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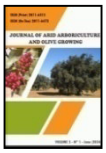

Fire Blight Disease Research Achievements in Tunisia: A Comprehensive Review

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Abstract

Fire blight disease, caused by *Erwinia amylovora* (Burrill), emerged as a devastating pathogen in Tunisia in spring 2012, causing catastrophic losses to the national pear and apple industry (Rhouma et al., 2014). Since its first detection in the Morneg region (Ben Arous governorate), the disease has spread rapidly throughout northern Tunisia, destroying more than 5,500 hectares of pear plantations (approximately 65% of total cultivated area) and reducing national pear production from 60,000 metric tons in 2011 to less than 20,000 metric tons by 2016 (Rhouma et al., 2014; Gaaliche et al., 2018). This review synthesizes significant research achievements by Tunisian scientists in understanding and combating fire blight since 2012. Major accomplishments include: establishment of pathogen identity through rigorous microbiological and molecular characterization (Rhouma et al., 2014; Bouazizi et al., 2025); comprehensive epidemiological assessment across all major production regions (Gaaliche et al., 2018); evaluation of cultivar susceptibility patterns and identification of resistance mechanisms (Gaaliche et al., 2018; Bouazizi et al., 2020); investigation of population genetic diversity and structure of *E. amylovora* isolates; characterization of virulence variability among pathogen populations; identification of molecular mechanisms underlying host defense responses including antioxidant systems and salicylic acid signaling (Bouazizi et al., 2020); isolation and characterization of bacteriophages with potential for biological control; and investigation of pathogenic determinants at the molecular level, including virulence gene regulation and metabolic signaling in the plant apoplast (Jeridi, 2022).

Keywords: *Erwinia amylovora*, fire blight, Tunisia, pathogen characterization, genetic diversity, cultivar susceptibility, host defense mechanisms, bacteriophage biocontrol, disease management, Mediterranean agriculture.

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1. Introduction

Fire blight disease, incited by the gram-negative bacterium *Erwinia amylovora* (Burrill), represents one of the most destructive bacterial diseases of economically important fruit crops worldwide (Rhouma et al., 2014). The disease primarily affects members of the Rosaceae family, with pear (*Pyrus communis* L.) and apple (*Malus domestica* Borkh.) serving as the most significant commercial hosts (Gaaliche et al., 2018; Johnson & Stockwell, 1998). Originally endemic to North America, the pathogen has progressively expanded its geographic range over more than two centuries, establishing pandemic status across North America, Europe, the Mediterranean region, the Middle East, North Africa, New Zealand, and East Asia (Rhouma et al., 2014; Vanneste, 2017).

Fire blight disease begins in spring when overwintering bacterial ooze infects blossoms during warm, humid, wet conditions. Warm temperatures (18-29°C), rainfall, and insect vectors facilitate bacterial spread to shoots and fruits, causing characteristic "shepherd's crook" wilting symptoms through vascular tissue colonization.

The Type III Secretion System (T3SS) serves as a molecular syringe to inject virulence effector proteins into host cells, triggering disease. This system is regulated by a cascade involving HrpL and sigma factors (σ^{54}, RpoN), which are activated by the bacterial alarmone (p)ppGpp. Host nitrogen status significantly modulates virulence gene expression—nitrogen-limited plants show enhanced hrp gene induction, with metabolites like linolenic acid promoting and citrate repressing T3SS expression.

E. amylovora secretes four Type III effector proteins (DspA/E, Eop1, Eop3, Eop4), with DspA/E being the major pathogenicity factor suppressing host defenses. The exopolysaccharide amylovoran protects bacteria from host immunity, facilitates tissue movement, and promotes biofilm formation. Tunisian isolates show variable biofilm formation correlating with virulence (Jeridi, 2022).

Bacterial motility, mediated by flagella and regulated by two-component signal systems, correlates with both biofilm production and virulence—highly virulent strains exhibit elevated motility. Infection success depends on complex regulatory networks, including quorum sensing (N-acylhomoserine lactone and autoinducer-2), c-di-GMP signaling, and RNA-binding proteins (CsrA, RprA) that coordinate early and late-stage virulence factor expression.

Before 2012, Tunisia maintained fire blight-free status through rigorous quarantine enforcement and careful agricultural protocols. The pear sector represented a significant component of the national agricultural economy, occupying approximately 8,250 hectares of cultivation distributed across northern, central, and southern regions. The three northern governorates of Manouba, Ben Arous, and Bizerte constituted the epicenter of pear production, providing approximately 74% of national production (Gaaliche et al. 2018). Indigenous pear varieties, exhibiting remarkable genetic diversity and exceptional adaptation to local conditions, coexisted alongside recently introduced European and American cultivars (Volk et al., 2020). Average annual production over the three years preceding disease emergence (2010-2012) reached 63,000 metric tons.

The catastrophic emergence of fire blight in Tunisia during spring 2012 marked an unprecedented agricultural crisis (Rhouma et al., 2014). Initial symptoms were observed on susceptible cultivars 'Alexandrine' and 'Williams' in the Morneg region of Ben Arous governorate during the flowering period (Rhouma et al., 2014). The pathogen demonstrated remarkable capacity for rapid dissemination, spreading during 2013 to encompass the governorates of Manouba, Tebourba, Bizerte, Zaghouan, and Beja, with documented disease incidence ranging from 10% to more than 75% depending on location and cultivar susceptibility (Gaaliche et al., 2018). Within four years, the epidemic had destroyed more than 5,500 hectares among the total 8,400 hectares of pear plantations, representing approximately 65-70% destruction of cultivated area (Gaaliche et al., 2018).

The economic consequences have been staggering (Gaaliche et al., 2018). Annual pear production collapsed from 60,000 metric tons in 2011 to less than 20,000 metric tons by 2016, representing a 66% reduction in national output. The cultivated area declined proportionally from 8,400 hectares to 3,260 hectares during the same period. Economic costs extended beyond immediate production losses to encompass expenses associated with orchard eradication, loss of invaluable genetic resources embodied in indigenous varieties, disruption of established market chains, and necessity for agricultural reconversion to alternative crops (Gaaliche et al., 2018).

