Fire Blight: Applied Genomic Insights of the Pathogen and Host

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Abstract

The enterobacterial phytopathogen *Erwinia amylovora* causes fire blight, an invasive disease that threatens a wide range of commercial and ornamental Rosaceae host plants. The response elicited by *E. amylovora* in its host during disease development is similar to the hypersensitive reaction that typically leads to resistance in an incompatible host-pathogen interaction, yet no gene-for-gene resistance has been described for this host-pathogen system. Comparative genomic analysis has found an unprecedented degree of genetic uniformity among strains of *E. amylovora*, suggesting that the pathogen has undergone a recent genetic bottleneck. The genome of apple, an important host of *E. amylovora*, has been sequenced, creating new opportunities for the study of interactions between host and pathogen during fire blight development and for the identification of resistance genes. This review includes recent advances in the genomics of both host and pathogen.

FIRE BLIGHT

Fire blight is a devastating necrogenic disease and the most important bacterial disease of apple, pear, and other plant members of the Rosaceae family, including important ornamental species. It was first reported in New York in the late 1700s on apple, pear, and quince, which had been introduced into North America during European settlement. In the late nineteenth and early twentieth centuries, pioneering research on fire blight by T.J. Burrill, J.C. Arthur, and M.B. Waite established that bacteria could cause disease on plants and insects could vector its spread (36). The current name of the pathogen, *Erwinia amylovora* (Burr.) Winslow et al., was established in 1920.

There are several distinct phases of the disease, including blossom blight, shoot blight, and rootstock blight (77, 118). *E. amylovora* cells overwinter in cankers, from which the bacterium emerges as ooze following warming temperatures in spring. Ooze consists of bacterial cells embedded in a polysaccharide matrix; this viscous substance protects cells from desiccation and other abiotic stress factors and is attractive to insects, such as flies, that can disseminate bacterial cells to flowers. The stigma surface of flowers acts as the major site of pathogen multiplication (**Figure 1*a*,*b***), with stigma exudates supporting pathogen growth to densities as large as 10^5–10^6 cells per flower (112). Growth of *E. amylovora* on stigmas is heavily dependent upon temperature, with maximal growth rates occurring between 21°C and 27°C. Moisture from rainfall or heavy dew enables bacterial cells to migrate downward along the outer surface of stigmas where infection occurs through the floral nectaries, resulting in blossom blight symptoms. Dissemination of bacteria among flowers can occur via wind and rain, but is most likely influenced by the action of pollinating insects, such as bees. Flower age also plays a role in infection, as the incidence of infection is usually higher in younger (one to three days old) rather than in older (five to eight days old) flowers (91).

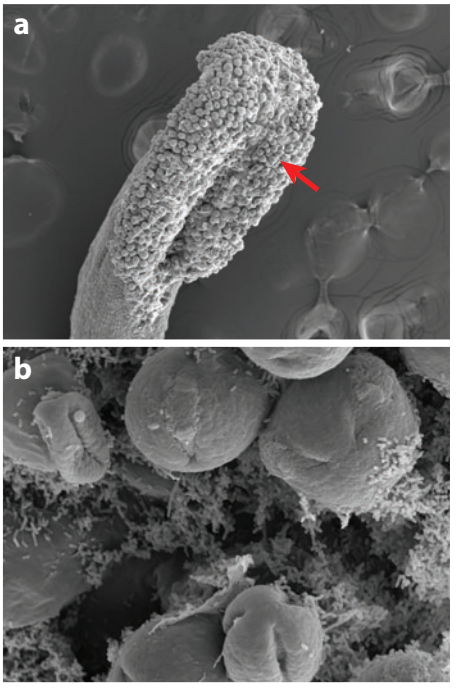

Figure 1

Scanning electron micrographs of (*a*) an apple flower stigma and (*b*) a close-up view of *Erwinia amylovora* cells growing in between papillae cells of stigma. The red arrow points to papillae cells in the region examined in closer detail.

After infection, *E. amylovora* systemically migrates through host tissues and can emerge as ooze, which provides inoculum for secondary cycles of blossom or shoot infection. Shoot infection occurs most readily on actively growing shoots, likely through microscopic wounds caused by a number of biotic or abiotic factors (118). *E. amylovora* cells can be spread via wind, rain, and insects, resulting in multiple infection cycles. Hail damage is a major facilitator of fire blight epidemics, leading to significant levels of shoot blight incidence. Rootstock crowns can also be infected by *E. amylovora* cells migrating internally downward from the scion or directly infecting rootstock suckers or wounds (73, 77).

Cultural control, including pruning and removal of infected tissues, is an important aspect of inoculum reduction (22). Populations

of *E. amylovora* on floral surfaces can be targeted for chemical control. The antibiotics streptomycin and oxytetracycline are the most efficacious tools available for blossom blight control (68). However, streptomycin resistance in *E. amylovora* occurs in several regions of the United States and in Canada, Israel, Lebanon, and New Zealand (68, 97). Other potential control materials for blossom blight that have been commercially utilized include several species of antagonistic bacteria and the agricultural antibiotic kasugamycin (12, 42, 67, 85, 118). The most effective control material for shoot blight is prohexadione-calcium, a compound that reduces apple shoot elongation (124).

Commercial apple cultivars vary in susceptibility to fire blight infection, and fire blight management is best accomplished on cultivars with decreased susceptibility. Resistance to fire blight in *Malus* (apple) ranges from highly susceptible to highly resistant. Pear is generally more susceptible to fire blight than apple. Several hundred apple and pear scion cultivars, apple rootstocks, and wild *Malus* and *Pyrus* species accessions have been evaluated for their resistance against fire blight by controlled inoculation of plant material (117).

CURRENT UNDERSTANDING OF FIRE BLIGHT HOST-PATHOGEN INTERACTIONS

During the past 30 years, extensive genetic studies have been performed to identify and characterize the genes involved in the ability of *E. amylovora* to cause fire blight (reviewed in 78). These studies determined that a protein secretion/translocation pathway, called the *hrp* type III secretion system (*hrp*-T3SS); the type 3 effector (T3E) DspA/E; and the exopolysaccharide amylovoran (*ams*) were each required for *E. amylovora* pathogenicity (78). This review mainly focuses on *hrp*-T3SS data obtained since 2005.

Both *ams* and *hrp*-T3SS mutants are unable to induce fire blight disease. Interestingly, an *hrp*-T3SS mutant and an *ams* mutant could complement each other, and co-inoculation of both mutants restores pathogenicity (126). Although an *hrp*-T3SS mutant is unable to elicit a hypersensitive response in tobacco or a local necrotic reaction when infiltrated in apple leaf mesophyll, an *ams* mutant is still able to elicit both reactions (71). This indicates that the *hrp*-T3SS mutant is altered in a basic attack mechanism that is required at the beginning of the infection process, whereas the *ams* mutant is blocked later on, as amylovoran is required for the bacterial progression in planta. It was further demonstrated that amylovoran is required for biofilm formation, which could explain why it is required for bacterial progression (49).

