2020) compared the efficacy of Blossom Protect to other yeasts strains, including *Cystofilobasidium infirmominiatum* YY6 and *Cryptococcus neoformans* C9 and C16. None of the tested yeast strains was as effective as Blossom Protect, whose mode of action is not related to reduction of *E. amylovora* populations in the flower (Temple et al. 2020).

As the mode of action of *A. pullulans* may rely on its ability to compete for space and nutrients, Slack et al. (2019) evaluated the impact of a preventive application of hydrogen dioxide and peroxyacetic acid before the application of Blossom Protect, in order to reduce the competition of the microbial populations residing in the apple flowers. However, the removal of the flower microbiota did not result in an increase of the Blossom Protect plant protection efficacy (Slack et al. 2019), an indication that the mode of action of *A. pullulans* might rely on multiple mechanisms. Accordingly, Zeng and colleagues (2023) reported flower treatment with *A. pullulans* would trigger the Systemic Acquired Resistance in the apple flowers by increasing the level of salicylic acid and inducing the expression of *PR1* and *PR2* genes. Interestingly, the immune response in the plant host would be active until five days after treatment. As this time period is also the life span of apple flowers, it is conceivable that fewer applications of Blossom Protect would be needed to have an effective plant protection efficacy (Zeng et al. 2023).

Despite the initial promises, years of experiments in open field showed the application of the above mentioned BCAs alone were not sufficient in controlling fire blight unless they were used together with streptomycin or other products (Sundin et al. 2009; Johnson et al. 2013, 2022). For instance, a significant increase in the fire blight control was achieved by applying Blossom Protect after fruit load thinning treatment with lime sulfur, reaching an efficacy comparable to streptomycin (Johnson et al. 2013). Recently, Johnson and colleagues (2022) suggested the different protection products may be applied at different bloom stage, e.g. Blossom Protect, copper and Serenade at 70% bloom, full bloom and petal fall, respectively. Notably, several trials showed this combination would reduce both disease severity and fruit russeting (Johnson et al. 2022).

Besides microbial BCAs, *E. amylovora* might be controlled using bacteriophages, viruses widespread in all habitats where their host, namely bacteria, live (Sieiro et al. 2020). The main characteristic of bacteriophages is they can infect and kill specific bacterial species or strains by lysing their cells, thus representing a valid alternative to antibiotics in plant disease management (Sieiro et al. 2020). Concerning fire blight, several bacteriophages isolated from apple trees in Germany and North America were characterized and tested against several *E. amylovora* strains (Müller et al. 2011). Müller and colleagues (2011) showed bacteriophages belonging to the *Myoviridae* family significantly reduced *E. amylovora* population on apple flowers in comparison to the members of the *Podoviridae* family.

In general, the use of bacteriophages has two important disadvantage such as the time of application. Since bacteriophage survival is strictly dependent on the presence of their host bacteria, there is reduction of bacteriophage population when flowers do not harbour *E. amylovora* (Ritchie and Klos 1979; Schnabel et al. 1999; Schnabel and Jones 2001). This issue can be solved by using a carrier like *Pa. agglomerans* Eh21-5 to deliver bacteriophages, as developed by Lehman (2007) and employed in later studies (Lehman 2007; Boulé et al. 2011; Geyder et al. 2020). The efficacy of this method might be negatively affected by the sensitivity of the bacterial carrier to the bacteriophages delivered, making the study of the bacteriophage-carrier interactions essential (Geyder et al. 2020). Moreover, *E. amylovora* may rapidly develop resistance to bacteriophages, as seen for other plant pathogenic bacteria (Dong et al. 2018; Fujiwara et al. 2011), thus limiting their efficacy. To overcome this problem, research has focussed on creating mixtures of several bacteriophages that can reduce the emergence of resistance in *E. amylovora* by using different infection strategies (Sieiro et al. 2020). The synergy between several bacteriophages was proved, but in some cases the validation *in planta* has not been tested yet (Born e al. 2011, 2015; Gayder et al. 2020; Jo et al. 2023).

## Future perspectives

As discussed so far, fire blight is influenced by many factors that make this disease complex and difficult to study and manage.

For instance, the growth of *E. amylovora* is strongly affected by environmental conditions such as humidity, rain, and temperature that also influence the efficacy of eco-sustainable strategies such as BCAs. These variables are impossible to control, especially nowadays that global warming causes more and more extreme climate events (Seneviratne et al. 2021) and for this reason it is important to test the response of new strategies to adverse environment phenomena.

In addition to heavy rain and winds, insects might play an important role in fire blight disease. *E. amylovora* can survive on the surface but also inside the body of pollinators and non-pollinators, thus becoming vectors able to spread the plant pathogen to healthy host plants. So far, most of the experiments were carried out in controlled laboratory

conditions and very few data were collected on the impact of insects' transmission in open field. This is an aspect to be investigated, as well as the heritability of *E. amylovora* over generations, which has been recently proved in *A. nitens* (Ribeiro et al. 2022), without testing the viability and the pathogenicity of the bacterial cells. It would be also of interest to dig into the complexity of the system "*E. amylovora* - host plant – vectors" by pursuing the work carried out by Cellini et al. (2019) to understand the attractive/repulsive effect of VOCs, in addition to the stimulation of plant defence.

The interaction between *E. amylovora* and host plants is also an open research field. Since PTI is the earliest immune response activated, it would be conceivable to focus on its implementation. However, since plant pathogens overcome this barrier by releasing effectors, ETI must not be forgotten. In this view, instead of concentrating only on wild apple varieties, looking in the genomes of other species belonging to the Rosaceae family might lead to identify QTLs harbouring new resistance candidate genes.

During the earliest phase of infection, when *E. amylovora* cells try to colonize flower stigmas, microbial communities residing in the flowers can influence the infective process, hindering or enhancing the establishment of *E. amylovora* population. By creating *E. amylovora* knock-out mutants in the genes related to QS and T6SS it will be possible to see their involvement in the interaction with the microbiota, expanding the knowledge on the mechanisms that *E. amylovora* might implement to manipulate the flower microbiota. Moreover, studying the bacterial community residing in apple flowers might lead to find new microbial species to use in the sustainable management of fire blight. BCAs so far commercialized are not sufficient to achieve a complete control of the disease. So, in the future, combining yeasts and bacteria may result in the development of new effective biopesticides.

The use of different strategies might possibly enhance the chance of reaching a complete fire blight control, avoiding environmental pollution, but further research is required.

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## Declarations

**Conflict of interest** The authors declare that there is no conflict of interest.

**Human and animal rights and informed consent** This article does not contain any studies with human participants / animals performed by any of the authors. All authors were informed about the study and agreed for publication. Informed consent was obtained from all individual participants included in the study.



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