2. Detection, Identification, and Initial Characterization of Erwinia amylovora in Tunisia

2.1. First Report and Pathogen Confirmation

The initial detection of fire blight in Tunisia occurred during spring flowering of 2012, when characteristic disease symptoms were observed on pear trees in the Morneg region of Ben Arous governorate (Rhouma et al., 2014). Affected cultivars exhibited classical fire blight symptomatology, including wilted and shriveled blossoms with characteristic brown discoloration, rapid wilting of young shoots forming characteristic "shepherd's crook" configurations, blackened necrotic leaves remaining attached to infected branches, and the presence of amber-colored bacterial exudate on affected tissues (Figure 1) (Rhouma et al., 2014).

Figure 1. Symptoms of blossom blight on pear (A); symptoms of shoot blight with bacterial exudate (B); symptoms of fruit blight on pear (C); and leaf blight symptoms (D). Canker symptoms observed on branches and scaffold limbs of pear trees.

Following EPPO diagnostic protocols, researchers at INRAT conducted systematic sampling and isolation from symptomatic tissues. Bacterial isolates were recovered on King's B (KB) medium, displaying morphological characteristics consistent with *Erwinia amylovora* (Figure 2A).White to cream-colored, circular, convex, mucoid colonies with diameters of 1.5-2.0 mm are visible after 48 hours of incubation at 25-27°C (Rhouma et al., 2014).

Rigorous confirmation involved multiple complementary methodological approaches (Rhouma et al., 2014). Biochemical characterization demonstrated that isolates were gram-negative, catalase-positive, oxidase-negative, capable of fermenting glucose while oxidizing sucrose (Rhouma et al., 2014). The isolates exhibited the biochemical profile of *E. amylovora* through API 20 E and API 50 CH systems. Pathogenicity assays using detached immature pear fruits (cv. Alexandrine) inoculated with bacterial suspensions (10^9 CFU/mL) consistently reproduced characteristic disease symptoms, including tissue browning, necrosis, and bacterial ooze production within 5-7 days, with successful re-isolation of bacteria from symptomatic tissues fulfilling Koch's postulates (Figure 2B) (Rhouma et al., 2014).

Molecular identification provided definitive species-level confirmation through PCR-based assays employing EPPO-recommended primer pairs targeting the 29-kb plasmid pEA29 (primers A/B or AJ75/AJ76), which amplified expected fragments of approximately 844-900 bp (Rhouma et al. 2014). Complementary PCR assays using chromosomal markers (primers FER1-F/rgER2-R) targeting conserved genomic regions produced the expected 458 bp amplicon (Figure 2C) (Bouazizi et al., 2025). Significantly, Tunisian researchers identified naturally occurring plasmid-deficient isolates lacking the pEA29 plasmid (designated Ea13 and Ea28), representing an important variant previously documented in Egypt, Spain, Germany, Iran, and other countries (Rhouma et al., 2014; Puławska and Sobiczewski, 2012). Purified PCR amplicons subjected to Sanger sequencing revealed 98-100% nucleotide sequence identity with authentic *E. amylovora* reference strains from diverse geographic origins, providing unequivocal molecular confirmation (Rhouma et al., 2014).

Real-time PCR assays employing TaqMan probes targeting the amsC gene (encoding a component of the amylovoran biosynthesis pathway) enabled sensitive and specific detection (Figure 2D) (Zhao et al., 2005; Bouazizi et al., 2025). This comprehensive multi-faceted diagnostic approach established beyond doubt that fire blight disease caused by *Erwinia amylovora* had emerged in Tunisia (Rhouma et al., 2014).

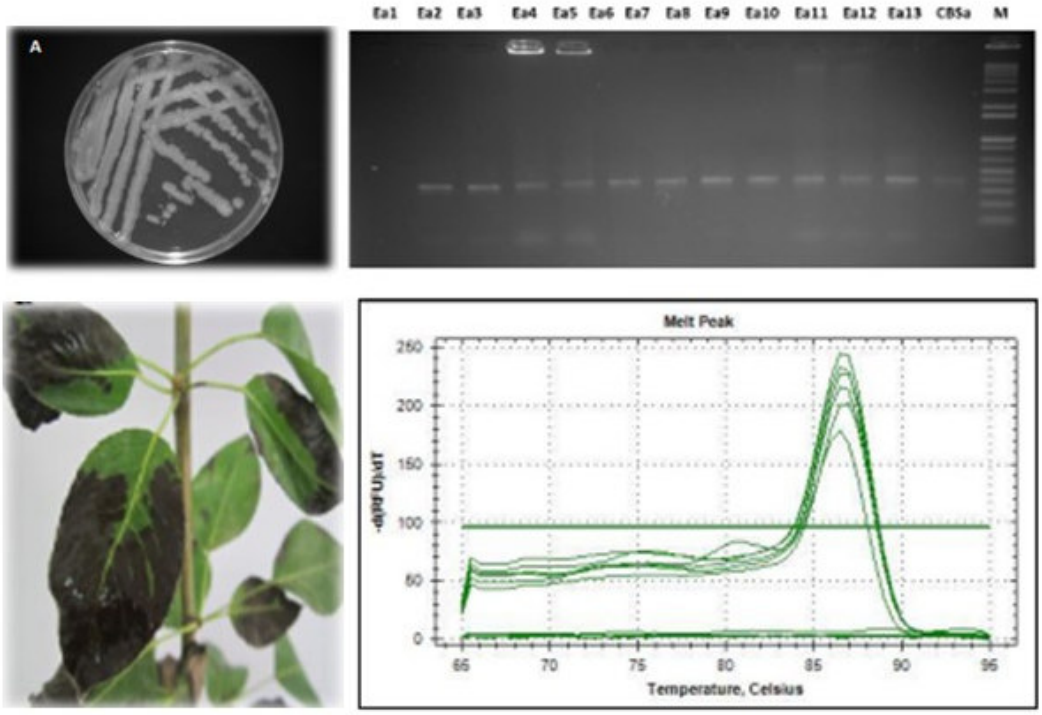

Figure 2. Components of the diagnostic protocol for symptomatic samples presenting typical symptoms of fire blight disease

2.2. Molecular and Biochemical Characterization

Among 59 isolates collected from various Tunisian regions between 2014 and 2018, researchers identified distinct molecular profiles (Bouazizi et al., 2025). The vast majority of isolates (57 of 59) retained the plasmid pEA29, while two naturally occurring plasmid-deficient strains were recovered, consistent with globally documented patterns of *E. amylovora* diversity (Bouazizi et al., 2025; Puławska and Sobiczewski, 2012).