The first T3Es identified in *E. amylovora* were harpins (HrpN and HrpW) and DspA/E, and their characterization is still an important challenge to understanding *E. amylovora* pathogenicity. DspA/E is central to the disease process, as *dspA/E* mutants do not grow on host plants and cannot induce disease symptoms. DspA/E is required to block callose deposition, a plant basal defense response that strengthens the plant cell wall at the site of infection (18), and to repress PR1 expression (13). Because these plant defenses are described as salicylic acid (SA)-dependent, this suggests that DspA/E contributes to disease development by inhibiting SA-dependent innate immunity. Ectopic expression of *dspA/E* induces necrosis in apple, tobacco, and *Nicotiana benthamiana*, whereas silencing of *NbSGT1*, the expression of which is required for programmed cell death mediated by several resistance proteins of plants, prevents DspA/E-induced necrosis in *N. benthamiana* (13, 81). Yeast two-hybrid assays performed with the N-terminal half of DspA/E as bait identified four serine/threonine receptor kinases of apple that were designated as DspE-interacting proteins from *Malus* (DIPM) (70). When the C-terminal half of DspA/E was used as bait, the only interactor identified was a cytoplasmic precursor of ferredoxin (78).

DspA/E secretion depends on a specific chaperone called DspB/F, which prevents intrabacterial DspA/E degradation (35, 80). Two independent groups identified DspA/E

secretion and translocation signals in the N-terminal part of the DspA/E protein and DspB/F-binding sites within amino acids 51 through 100 of DspA/E (80, 114). A binding site at this location is classically detected for almost all reported T3SS chaperones with their cognate T3Es (84, 115). Structural modeling of DspB/F and identification of candidate DspB/F residues involved in virulence and DspA/E interaction confirm that DspB/F functions as a classical T3SS chaperone (115). Neither disruption of the N-terminal DspA/E chaperone-binding domain nor deletion of the *dsp*B/F gene had a significant impact on translocation levels of N-terminal DspA/E-CyaA fusions (114), but the presence of DspB/F strongly enhances the translocation of a DspA/E-AvrRpt2 fusion protein into plant cells (80). These contradictory results indicate that the requirement for the DspB/F chaperone is influenced by the C-terminal part of the protein. In accordance with this, it was found that DspB/F also interacts with the C-terminal half of DspA/E (80).

HrpN and HrpW effectors belong to the harpin family, and their effects on *E. amylovora* pathogenesis have been characterized (78). HrpN was localized in the apoplast in planta during the infection process (89), suggesting that HrpN is not injected into the plant cytoplasm by the T3SS but acts as an accessory protein of the T3SS. Recent data challenging this view identified functional N-terminal type three translocation signals in HrpN and HrpW, suggesting that harpins could also be injected inside the plant cell (6, 14). Furthermore, HrpN was shown to play a critical role in the translocation process into the host plant cell because translocation of DspA/E::CyaA, HrpN::CyaA, and HrpW::CyaA fusion proteins was drastically reduced in a *hrp*N mutant background (6, 14). HrpN was also found to form an ion-conducting pore in the artificial lipid bilayer (24). These data suggest that HrpN localizes in the plant plasma membrane as part of the translocon apparatus.

Purified HrpN triggers opposite ion flux responses in nonhost *Arabidopsis thaliana* cells and host apple cells, underscoring the fact that HrpN is perceived differently by host and nonhost plants (93). Most importantly, HrpN does not trigger cell death on apple leaves or cells (62, 93). Compared with the wild-type strain, an *hrp*N mutant provokes a reduced oxidative burst in apple, suggesting that HrpN contributes to the generation of oxidative stress in host plants (120). However, purified HrpN fails to induce oxidative stress in apple leaves or cells, which suggests that oxidative stress induction is indirect and depends on HrpN-dependent translocation of other T3Es (14).

Analysis of the secretome of *E. amylovora* allowed the identification of several new proteins whose secretion in vitro is dependent on a functional T3SS (74). Among these, HrpK and HrpJ may participate together with HrpN to form the translocon apparatus. Several of the new proteins identified in the secretome are homologous to already known T3Es identified in other phytopathogenic bacteria and are therefore likely injected into the plant cells during the interaction. Eop1 (formerly called orfB or EopB) has significant homology to the AvrRXv/YopJ effector family. On the basis of this homology, Eop1 is believed to act as a cysteine protease, yet an *eop*1 mutant is not reduced in virulence on apple (78). The Eop1 sequence from *Rubus* strains is quite divergent from Eop1 sequences from *Malus* strains, and it was shown that Eop1 from *Rubus* strains functions as a host-range limiting factor (2). Eop2 shares homology with HopPmaH/HopAK family proteins, and Eop3 is homologous to AvrPphE (HopX). Eop3 (also called HopX1ea) functions as an avirulence gene in apple (8). The individual contribution of these T3Es to *E. amylovora* virulence remains to be determined. Traces of a protein with homology to the AvrRpt2 effector of *Pseudomonas syringae* were also identified by MS/MS (mass spectrometry) spectra analysis in *E. amylovora* supernatant, indicating that this T3E is secreted in very low amounts in vitro (74). AvrRpt2$_{Ea}$ is homologous to AvrRpt2$_{Ps}$, a *P. syringae* cysteine protease whose activity is detected by the *A. thaliana* resistance gene RPS2. *E. amylovora* AvrRPt2$_{Ea}$ is also recognized by RPS2 in *A. thaliana*, indicating that it

performs the same function as AvrRpt2$_{Ps}$ (128). An *avr*RPt2$_{Ea}$ mutant is affected in virulence; therefore, this T3E participates in *E. amylovora* virulence (127). Another T3E, HopPtoC$_{Ea}$, was not detected in the *E. amylovora* secretome but was identified in a general screening for *E. amylovora* genes activated during infection. An insertional mutation within *hopPtoC*$_{Ea}$ did not result in reduced virulence (127).

GENOMICS OF *ERWINIA AMYLOVORA*

The first published genome of *E. amylovora* was that of strain CFBP1430 (106), isolated in France from *Crataegus* in 1972. The genome of this strain consists of the 3.8 Mb circular chromosome and the nearly ubiquitous plasmid pEA29. Soon after, the complete genome of a North American strain isolated from apple, Ea273 (100), and the draft genome of a *Rubus* strain, ATCC BAA-2158, were published (90, 100). Recently, draft sequences of an additional seven isolates from Maloideae and two additional *Rubus* strains were generated (63), and raw assemblies for eight further Maloideae isolates are available (105). Although the sequenced strains are biased toward strains of European origins, which are known to be less diverse than North American strains, the total collection provides a nice overview of the diversity within the species.

From the genome data, two clear groups can be distinguished that correspond to the host range of the strains. One group represents the Maloideae isolates that are very monomorphic, sharing more than 99.99% sequence identity over the complete genome (106). This corresponds to the description of diversity within different populations of *E. amylovora* strains, in which only minor differences were detected within the strains tested (4). Small differences within the near-identical Maloideae genomes have been observed, including different internal transcribed spacer regions (66), an *rhs* gene region (106), VNTR (variable number of tandem repeats) (21), and CRISPR (clustered regularly interspaced short palindromic repeats) repeat regions (CRRs) (96, 107). The CRRs are capable of distinguishing groups of *E. amylovora* isolates; however, the majority of these groupings are limited to strains isolated in North America, and the overall distinguishing power is relatively small (96).