Biochemical characterization through standardized API systems revealed that Tunisian isolates display the classical biochemical profile of *E. amylovora*: ability to ferment glucose, oxidize sucrose, and utilize diverse carbon sources (Bouazizi et al., 2025). Antibiotic resistance testing revealed that all Tunisian isolates exhibited a streptomycin-sensitive phenotype with a single exception (isolate Ea34) recovered from Ben Arous in 2017, representing an important finding given that streptomycin is not registered for fire blight control in Tunisia (Jeridi, 2022). The emergence of streptomycin resistance in *E. amylovora* populations has been documented globally through mutations in the rpsL gene encoding ribosomal protein S12 (Manulis et al., 1998 ; Vanneste and Voyle, 1999 ; Russo et al., 2008 ; de Leon Door et al., 2013).

3. Pathogen Diversity, Virulence Variation, and Population Genetic Structure

3.1. Virulence Characterization

Research documenting virulence variation among Tunisian *E. amylovora* isolates represents a significant contribution to understanding pathogen population heterogeneity (Bouazizi et al., 2025). Virulence assessment conducted on detached pear fruitlets (cv. Alexandrine) using 59 isolates revealed substantial virulence variability, with three virulence categories identifiable: weakly virulent isolates (13.56% of population) producing lesion diameters <7 mm; moderately virulent isolates (55.93% of population) with lesion diameters of 8.0-17.99 mm; and highly virulent isolates (30.51% of population) producing lesions >18 mm (Bouazizi et al., 2025). This virulence stratification suggests that Tunisian *E. amylovora* populations comprise diverse strains exhibiting differential aggressive capacity.

Molecular investigations revealed that virulence variation correlates partially with genetic clustering patterns. Notable linkage was observed between ISSR genetic markers and virulence phenotypes, with all weakly virulent isolates and most moderately virulent isolates clustering within a single genetic clade (Clade II), while highly virulent isolates were distributed across both major genetic clusters (Bouazizi et al., 2025).

The plasmid pEA29 contributes significantly to virulence expression but is not essential for pathogenicity (Bouazizi et al., 2025). The plasmid-deficient isolate Ea13 ranked among the weakest virulent strains (mean lesion diameter 7.9±1.8 mm), while plasmid-deficient isolate Ea28 exhibited high virulence (19.9±1.7 mm), indicating that chromosomal virulence factors can compensate for pEA29 absence (Bouazizi et al., 2025; Khan et al., 2012).

3.2. Genetic Diversity and Population Structure

Comprehensive investigation of *E. amylovora* population genetic structure using Internal Short Sequencing Repeat (ISSR) molecular markers represents the first such investigation in North Africa. Analysis of 59 Tunisian isolates along with 8 reference strains from Morocco and Algeria using 40 polymorphic ISSR loci generated comprehensive population genetic data (Bouazizi et al., 2025).

UPGMA cluster analysis assigned all 67 isolates into two major genetic clades (defined at 45% similarity threshold), further subdivided into 12 sub-clades at ≥69% similarity. Notably, genetic clustering exhibited no correlation with the geographic origin of isolates, as strains from diverse Tunisian regions, Algeria, and Morocco intermixed within both major clades. This geographic admixture suggests either frequent gene flow among regional populations or multiple independent introduction events from geographically diverse sources into North Africa (Bouazizi et al., 2025).

Bayesian STRUCTURE analysis confirmed the UPGMA-identified two-cluster population structure, with assignment of K=2 as optimal cluster number. Analysis of molecular variance (AMOVA) identified significant genetic differentiation between the two subpopulations, with 85% of total genetic variance partitioned among subpopulations and only 15% within them ($p<0.001$), indicating that the two subpopulations represent genetically distinct entities rather than components of a single clonal population (Bouazizi et al., 2025).

Mean Shannon Index across populations was 0.384, with unbiased gene diversity of 0.254, indicating moderate genetic heterogeneity within the Tunisian *E. amylovora* population. Comparison of genetic diversity metrics between Tunisian isolates and those from neighboring countries revealed that Algerian and Moroccan strains exhibited genetic similarity to particular Tunisian isolates rather than forming distinct geographic clusters (Bouazizi et al., 2025).

4. Cultivar Susceptibility and Host Defense Mechanisms

4.1. Cultivar Susceptibility Assessment

Comprehensive evaluation of pear and apple cultivars widely grown in Tunisia revealed significant variability in disease response patterns (Figure 3) (Bouazizi et al., 2020). Assessment of 11 cultivars (eight pear and three apple) identified two cultivars as least susceptible (LS), six as moderately susceptible (MS), and three as susceptible (S) (Bouazizi et al., 2020).

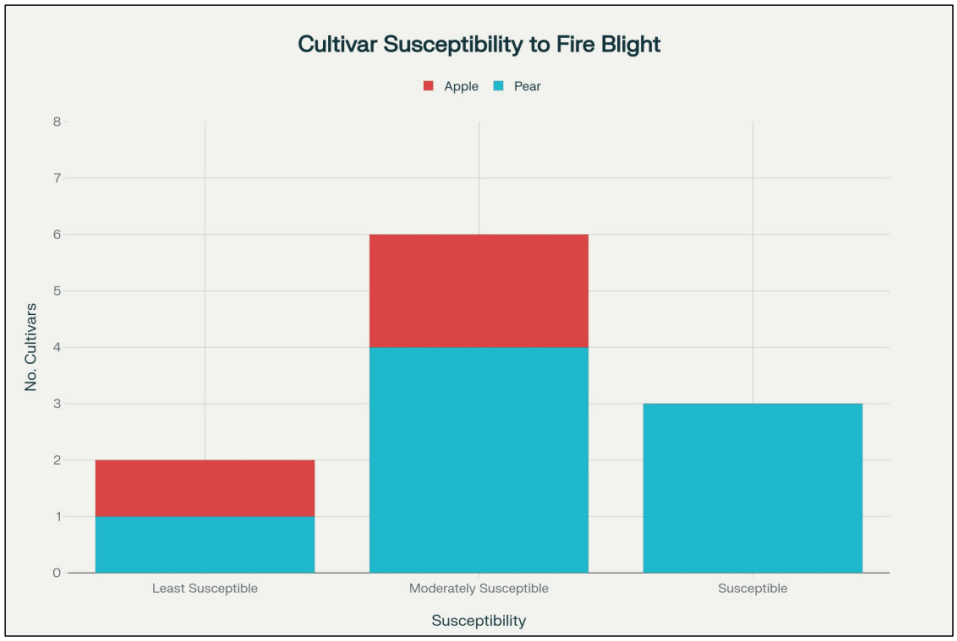

Figure 3. Distribution of tested cultivars based on their susceptibility level to *E. amylovora* infection

Among pear cultivars, the local variety 'Ambri' emerged as the only least susceptible genotype, exhibiting the lowest disease severity scores throughout the observation period and zero percent plant mortality. Foreign cultivars 'Alexandrine,' 'Meski Ahrech,' and 'Bohème' were classified as susceptible, with 44.44-55.55% plant mortality by experiment conclusion. Moderately susceptible pear cultivars included 'Williams,' 'Bouguedma,' 'AIDA1,' and 'PACA1,' exhibiting intermediate disease responses (Table 1) (Bouazizi et al., 2020).

Apple cultivars demonstrated generally better resistance than pear cultivars. 'Lurka' and 'Anna' were classified as least susceptible and moderately susceptible, respectively, while 'Meski' was moderately susceptible (Gaaliche et al., 2018). The identification of local pear cultivar 'Ambri' as displaying substantial resistance to *E. amylovora* represents a significant discovery with profound implications for breeding programs aimed at developing durable resistance (Peil et al., 2021; Emeriewen et al., 2019).

Table 1. Susceptibility evaluation of olive varieties to Xylella fastidiosa. Values represent symptom development percentages at different days post-infection (dpi). FMS: Final Mean Score; PDP: Percentage of Dead Plants; Classification: R = Resistant, MS = Moderately Susceptible, S = Susceptible

Group	Variety	7 dpi	14 dpi	21 dpi	28 dpi	FMS (%)	PDP (%)	Classification
Apple	Lurka	00.00	18.36	27.08	33.05	19.64	00.00	R
	Meski	00.00	50.66	61.00	64.76	45.83	11.11	MS
Pear	Anna	00.38	47.72	53.79	61.36	40.81	11.11	MS
	Williams	14.69	48.87	63.27	66.10	48.23	22.22	MS
	Alexandrine	16.58	51.58	64.78	71.28	60.68	44.44	S
	Bouguedma	05.71	24.42	40.70	57.36	32.04	00.00	MS
	Meski Ahrech	23.00	62.34	66.97	66.37	54.23	55.55	S
	Ambri	08.64	16.45	21.72	28.52	19.14	00.00	S
	AIDA1	13.58	16.58	36.93	54.88	30.49	11.11	MS
	PACA1	11.25	43.85	62.36	70.12	46.89	11.11	MS
	Bohème	17.25	42.23	66.25	74.78	50.10	33.33	S

Field observations documented differential susceptibility patterns among cultivars (Gaaliche et al., 2018). Foreign pear cultivars including Santa Maria, Alexandrine, Douillard, Dr. Jules Guyot, and Williams consistently exhibited severe disease symptoms and high infection rates (Gaaliche et al., 2018). Local pear varieties including Radsi, Ambri, Fayeli, and Meski Bouguedma showed appreciable tolerance and reduced disease severity (Gaaliche et al., 2018).

4.2. Host Defense Mechanisms and Molecular Basis of Resistance

Investigation of physiological and molecular mechanisms underlying differential susceptibility to *E. amylovora* in contrasting pear cultivars 'Ambri' (least susceptible) and 'Alexandrine' (susceptible) has elucidated multiple defense pathways contributing to resistance expression (Figure 4) (Bouazizi et al., 2020).