The three sequenced *Rubus* isolates are more divergent from and also more genetically diverse than the Maloideae group (63). Among the *Rubus* isolates, the sequence identity to the Maloideae strains can be as low as 98.5% over the complete genomes, indicating that the genomes of *Rubus* isolates contradict the description of a monomorphic species (63). Whereas the Maloideae strains have only limited differences within the CRR, indicating that they all originate from a single genotype, all sequenced CRRs of *Rubus* strains were completely divergent from each other and from the Maloideae group (96). Additionally, genomic islands, e.g., 20-kb nonribosomal peptide synthase–polyketide synthase clusters, always observed in the CRR of *Rubus* isolates, are absent in Maloideae strains (96). Furthermore, there are large differences in the island transfer (IT) region between each of the *Rubus* strains and the Maloideae strains, all of which have a small, identical IT region (63).

One of the most obvious differences among strains of *E. amylovora* is the presence of different plasmids. Multiple plasmids were previously described (30, 54, 64, 66), and new ones were detected during genome sequencing (90, 100). Currently, this is the largest factor influencing the pan-genome size of *E. amylovora*.

Plasmid pEA29 is near ubiquitous in *E. amylovora*, but strains lacking this plasmid can be observed as well (55, 56). The primary trait associated with pEA29 is thiamine biosynthesis via the plasmid-encoded *thiOSGF* genes, which contribute to pathogen fitness (65). No clear virulence functions have been described for any other *E. amylovora* plasmids (54). Notably, most studies have identified and sequenced the plasmids in a limited number of strains from a limited geographical origin. A global distribution study of these plasmids, as recently reported for pE170 (54), may shed more light on the

evolutionary significance of plasmids in pathogen adaptation to environments and/or hosts.

Comparison of the *E. amylovora* genomes has not provided clear indications of possible factors affecting either host range (except for *Rubus* isolates) or virulence. A useful alternative is to compare the *E. amylovora* genome to various related species within the genus *Erwinia*, which differ from *E. amylovora* in host range or virulence. Currently available genomes from other *Erwinia* spp. include two *Erwinia pyrifoliae* strains, DSM 12163^T and Ep1/96 (50, 103); *Erwinia* sp. Ejp617 (82); *Erwinia tasmaniensis* Et1/99 (51); and *Erwinia billingiae* Eb661 (50) as well as unpublished draft genomes for *Erwinia piriflorinigrans* CFBP 5888^T (104) and *Erwinia persicina* CFBP 3622^T (T.H.M. Smits & B. Duffy, unpublished results).

Plasmids have been detected in all genome-sequenced *Erwinia* spp. (44). All *E. pyrifoliae* strains, *Erwinia* sp. Ejp617, and *E. piriflorinigrans* CFBP 5888 contain a similar-sized plasmid of approximately 30 kb related to pEA29 with the thiamine biosynthesis genes as a common feature (50, 82, 103, 104). However, the organization of the genes indicates that this plasmid has undergone several recombinational events. Multiple small cryptic plasmids are also present in these species and in *E. tasmaniensis* Et1/99 (44, 51, 53, 58, 103, 106). However, the epiphytes *E. billingiae* EB661 and *E. persicina* CFBP3622 contain two large plasmids (50). The relationships and potential functions of these plasmids have been reviewed recently (54). Comparative genomics indicates the *Erwinia* spp. can be divided into a group of patho-adapted species, including *E. amylovora*, *E. pyrifoliae*, *E. tasmaniensis*, and *E. piriflorinigrans*, and an epiphytic group that includes *E. persicina* and *E. billingiae*, which are more closely related to *Pantoea* spp. (44). The patho-adapted group is distinguished by a reduction of chromosome size resulting from a significant amount of gene loss and the acquisition of a large range of pathogenicity and virulence factors (44, 105). The epiphytic group has a broader host range and a wider lifestyle in the environment.

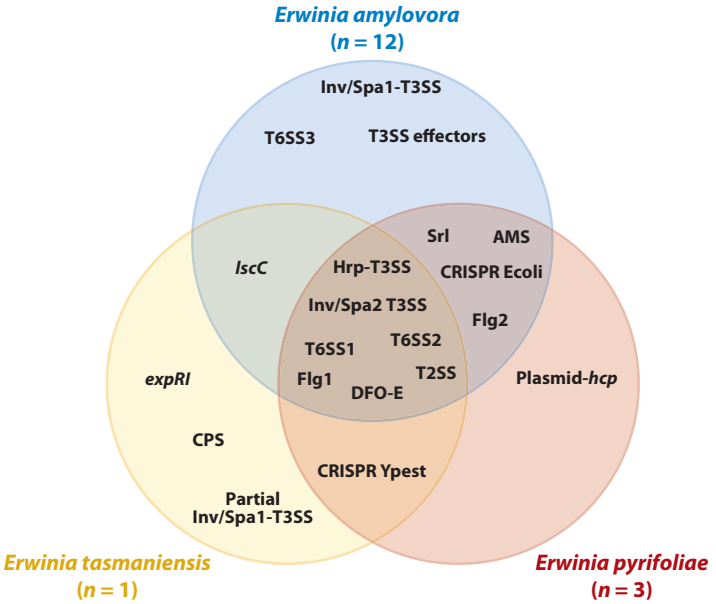

Figure 2

Venn diagram indicating selected features relevant to biology, fitness, and plant-adapted life styles in the three best-studied genome sequences of *Erwinia* species.

Comparative genomics also allowed determination of specific features acquired within the genomes of patho-adapted and epiphytic *Erwinia* spp. since their last common ancestor. A major pathogenicity factor, Hrp T3SS, is shared by all members of the patho-adapted group (**Figure 2**) (106), but minor modifications are present in this region. The genes *orfU1/U2* are only present in *E. amylovora* strains (106), with the *orfU1* gene present in *Rubus* isolates, and *orfU2* and a pseudogene of *orfU1* present in Maloideae isolates (63). *E. tasmaniensis* Et1/99 lacks the HAE region that includes the effector gene *hrpK* (51). Additional T3E located elsewhere in the genome have been identified only in the genome of *E. amylovora* (106). Two additional T3SSs are present in the genome of *E. amylovora* CFBP 1430 (106). The core genome of the four patho-adapted species also includes the Inv/Spa2 T3SS-containing PAI3 and the type II secretion system (44). A second orthologous gene cluster located on PAI2, Inv/Spa1 T3SS,

is completely preserved only in *E. amylovora* and *E. piriflorinigrans*, and partially present in *E. tasmaniensis* (44).

The second major pathogenicity factor of *E. amylovora*, the *ams* gene cluster involved in the biosynthesis of the exopolysaccharide (EPS) amylovoran, is present only in the genomes of *E. amylovora*, *E. pyrifoliae*, *Erwinia* sp. Ejp617, and *E. piriflorinigrans* CFBP 5888. In *E. tasmaniensis* Et1/99, *E. billingiae* Eb661, and *E. persicina* CFBP 3622, the *amsDE* genes are exchanged for two other glycosyl transferases, potentially yielding an EPS that is more closely related to stewartan of *Pantoea stewartii* subsp. *stewartii* (17, 44). In *E. tasmaniensis* Et1/99, the lack of amylovoran biosynthesis was seen by Kube et al. (51) as a determining factor for this strain to be an epiphyte (51). The biosynthetic gene, *lscC*, required for production of the second *E. amylovora* EPS is absent from the *E. pyrifoliae* strains (50, 103) but present in the other patho-adapted species. It has not been found in the epiphytic species.

Quorum sensing mediated by *N*-acyl-homoserine lactone pheromones, referred to as AI-1 autoinduction, is a common strategy utilized by a wide diversity of bacterial species to coordinate expression of genes that are critical for virulence, biocontrol activity, and epiphytic fitness (57). Moreover, AI-1 can have transcriptome-wide regulatory effects (5, 45, 125). The phytopathogen *E. amylovora* does not rely on a quorum sensing system for its virulence (94, 95, 106). Within the *Erwinia* spp., only *E. tasmaniensis* Et1/99 and *E. piriflorinigrans* CFBP 5888 contain an *expRI* quorum sensing system (51, 104, 105). However, this system has not been studied in detail. It is located at the syntenous position in the chromosome, as is the complete second flagellar gene cluster in *E. amylovora* CFBP 1430 and *E. pyrifoliae* DSM 12163 (105).

PATHOGEN TRANSCRIPTOMICS

The availability of complete genome sequences of *E. amylovora* has enabled genome-level transcriptomic studies utilizing an oligonucleotide microarray developed at the James Hutton Institute (Dundee, U.K.) and synthesized by Agilent Technologies (Palo Alto, CA). The microarray consists of 3,483 chromosomal sequences from *E. amylovora* ATCC 49946 and 483 sequences from known *E. amylovora* plasmids (69). An *hrpL* mutant of *E. amylovora* Ea1189 was used to validate the array and revealed that 19 genes exhibited positive direct or indirect regulation by HrpL and five genes were negatively regulated (69). This work also identified novel genes of the HrpL regulon of *E. amylovora*, including EAM_2938, which encodes a putative membrane protein and has a strong virulence phenotype when mutationally interrupted (69). A second study has utilized this same microarray to define the RcsB and RcsC regulons of *E. amylovora* during immature pear infection, identifying 648 differentially regulated genes, including amylovoran biosynthesis genes, cell wall proteins, and cell membrane proteins (123). Of particular interest was the identification that the RcsBCD phosphorelay system regulates the expression of EAM_2938, also regulated by HrpL as described above (123). It is anticipated that microarray studies will facilitate experiments that yield a deeper, more comprehensive understanding of *E. amylovora* pathogenesis, broader identification of virulence gene regulatory circuitry, and increased discovery of new virulence genes.

WHOLE-GENOME SEQUENCE OF *MALUS*

The availability of the apple whole-genome sequence (WGS) (118) has created an excellent opportunity to understand host genes involved in the development of fire blight disease. Both Sanger dye primer sequencing of paired reads (26% of data) and 454 sequencing of both paired and unpaired reads of diploid *Malus* × *domestica* "Golden Delicious" resulted in 16.9-fold genome coverage. The total contig length (603.9 Mb) of the assembled sequence covers all 17 chromosomes and approximately 81.3% of the genome, which is estimated at 742.3 Mb. The apple genome shows evidence of a recent

duplication that may have spawned the additional gene family members needed for the evolution and development of the unique structure of apple and pear fruit, termed the pome (118). Annotation of the genome predicted 57,386 genes, which is the largest number of genes predicted for a plant genome to date. More than 800 genes are described as resistance gene analogs of the nucleotide-binding site–leucine-rich repeat (NBS-LRR) family.

The "Golden Delicious" WGS has been used as a reference for resequencing the genomes of other apple cultivars, whereby short DNA sequence reads (35–100 bp) are assembled to the WGS in order to detect sequence differences between individual cultivars and "Golden Delicious" (89). This has resulted in the identification of more than two million candidate SNPs (single nucleotide polymorphisms) in apple (**http://www.rosaceae.org/node/454**), many of which have been validated by hybridization to SNP arrays (48). Because resequencing data can locate heterozygous SNPs in bi-parental mapping populations that will segregate at a defined genome location in the population, the efficiency of marker development for quantitative trait loci (QTLs) analysis has increased greatly. It has also facilitated precise allelotyping of most apple genes that is useful in the identification of candidate resistance alleles.

GENOMIC INSIGHTS OF HOST PLANTS OF *ERWINIA AMYLOVORA*

To date, genome-wide studies of the interaction between host and pathogen are not completely available. We know that *E. amylovora* induces lipid peroxidation, electrolyte leakage, and modulation in antioxidant status in its susceptible host, an effect that is more characteristic of the oxidative burst generally associated with the hypersensitive reaction in nonhosts than susceptible in a host (121). Adjacent host cells surrounding the infection site are associated with localized acquired resistance that involves cell wall reinforcement (i.e., callose deposition), accumulation of phytoalexins, and activation of three pathogenesis-related (PR) protein genes of apple, PR2, PR5, and PR8 (3, 7, 76, 122). PR proteins can also be synthesized throughout the plant and lead to systemic acquired resistance (SAR), which represents the third line of defense mediated by SA (122). *E. amylovora* has been shown to delay the expression of the phenylpropanoid genes during infection (3, 72, 76, 122).

Transcriptomics

Several studies utilizing the large-scale transcriptomic assays of suppression subtractive hybridization (76), cDNA-AFLP (4), and microarray analysis (7, 9, 40, 41, 99, 111) have been conducted to identify differential gene regulation during interactions of *E. amylovora* and *Malus*. To date, the microarrays used in transcriptomic studies have been designed from *Malus* EST (expressed sequence tag) data. A 40,000-feature *Malus* 70-mer oligonucleotide microarray was developed at the University of Illinois on the basis of the assembly of approximately 184,000 *Malus* ESTs into 33,825 predicted transcripts (34, 47) and was used to identify genes differentially regulated during the early stages of apple flower infection with *E. amylovora* (99). A NimbleGen™ array consisting of 55,230 probes was developed on the basis of *Malus* ESTs from multiple sources and used to study the rootstock-regulated gene expression patterns in apple tree scions that can affect fire blight susceptibility (40, 41). Recently, a complete array of the transcriptome, called AryAne, was developed on the basis of the annotation of the WGS. A new version of this microarray, AryAne2, with a more accurate annotation integrating data obtained from RNA-Seq, will be available by the end of 2012 (J.M. Celton, S. Gaillard, M. Bruneau, S. Pelletier, M.C. Lepaslier, J. Rees, C. Gourdon, E. Costes, J.J. Kellner, M. Orsel, S. Mikol-Segonne, F. Laurens, and J.P. Renou, unpublished results). An alternative approach to microarray analysis also made available by the WGS is the application of next-generation sequencing methods

for transcript profiling, which can interrogate allele-specific expression, determine the structures of RNA splice isoforms, garner mutation information, identify novel transcripts, examine gene fusion events, and better quantify gene expression levels. Analysis of RNA-Seq data allowed the identification of approximately 1,000 genes differentially expressed during the interaction between *E. amylovora* and apple (L. Righetti & M. Malnoy, unpublished results).