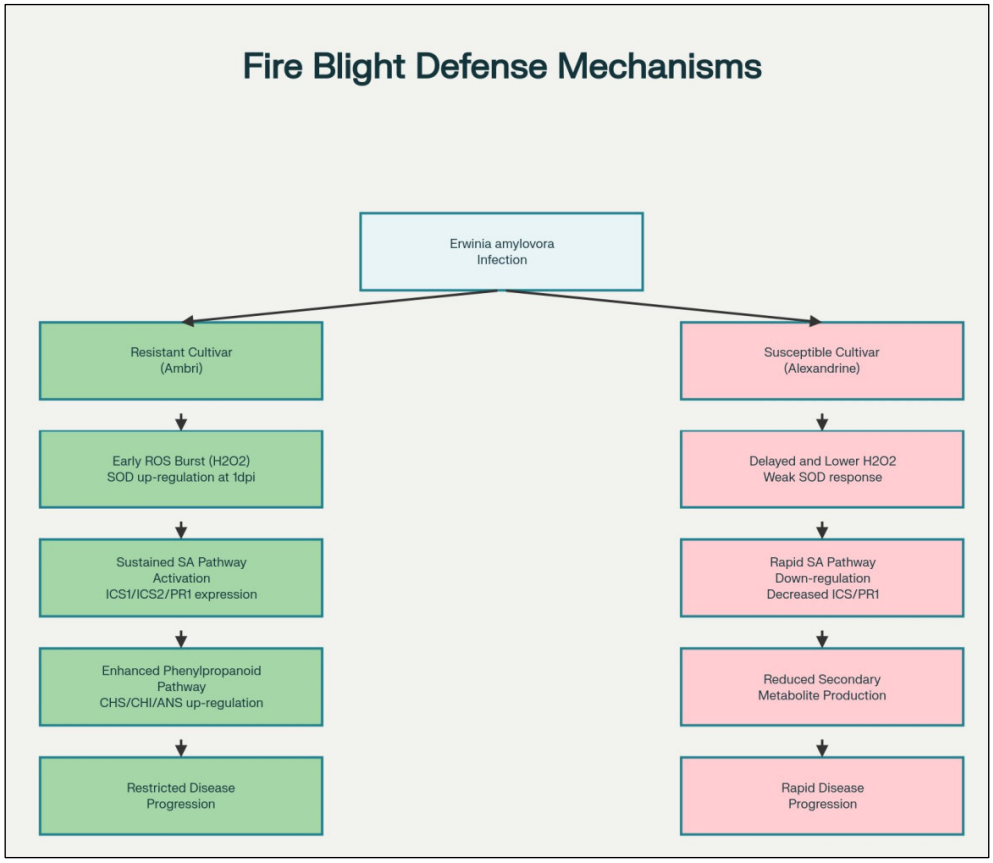

Figure 4. differential plant defense responses between resistant 'Ambri' and susceptible 'Alexandrine' cultivars following *E. amylovora* infection,

4.2.1. Reactive Oxygen Species and Antioxidant Defense

Monitoring of hydrogen peroxide (H_2O_2) accumulation in leaf tissues following *E. amylovora* infection revealed differential temporal dynamics between resistant and susceptible cultivars. The least susceptible cultivar 'Ambri' exhibited a rapid H_2O_2 burst beginning at 1 day post-infection (dpi), achieving peak concentration of 6.89 mmol/g fresh weight at 2 dpi, and maintaining elevated H_2O_2 levels through 4 dpi (Bouazizi et al., 2020). In contrast, the susceptible cultivar 'Alexandrine' achieved lower peak H_2O_2 concentration (5.36 mmol/g FW) delayed until 2 dpi, with rapid decline thereafter (Bouazizi et al., 2020).

Reactive oxygen species (ROS) play critical dual roles in plant-pathogen interactions, serving both as antimicrobial agents and as signaling molecules activating defense responses (Venisse et al., 2001; Mhamdi & Van Breusegem, 2018). Evidence suggests that *E. amylovora* can induce oxidative stress even in compatible interactions through its functional hrp (hypersensitive reaction and pathogenicity) cluster, using ROS production as a tool to provoke host cell death during pathogenesis (Venisse et al., 2001; Degrave et al., 2008).

Gene expression analysis revealed coordinated regulation of antioxidant enzymes contributing to differential H_2O_2 dynamics (Bouazizi et al., 2020). Superoxide dismutase (SOD), the primary enzyme converting superoxide radicals to H_2O_2, was significantly up-regulated at 1 dpi in resistant 'Ambri', maintaining elevated expression through 4 dpi. Susceptible 'Alexandrine' exhibited lower SOD up-regulation magnitude (Bouazizi et al., 2020). Catalase (CAT), the enzyme responsible for H_2O_2 decomposition, displayed complex regulation: early up-regulation at 1-2 dpi in both cultivars, followed by maintenance in 'Ambri' through 8 dpi but dramatic decline in 'Alexandrine'. Ascorbate peroxidase (APX), another H_2O_2-metabolizing enzyme, was up-regulated at 1 dpi in both cultivars, with sustained higher expression in 'Ambri' compared to 'Alexandrine' (Bouazizi et al., 2020).

The coordinated regulation of SOD (H_2O_2-generating), CAT, and APX (H_2O_2-consuming) activities suggests that resistant 'Ambri' maintains optimal H_2O_2 homeostasis, balancing ROS antimicrobial activity with cell protection from oxidative damage (Mhamdi & Van Breusegem, 2018 ; Bouazizi et al., 2020).

4.2.2. Salicylic Acid Pathway Activation

The salicylic acid (SA) signaling pathway showed differential activation between resistant and susceptible cultivars. The gene encoding phenylalanine ammonia lyase (PAL), catalyzing the first committed step of the phenylpropanoid pathway leading to SA synthesis, was up-regulated at 1 dpi in both cultivars, with more dramatic down-regulation in susceptible 'Alexandrine' compared to resistant 'Ambri' (Bouazizi et al., 2020).

Genes encoding isochorismate synthase isoforms (ICS1 and ICS2), catalyzing an alternative SA biosynthetic pathway, exhibited rapid up-regulation through 2 dpi in both cultivars, with significantly higher induction magnitude in resistant 'Ambri' (Bouazizi et al., 2020; Shine et al., 2016). Notably, pre-inoculation expression levels of ICS1 and ICS2 were significantly higher in 'Ambri' compared to 'Alexandrine', suggesting that baseline SA pathway priming contributes to resistance expression (Bouazizi et al., 2020).

The gene encoding pathogenesis-related protein 1 (PR1), a canonical marker of SA-pathway activation and systemic acquired resistance (SAR), was significantly up-regulated in both cultivars following infection, with up-regulation sustained through 4 dpi before declining (Van Loon et al., 2006 ; Bouazizi et al., 2020). Higher baseline and infection-induced PR1 expression in 'Ambri' suggested stronger SA-pathway engagement in the resistant cultivar (Bouazizi et al., 2020). Salicylic acid plays a central role in establishing SAR, a state of heightened defense throughout the plant following local infection, providing long-lasting, broad-spectrum resistance to subsequent pathogen challenge (Vlot et al., 2009; Fu & Dong, 2013).