In summary, these preliminary transcriptomic analyses have revealed approximately 3,000 genes differentially modulated in susceptible plants in response to *E. amylovora*, similar to previous observations in other host-pathogen interactions (116). In general, the transcripts differentially regulated following *E. amylovora* infection encode enzymes involved in various metabolic pathways, transcription factors, signaling components, defense-related proteins, and transporters, many of which have also been associated with disease responses in *Arabidopsis* and rice, which suggests that similar response pathways are involved in apple. A number of the transcripts regulated by *E. amylovora* have also been associated with drought and other abiotic stresses.

Identification of Candidate Resistance Genes

In general, resistance to *E. amylovora* is quantitative, and several QTLs for resistance have been identified in apple and pear by genetic analysis (16, 20, 23, 48, 53; reviewed in 46). It has not been determined if these QTLs are the result of several partial resistance genes concentrated at specific genomic loci or represent the general location of a major monogenic resistance gene. Both scenarios are possible, and results of QTL analysis and other evidence exist to support them (33, 83, 88) (**Figure 3**). Currently, no specific interactions between pathogen avirulence genes and host resistance genes have been defined.

Powerful genomics technologies are now available for both identification of candidate resistance genes and molecular marker development from these QTLs (86, 119). In cases in which QTLs are likely to be the result of several genes contributing to resistance, possible candidates can be inferred by integrating genetic mapping studies with bioinformatics analysis of transcript profiling data and genome sequence databases (33). The markers that genetically define the position of the QTL are used to locate it within the WGS and thereby determine the predicted coding sequences within the QTL. The potential impact of these predicted coding regions on resistance is then inferred by their expression in transcriptomic studies, similarity to potential receptor databases, bioinformatics analysis, and inferences drawn from model species and the scientific literature. SNP analysis of population parents and resistant and susceptible progeny can then be used to identify candidate resistance alleles genetically associated with resistance. Using this type of approach, Gardiner et al. (33) identified several possible candidate resistance genes on linkage groups (chromosome) 3 and 7 of *Malus* robusta 5. Proof that the candidate genes play a functional role in resistance requires additional plant transformation studies; however, given that the candidate alleles are genetically correlated and tightly linked with resistance, they are useful for the development of markers. Additionally, because the candidates are derived from known coding regions (ESTs), they are likely to be present in different genetic backgrounds and thereby be useful for marker-assisted breeding (MAB) of fire blight resistance.

In cases in which QTLs with large effects on resistance are thought to be due to major resistance genes, positional cloning can be used to identify likely candidate genes for marker development. Using this approach, Parravicini et al. (83) defined the resistance locus of the QTL on linkage group 12 of apple "Evereste" to a 78-kb region containing a cluster of eight genes with homologies to known bacterial resistance genes. From this cluster, the two most probable fire blight resistance genes show homology with the *Pto/Prf* complex in

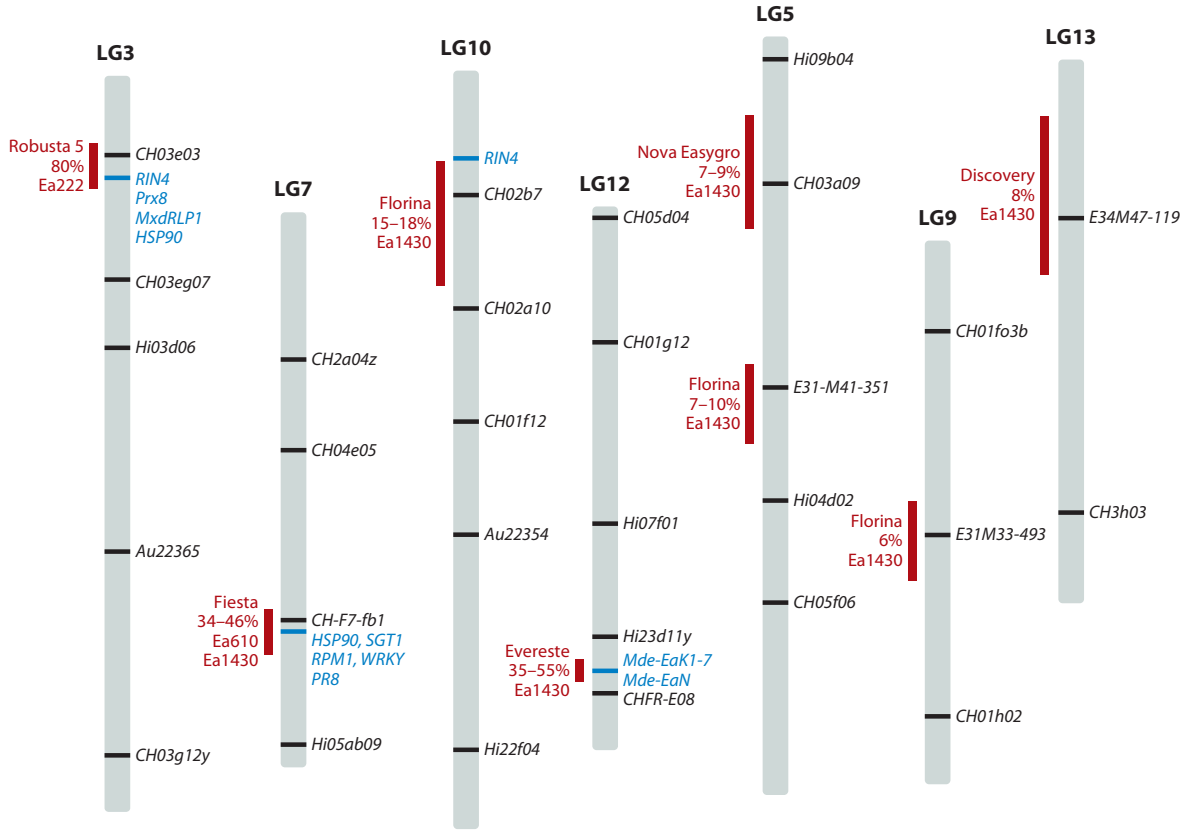

Figure 3

Position of fire blight resistance quantitative trait loci (QTLs) (*red*) in the apple genome with source cultivar, their effect on resistance and the strain of *Erwinia amylovora* used for phenotyping, and the position of the candidate gene(s) (*blue*) involved in resistance within the QTL. The genetic framework map is based on the integrated map used to anchor the apple whole-genome sequence (119). Abbreviations: HSP, heat shock protein; LG, linkage group (chromosome); MdE-EaK, *Malus domestica* cv. Evereste *E. amylovora kinase*; MdE-EaN, MdE-Ea nucleotide-binding site–leucine-rich repeat; Prx, peroxidase; MxdRLP, *Malus* × *domestica* leucine-rich repeat receptor-like protein; RPM, resistance to *Pseudomonas syringae* pv. *maculicola*; SGT1, suppressor of G2 allele of skp1.

tomato (83). As in the above example, these genes require further analysis to demonstrate their role in resistance but can be used in the immediate future for MAB and could also be used to improve the resistance of susceptible apple or pear cultivars via cisgenesis.