4.2.3. Phenylpropanoid Pathway and Secondary Metabolite Accumulation

The phenylpropanoid (PP) pathway, producing diverse secondary metabolites with antimicrobial properties, showed differential regulation between cultivars (Bouazizi et al., 2020). Genes encoding key PP pathway enzymes such as, chalcone synthase (CHS), chalcone isomerase (CHI), anthocyanidin synthase (ANS), and flavanone 3-hydroxylase (FHT) were early up-regulated following infection in both cultivars, beginning at 1 dpi, with up-regulation more pronounced in resistant 'Ambri' (Bouazizi et al., 2020).

From 4 dpi onward, PP pathway genes exhibited significant down-regulation in both cultivars, with substantially greater repression magnitude in susceptible 'Alexandrine' (Bouazizi et al., 2020). This pathogen-induced suppression in susceptible cultivars may facilitate nutrient accumulation and enhanced cell-wall permeability supporting pathogen growth (Bouazizi et al., 2020). In contrast, maintained higher expression of PP genes in 'Ambri' promotes continued synthesis of antimicrobial secondary metabolites including flavonoids and phenolic compounds that exhibit significant antibacterial activity (Cushnie & Lamb, 2005 ; Vogt, 2010 ; Zaynab et al., 2018 ; Bouazizi et al., 2020).

5. Economic Impact and Disease Epidemiology

5.1. Geographic Spread and Regional Impact

Comprehensive four-year surveys (2012-2016) documented fire blight disease distribution across northern Tunisia's primary pear production regions (Gaaliche et al., 2018). Initial disease symptoms first appeared in May 2012 in the region of Mornag (Ben Arous governorate) affecting both 'Alexandrine' and 'Williams' cultivars (Gaaliche et al., 2018). By May 2013, the pathogen had spread to encompass pear orchards in multiple governorates, with documented disease incidence of 76% in Beja, 73% in Manouba, 62% in Ben Arous, 20% in Zaghouan, 14% in Ariana, 2.5% in Bizerte, and 1.5% in Nabeul (Figure 5) (Gaaliche et al., 2018).

By late 2013, fire blight had ravaged between 5,000 and 6,000 hectares among the total 8,400 hectares of cultivated pear plantations (70-80% of cultivated area). By 2016, cumulative damage had reached destruction of more than 5,500 hectares (Gaaliche et al., 2018).

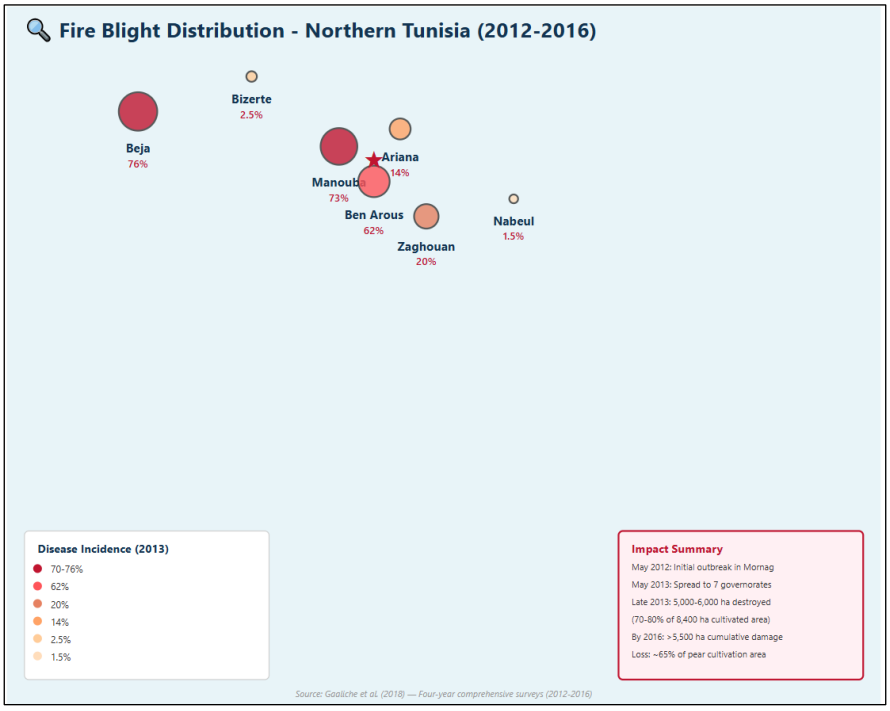

Figure 5. Comprehensive surveys documenting *E. amylovora* spread across pear production regions

5.2. Production Losses and Economic Consequences

Quantitative documentation of production losses reveals the catastrophic economic impact of fire blight. Annual average pear production declined dramatically from 60,000 metric tons in 2011 to less than 20,000 metric tons in 2016, representing a 66% reduction in national output within five years of disease emergence (Figure 6). Total cultivated area contracted from 8,400 hectares in 2011 to 3,260 hectares by 2016 (Figure 6) (Gaaliche et al., 2018). The decline in per-hectare productivity proved equally severe: average yields dropped from 7.7 tons/hectare in 2010 to 4.3 tons/hectare post-epidemic emergence (Gaaliche et al., 2018).

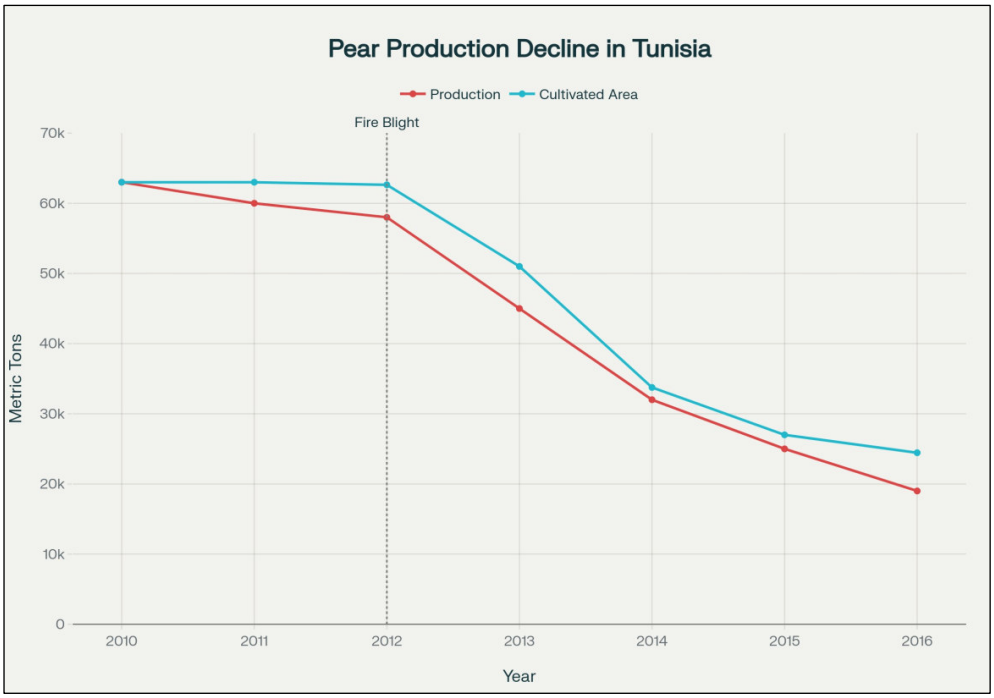

Figure 6. Pear production and cultivated area decline in Tunisia (2010-2016) following fire blight (*E. amylovora*) epidemic (Bouazizi et al. 2020).

6. Bacteriophage Isolation and Biological Control Potential

6.1. Phage Discovery and Characterization

Research into bacteriophage-based biological control represents an innovative contribution emerging from Tunisian fire blight investigations. Initial surveys conducted on Tunisian soil and infected pear tree tissues recovered bacteriophages capable of lysing *E. amylovora*. A total of nine phages were isolated from environmental and plant samples, with four phages (designated pHEa1, pHEa2, pHEa3, and pHEa4) demonstrating clear lytic activity against tested *E. amylovora* strains.

Morphological analysis using electron microscopy classified pHEa1 and pHEa2 as Myovirus-like morphotypes (characteristic contractile tails), while pHEa3 and pHEa4 were identified as Podovirus-like (characteristic short, non-contractile tails). Genetic analysis revealed that these phages possess distinct genomic profiles, suggesting that lytic capability against *E. amylovora* has evolved through multiple independent bacteriophage lineages.
Bacteriophages have been recognized as promising biological control agents for management of *E. amylovora*, representing alternatives to antibiotics that avoid development of resistance and harmful effects on beneficial microorganisms (Born et al., 2017; Roach et al., 2015). Engineering of bacteriophages through recombinant DNA technology can enhance biocontrol efficacy, as demonstrated by combining desired properties of different phages in single recombinant phages with broad host range and depolymerase activity for capsule degradation (Born et al., 2017).

6.2. Phage Host Range and Specificity

Host range testing of the four lytic Tunisian phages revealed that each phage demonstrated lytic activity against multiple Tunisian *E. amylovora* isolates, though with variable efficiency. Phage host range appeared broader when tested against strains from the same geographic region of origin compared to geographically distant isolates, suggesting potential co-evolution of phages and bacterial populations. Importantly, lytic specificity to *E. amylovora* suggests that phage-based treatments would not affect beneficial microflora in orchard environments (Gayder et al., 2019).

Recent advances in phage-based biocontrol have demonstrated effectiveness in European settings, with preventive application of bacteriophage cocktails delaying onset of fire blight symptoms and reducing disease severity in plant material (Born et al., 2017). Optimization of formulation with adjuvants enhances UV stability and adsorption on plant surfaces, critical factors for field efficacy under environmental stress (Born et al., 2017).

7. Integrated Disease Management Perspectives

7.1. Current Management Challenges

Field experience demonstrated that mineral oil and copper-based fungicides cannot adequately suppress disease when environmental conditions strongly favor pathogen multiplication and spread (Gaaliche et al., 2018; Sundin et al., 2016). Sanitary measures alone cannot contain systemic bacterial infections spreading through vascular tissues (Gaaliche et al., 2018). Conventional antibiotic sprays raise concerns regarding antibiotic resistance development (Jeridi, 2022; Manulis et al., 1998; Russo et al., 2008).

Copper-based control agents (CBCAs) including copper oxychloride, copper sulfate basic, and tribasic copper sulfate have been analyzed for disease severity reduction efficacy against *E. amylovora*, though their effectiveness is limited and *E. amylovora* can enter a viable-but-nonculturable (VBNC) state in the presence of copper as a survival strategy (Ordax et al., 2006). Development of glycine-copper(II) hydroxide nanoparticles with improved biosafety represents a potential advancement for sustainable plant disease management (Sundin et al., 2016).

7.2. Breeding for Resistance

The identification of local pear cultivar ‘Ambri’ as displaying substantially enhanced resistance to *E. amylovora* provides a foundation for rational breeding program development (Gaaliche et al., 2018). The mechanistic research documenting enhanced antioxidant enzyme expression, elevated salicylic acid pathway activation, and maintained phenylpropanoid pathway gene expression in resistant ‘Ambri’ suggests specific traits to target in breeding programs (Bouazizi et al., 2020; Peil et al., 2021).

Direct selection of Ambri germplasm for orchard redevelopment represents one approach, potentially grafting resistant Ambri onto rootstocks of commercially-preferred cultivars (Gaaliche et al., 2018). Crossing programs between resistant local cultivars (Ambri, Radsi, Fayeli, Meski Bouguedma) and widely-cultivated foreign cultivars offer another avenue to pyramid resistance alleles into commercially-acceptable genetic backgrounds (Gaaliche et al., 2018).