APPLICATION OF GENOMICS TO IMPROVE FIRE BLIGHT MANAGEMENT

Biocontrol

Although antibiotics are effective in the control of blossom blight, they are prohibited in the European Union and Switzerland because of the risk of transferring antibiotic resistance genes from agricultural to clinical bacteria. An alternative to antibiotics is biocontrol via the application of natural antagonists. A few commercial products are currently available based on *Pseudomonas fluorescens* (BlightBan®), *Bacillus subtilis* (BioPro®, Serenade®), and *Aureobasidium pullulans* (BlossomProtect®) (15, 42). New products are at various stages of development in Europe, North America, and New Zealand (BlossomBless®) based on *Pantoea agglomerans* (formerly *Erwinia herbicola*, *Enterobacter agglomerans*) (92). *P. agglomerans* is

one of the most common bacteria isolated from fire blight–susceptible plants and functions in biocontrol via competition and antibiosis (42, 123). Plant isolates of this species are among the most promising options for biological control of fire blight. *P. agglomerans* colonizes and offers protection to other plant diseases (10, 31), giving it the potential added value of multiple agricultural applications.

Genomes of biocontrol strains and related species allow the identification of biocontrol factors in the respective strains. The genome of *Pantoea vagans* C9-1, formerly known as *P. agglomerans* (97), consists of a circular 4.025-Mb chromosome and three large plasmids 530 kb, 166 kb, and 168 kb in size (38, 107, 108). The 530-kb megaplasmid pPag3 encodes the biosynthetic gene cluster for the carotenoid pigment, the quorum sensing system gene *pagRI*, and the desferrioxamine E biosynthetic gene cluster (97, 102, 109). The nonpigmented strain lacking pPag3 produces small rather than large halos on CAS agar plates but only a small halo close to the colony, which indicates the production of the chromosomally encoded enterobactin (26, 107, 109).

Plasmid pPag2 is found in *P. vagans* C9-1 but not in other *P. vagans* isolates (107). This plasmid contains two sorbitol biodegradation gene clusters, resistance genes against tellurite, and the biosynthesis cluster for the dapdiamide antibiotic herbicolin I (43), suggesting this plasmid could constitute a biocontrol-specific plasmid (107). The chromosome contains an antibiotic biosynthesis gene cluster as well (107, 108). This cluster, encoding the peptide antibiotic pantocin A, is located on a 28-kb genomic island inserted in the N-terminal region of the *mutS* gene.

Comparison of multiple *Pantoea* spp. genomes was used for determination of the biocontrol-specific genes in *P. vagans* C9-1 and *P. agglomerans* E325. As expected for *P. vagans* C9-1, the herbicolin I and pantocin A gene clusters were indicated as singletons (43, 44, 107). The antibiotic biosynthesis gene cluster of *P. agglomerans* E325 was identified from the list of singletons for this strain and later confirmed by sequencing the flanks of two Tn5-EZ mutants negative for antibiotic biosynthesis (T.H.M. Smits & B. Duffy, unpublished results).

Transgenic and Cisgenic Applications

A number of genes have been transgenically expressed in apple to improve bacterial resistance, with varying degrees of success (reviewed in 1). Researchers have followed different strategies, including production of antimicrobial proteins, inhibition of bacterial pathogenicity factors, and silencing or overexpression of related defense genes of the plant hosts (pear and apple). The availability of both host and pathogen genomes will facilitate development of new applications for fire blight management based on the transgenic expression of both host and pathogen genes that induce resistance.

One approach to enhance resistance is to express an *E. amylovora* effector gene to either create local and limited cell death at the point of infection or induce SAR. Expression in apple and pear of *E. amylovora* HrpN did not cause any detectable damage (62); however, when expressed in "Malling 26" rootstock, it resulted in a significant reduction in susceptibility to fire blight. Two HprN transgenic lines were observed to be resistant, and resistance was confirmed after several years of field evaluation (M. Malnoy, unpublished results). Analogous results were also obtained by overexpression of the apple *NPR1* gene, a regulator of SAR (60).

Using genetic engineering to alter the expression of apple proteins that interact with *E. amylovora* is also likely to influence interaction of host and pathogen. DspA/E- and HrpN-interacting proteins of *M.* × *domestica* (DIPM and HIPM, respectively) interact directly with two of the main *E. amylovora* effectors (70, 81). Four known DIPM genes interact with DspA/E (70). Silencing the four DIPM genes through genetic engineering also leads to a partial resistance to fire blight (11). Fibrillin (also known as FIBRILLIN4 and harpin-binding protein1) is associated with the photosystem II (PSII) light-harvesting complex (32) and physically interacts with the HrpN

protein (110). Silencing the *fib4* genes in apple increased the susceptibility of the transgenic lines compared with the control (101). Although this interaction needs to be confirmed in vivo, these results are consistent with the hypothesis that FIB4 is an HrpN target given that reduction of FIB4 expression could amplify the impact of HrpN and lead to increased disease susceptibility (101). Surprisingly, HrpN is also reported to enhance plant growth (39, 93). This effect is likely to be mediated by HIPM, a 6.5-kDa plasma membrane–localized protein that interacts with HrpN in yeast and in vitro and functions as a negative regulator of plant growth (79). It is thus tempting to speculate that HrpN could interact with HIPM at the plant plasma membrane and that this could lead to enhanced growth. Preliminary data show that silencing of this gene leads to partial resistance to fire blight (M. Malnoy, unpublished results). A high level of resistance to fire blight was also obtained in pear by decreasing the amount of iron available in the plant (19, 61).

Phenylpropanoid-derived polyphenols may be involved in fire blight defense (113). Overexpression of the *Zea mays leaf color* (*Lc*) gene in transgenic apple plants resulted in increased accumulation of anthocyanins and flavan-3-ols, altered growth habit, and created a significant increase in resistance to both apple scab and fire blight (29, 52). However, although silencing the flavanone-3β-hydroxylase gene of apple led to an accumulation of flavanones, it did not increase the resistance to fire blight, suggesting that redirection toward antibacterial flavan-3-ols does not happen and other flavonoids do not play a major role in resistance (27).

The unraveling of the apple genome combined with functional genomics studies has resulted in the cloning of a number of possible fire blight resistance determinants that may be involved in the mechanism of resistance or susceptibility. Gardiner et al. (33) identified several candidate resistance genes, including a class III peroxidase, a leucine-rich repeat protein, and a heat shock protein (HSP90) associated with the major fire blight QTL on linkage group 3. The HSP90 genes are interesting candidates because they were found to be differentially expressed in three separate studies of genes differentially regulated following inoculation of apple with *E. amylovora* (3, 41, 76) and were found associated with two of the three major fire blight QTLs. A complex of HSP90, SGT1, and RAR1 is known to be important in the regulation of NBS-LRR resistance proteins in planta and is essential for disease resistance triggered by a number of NBS-LRR resistance proteins, such as RPM1 (37). Interestingly, SGT1, RPM1, and HSP90 were identified as differentially expressed in response to *E. amylovora*. We can speculate that this complex can also modulate the expression of the candidate NBS-LRR, kinase genes isolated in the LG12, and the RIN4-like gene found as a candidate resistant gene in *Malus* robusta 5.