Fire blight resistance in *Malus* species is controlled by quantitative trait loci (QTLs), with major QTLs identified on various linkage groups including FB_Mfu10 on linkage group 10 from *Malus fusca* and resistance loci on linkage group 12 from *Malus × arnoldiana* (Emeriewen et al., 2018; Peil et al., 2021). Candidate genes with serine/threonine kinase and leucine-rich repeat domains have been identified, providing targets for marker-assisted breeding and biotechnological approaches to develop cultivars with decreased fire blight susceptibility (Peil et al., 2021; Emeriewen et al., 2019).

Root system traits also impact fire blight susceptibility, with resistant rootstocks facilitating disease management through systemic defense mechanisms that activate carbohydrate metabolic pathways reciprocally interacting with plant immune system genes (Emeriewen et al., 2018).

7.3. Biological Control Development

The isolation and characterization of *E. amylovora*-specific bacteriophages from Tunisian sources represents a novel biological control approach warranting further development (Jeridi, 2022; Born et al., 2017). In planta efficacy testing under controlled greenhouse conditions and field trials must be conducted to establish phage-mediated disease suppression (Jeridi, 2022). Optimization of application protocols including timing relative to pathogen infection windows, inoculum concentrations, and delivery methods represents an essential research priority (Jeridi, 2022; Born et al., 2017).

Additional biological control strategies include exploitation of flower-inhabiting bacterial commensals such as *Pseudomonas* and *Pantoea* species that naturally colonize apple and pear blossoms, with co-inoculation of multiple beneficial strains reducing disease incidence through inter-species interactions that suppress pathogen activity (Johnson & Stockwell, 1998).

7.4. Germplasm Conservation and Utilization

Documentation and preservation of local pear germplasm exhibiting resistance traits should be undertaken to protect these valuable genetic resources (Bouazizi et al., 2020; Volk et al., 2020). Comprehensive genetic characterization of germplasm collections provides relevant information to optimize conservation strategies, support use in breeding programs, and increase knowledge of *Pyrus* taxonomy, evolution, and domestication (Volk et al., 2020; Höfer et al., 2023). Cryopreservation techniques offer methods for establishing duplicate collections to preserve material safely from biotic and abiotic stress factors (Höfer et al., 2023).

7.5. Integrated Approach Framework

Sustainable fire blight management in Tunisia requires coordinated integration of multiple complementary tactics (Gaaliche et al., 2018; Johnson & Stockwell, 1998). Immediate priority measures should include quarantine enforcement to prevent spread to disease-free regions (Gaaliche et al., 2018). Deployment of identified resistant cultivars in replanting programs represents an urgent priority (Gaaliche et al., 2018). Documentation and preservation of local pear germplasm exhibiting resistance traits should be undertaken to protect these valuable genetic resources (Bouazizi et al., 2020).

Medium-term research initiatives should focus on acceleration of breeding programs to develop cultivars combining disease resistance with commercial fruit quality (Gaaliche et al., 2018; Peil et al., 2021). Field evaluation of bacteriophage-based biological controls must be prioritized (Jeridi, 2022; Born et al., 2017). Investigation of plant defense activators and other non-chemical disease suppression tactics warrants exploration (Jeridi, 2022; Vlot et al., 2009).

Long-term strategic initiatives must encompass establishment of fire blight-resistant pear orchards as foundation for industry recovery (Gaaliche et al., 2018). Development of national fire blight management strategy informed by epidemiological research and forecasting systems is essential for coordinated response (Gaaliche et al., 2018; Steiner, 1990). International collaboration for knowledge exchange and technology transfer with other Mediterranean countries will accelerate progress (Rhouma et al., 2014; Vanneste, 2017).

8. Conclusion

Research conducted by Tunisian scientists in response to the fire blight epidemic has generated significant contributions advancing national disease management capacity and global understanding of fire blight pathology (Rhouma et al., 2014; Bouazizi et al., 2025). Establishment of pathogen identity through comprehensive microbiological and molecular characterization formally documented fire blight's emergence in Tunisia (Rhouma et al., 2014). Documentation of epidemiological patterns, including geographic spread, cultivar susceptibility, and production impacts provided essential baseline data (Gaaliche et al., 2018).

Characterization of pathogen genetic diversity using ISSR methodology revealed evidence of multiple independent *E. amylovora* introduction events and identified two genetically distinct subpopulations (Bouazizi

et al., 2025). Investigation of virulence variation demonstrated that Tunisian *E. amylovora* populations comprise isolates with substantially different aggressive capacity (Bouazizi et al., 2025). Elucidation of host defense mechanisms at molecular level identified specific genes and pathways contributing to differential susceptibility and provided insights guiding rational breeding program development (Bouazizi et al., 2020; Peil et al., 2021).

Discovery of bacteriophages with lytic activity against *E. amylovora* opened novel biological control research directions (Jeridi, 2022; Born et al., 2017). Investigation of virulence regulation revealed how host nutritional status modulates pathogen virulence gene expression through apoplastic metabolite signaling (Jeridi, 2022). These significant accomplishments represent meaningful progress toward understanding fire blight under Mediterranean conditions and provide foundation for sustainable management strategies (Rhouma et al., 2014).

Future research priorities include: complete genome sequencing and comparative genomics of Tunisian isolates (Bouazizi et al., 2025; Puławska and Sobiczewski, 2012); detailed functional analysis of virulence factors including type III effectors and their regulation (Jeridi, 2022; McNally et al., 2012; Zhao et al., 2009); investigation of bacteriophage-mediated disease suppression through in planta and field efficacy testing (Jeridi 2022; Born et al. 2017); comparative effectiveness studies of integrated disease management approaches combining resistant cultivars, biological control, forecasting-based interventions, and cultural practices (Gaaliche et al., 2018; Johnson & Stockwell, 1998); and establishment of fire blight research coordination among Tunisian research institutions to eliminate redundancy and facilitate integrated investigation (Bouazizi et al., 2020).

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