Over the next few years, genomics will enable identification of many fire blight resistance genes, and pathogen-derived genes will induce host resistance. The use of native apple promoters will facilitate gene expression where and when it is desired. With clean vector technology (59) allowing the removal of the selection marker, it will be possible to produce cisgenic apples, i.e., apple plants that will be exclusively modified with *Malus* DNA, alleviating some of the concerns associated with transgenic crops. However, the insertion site of these transgenes will not correspond to the original site of the gene, which could lead to epigenetic effects. The possibilities offered by recombinant DNA technology in apple can be used to benefit the producer, the environment, and the consumer. It remains to be seen how long it will take until a broad acceptance by the public is achieved.

Genomics Strategies to Facilitate Marker-Assisted Breeding

The use of fire blight–resistant apple cultivars is one of the most effective methods available to manage this disease. Breeding apple by sexual hybridization is costly, time consuming, and relatively inefficient because of apple's large plant size, its long juvenile phase, and the necessity for repeated phenotypic evaluations

over different years and at different locations. In MAB, the markers flanking a QTL region following QTL analysis, or functional markers representing the resistance genes themselves following candidate gene analysis, are used as the predictive genetic tests of performance by screening DNA obtained from young seedlings (86). Breeding efficiency is thereby increased through evaluation of young seedlings for multiple positive and negative traits, and then through selection for desirable recombination events prior to resource-intensive planting in the field. MAB is highly desirable in breeding apples and pears for resistance to fire blight, as phenotypic screening with *E. amylovora* is a destructive assay that is highly variable because of the effect of the environment, whereas genetic tests are nondestructive and environmentally neutral. Additionally, many countries prohibited phenotypic screening with *E. amylovora* under field conditions because of quarantine regulations. Furthermore, when pyramiding multiple sources of resistance to enhance durability, it is often difficult to distinguish the phenotype of resistant progeny containing one versus two QTLs.

To date, five QTL-based studies in six pedigrees have reported on the presence of 27 genomic regions that are significantly associated with fire blight resistance in apple (47). The three major QTLs on linkage group 3 (robusta 5), linkage group 7 ("Fiesta" and a U.S. accession of robusta 5), and linkage group 12 ("Evereste" and *M. floribunda* 821) have been validated in multiple studies and in different genetic backgrounds and thus could be useful in MAB. However, it should be noted that except for the QTL in "Fiesta" and the minor QTL in "Florina," the major fire blight resistance QTLs have been identified in crab apples that have poor fruit quality. Therefore, utilizing these would require several generations of backcrossing before resistance could be introgressed into a new cultivar of superior fruit quality. The long juvenile phase of apple can be shortened by transgenic expression of transcription factors controlling flowering, such as *BpMADS4*, to create early flowering lines for use in rapid-cycle or high-speed backcross programs (28). After introgression of resistance into a favorable genetic background, the early flowering trait is eliminated by selection of progeny with a normal flowering trait (transgene segregates 1:1). The combination of transgenic early flowering–accelerated breeding and MAB is currently being used to introgress the fire blight resistance of "Evereste" into prebreeding material of superior fruit quality (52).

Because of the large investment required to establish apple orchards and their long period of productivity (approximately 20–25 years), the development of cultivars with durable resistance is critical for successful disease management. The poor definition of host gene by pathogen gene interactions among the existing fire blight resistance QTLs remains a challenge to the application of MAB in the development of durable resistance. It has long been known that strains of *E. amylovora* are capable of overcoming the resistance of *Malus* × *robusta*, Robusta 5 (75), and recent research suggests that the major QTLs for fire blight resistance on linkage group 3 of Robusta 5 are strain specific (33, 87). Defining *E. amylovora* strain by *Malus* resistance and QTL specificity will be necessary to develop QTL pyramiding strategies for durable resistance. The limited diversity among *E. amylovora* strains should make it feasible to use a genomic approach to identify pathogen avirulence gene candidates and thereby accelerate the discovery of gene-for-gene interactions. A combined early flowering–accelerated breeding and MAB could then be used to rapidly create breeding lines that pyramid several mechanisms of resistance to fire blight to obtain durable resistance.

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LITERATURE CITED

1. Aldwinckle H, Malnoy M. 2009. Plant regeneration and transformation in the Rosaceae. *Spec. Issue Transgen. Plant J.* 1:1–39
2. Asselin JE, Bonasera JM, Kim JF, Oh CS, Beer SV. 2011. Eop1 from a *Rubus* strain of *Erwinia amylovora* functions as a host-range limiting factor. *Phytopathology* 101:935–44
3. Baldo A, Norelli JL, Farrell RE Jr, Bassett CL, Aldwinckle HS, Malnoy M. 2010. Identification of genes differentially expressed during interaction of resistant and susceptible apple cultivars (*Malus* × *domestica*) with *Erwinia amylovora*. *BMC Plant Biol.* 10:1
4. Barionovi D, Giorgi S, Stoeger AR, Ruppitsch W, Scortichini M. 2006. Characterization of *Erwinia amylovora* strains from different host plants using repetitive-sequences PCR analysis, and restriction fragment length polymorphism and short-sequence DNA repeats of plasmid pEA29. *J. Appl. Microbiol.* 100:1084–94
5. Bernard CS, Brunet YR, Gueguen E, Cascales E. 2010. Nooks and crannies in type VI secretion regulation. *J. Bacteriol.* 192:3850–60
6. Bocsanczy AM, Nissinen RM, Oh C-S, Beer SV. 2008. HrpN of *Erwinia amylovora* functions in the translocation of DspA/E into plant cells. *Mol. Plant Pathol.* 9:425–34
7. Bocsanczy AM, Phillips JG, Dardick CD, Korban SS, Bassett CL, et al. 2009. Analysis of apple (*Malus*) responses to bacterial pathogens using an oligo microarray. *Phytopathology* 99:S514
8. Bocsanczy AM, Schneider DJ, Declerck GA, Cartinhour S, Beer SV. 2012. HopX1ea (Eop3) in *Erwinia amylovora* functions as an avirulence gene in apple and is regulated by HrpL. *J. Bacteriol.* 194:553–60
9. Bonasera JM, Kim JF, Beer SV. 2006. PR genes of apple: identification and expression in response to elicitors and inoculation with *Erwinia amylovora*. *BMC Plant Biol.* 6:23
10. Bonaterra A, Camps J, Montesinos E. 2005. Osmotically induced trehalose and glycine betaine accumulation improves tolerance to dessication, survival and efficacy of the postharvest biocontrol agent *Pantoea agglomerans* EPS125. *FEMS Microbiol. Lett.* 250:1–8
11. Borejsza-Wysocka E, Malnoy M, Meng X, Bonasera JM, Nissinen RM, et al. 2006. The fire blight resistance of apple clones in which DspE-interacting proteins are silenced. *Acta Hortic.* 704:509–13
12. Born Y, Fiesler L, Marazzi J, Lurz R, Duffy B, Loessner MJ. 2011. Novel virulent and broad host range *Erwinia amylovora* bacteriophages reveal a high degree of mosaicism and relationship to *Enterobacteriacea* phages. *Appl. Environ. Microbiol.* 77:5945–54
13. Boureau T, El Maarouf-Bouteau H, Garnier A, Brisset MN, Perino C, et al. 2006. DspA/E, a type III effector essential for *Erwinia amylovora* pathogenicity and growth in planta, induces cell death in host apple and nonhost tobacco plants. *Mol. Plant-Microbe Interact.* 19:16–24
14. Boureau T, Siamer S, Perino C, Gaubert S, Patrit O, et al. 2011. The HrpN effector of *Erwinia amylovora*, which is involved in type III translocation, contributes directly or indirectly to callose elicitation on apple leaves. *Mol. Plant-Microbe Interact.* 24:577–84
15. Broggini GAL, Duffy B, Holliger E, Schärer H-J, Gessler C, Patocchi A. 2005. Detection of the fire blight biocontrol agent *Bacillus subtilis* BD170 (Biopro®) in a Swiss apple orchard. *Eur. J. Plant Pathol.* 111:93–100
16. Calenge F, Drouet D, Denance C, Van de Weg WE, Brisset MN, et al. 2005. Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. *Theor. Appl. Genet.* 111:128–35

17. Coplin DL, Majerczak DR, Bugert P, Geider K. 1996. Nucleotide sequence analysis of the *Erwinia stewartii cps* gene cluster for synthesis of stewartan and comparison to the *Erwinia amylovora ams* cluster for synthesis of amylovoran. *Acta Hortic.* 411:251–57
18. DebRoy S, Thilmony R, Kwack YB, Nomura K, He SY. 2004. A family of conserved bacterial effectors inhibits salicylic acid–mediated basal immunity and promotes disease necrosis in plants. *Proc. Natl. Acad. Sci. USA* 101:9927–32
19. Djennane S, Cesbron C, Sourice S, Cournol R, Dupuis F, et al. 2011. Iron homeostasis and fire blight susceptibility in transgenic pear plants overexpressing a pea ferritin gene. *Plant Sci.* 180:694–701
20. Dondini L, Pierantoni L, Gaiotti F, Chiodini R, Tartarini S, et al. 2004. Identifying QTLs for fire blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. *Mol. Breed.* 14:407–18
21. Dreo T, Ravnikar M, Frey JE, Smits THM, Duffy B. 2011. In silico analysis of variable number of tandem repeats in *Erwinia amylovora* genomes. *Acta Hortic.* 896:115–18
22. Duffy B, Schärer H-J, Vogelsanger J, Schoch B, Holliger E. 2005. Regulatory measures against *Erwinia amylovora* in Switzerland. *EPPO Bull.* 35:239–44
23. Durel C-E, Denance C, Brisset MN. 2009. Two distinct major QTL for resistance to fire blight co-localize on linkage group 12 in apple genotypes "Evereste" and *Malus floribunda* clone 821. *Genome* 52:139–47
24. Engelhardt S, Lee J, Gaebler Y, Kemmerling B, Haapalainen M-L, et al. 2009. Separable roles of the *Pseudomonas syringae* pv. *phaseolicola* accessory protein HrpZ1 in ion-conducting pore formation and activation of plant immunity. *Plant J.* 57:706–17
25. Fahrentrapp J, Broggini GAL, Kellerhals M, Peil A, Richter K, et al. 2012. *Malus* × *robusta* 5 fire blight candidate resistance gene coding for a CC-NBS-LRR. *PloS ONE.* In press
26. Feistner GJ, Ishimaru C. 1996. Proferrioxamine profiles of *Erwinia herbicola* and related bacteria. *BioMetals* 9:337–44
27. Flachowsky H, Halbwirth H, Treutter D, Richter K, Hanke MV, et al. 2012. Silencing of flavanone-3-hydroxylase in apple (*Malus* × *domestica* Borkh.) leads to accumulation of flavanones, but not to reduced fire blight susceptibility. *Plant Physiol. Biochem.* 51:18–25
28. Flachowsky H, Le Roux PM, Peil A, Patocchi A, Richter K, Hanke MV. 2011. Application of a high-speed breeding technology to apple (*Malus* × *domestica*) based on transgenic early flowering plants and marker-assisted selection. *New Phytol.* 192:364–67
29. Flachowsky H, Szankowski I, Fischer TC, Richter K, Peil A, et al. 2010. Transgenic apple plants over-expressing the *Lc* gene of maize show an altered growth habit and increased resistance to apple scab and fire blight. *Planta* 231:623–35
30. Foster GC, McGhee GC, Jones AL, Sundin GW. 2004. Nucleotide sequences, genetic organization, and distribution of pEU30 and pEL60 from *Erwinia amylovora*. *Appl. Environ. Microbiol.* 70:7539–44
31. Francés J, Bonaterra A, Moreno MC, Cabrefiga J, Badosa E, Montesinos E. 2006. Pathogen aggressiveness and postharvest biocontrol efficiency in *Pantoea agglomerans*. *Postharvest Biol. Technol.* 39:299–307
32. Galetskiy D, Susnea I, Reiser V, Adamska I, Przybylski M. 2008. Structure and dynamics of photosystem II light-harvesting complex revealed by high-resolution FTICR mass spectrometric proteome analysis. *J. Am. Soc. Mass Spectrom.* 19:1004–13
33. Gardiner SE, Norelli JL, de Silva N, Fazio G, Peil A, et al. 2012. Candidate gene markers associated with quantitative trait loci for fire blight resistance in *Malus* "robusta 5" accessions. *BMC Genet.* 13(1):25
34. Gasic K, Gonzalez DO, Thimmapuram J, et al. 2009. Comparative analysis and functional annotation of a large expressed sequence tag collection of apple. *Plant Genome* 2:23–38
35. Gaudriault S, Paulin JP, Barny MA. 2002. The DspB/F protein of *Erwinia amylovora* is a type III secretion chaperone ensuring efficient intrabacterial production of the Hrp-secreted DspA/E pathogenicity factor. *Mol. Plant Pathol.* 3:313–20
36. Griffith CS, Sutton TB, Peterson PD. 2003. *Fire Blight: The Foundation of Phytobacteriology*. St. Paul MN: APS Press. 144 pp.
37. Hubert DA, He Y, McNulty BC, Tornero P, Dangl JL. 2009. Specific *Arabidopsis* HSP90.2 alleles recapitulate RAR1 cochaperone function in plant NB-LRR disease resistance protein regulation. *Proc. Natl. Acad. Sci. USA* 106:9556–63

38. Ishimaru CA, Klos EJ, Brubaker RR. 1988. Multiple antibiotic productions by *Erwinia herbicola*. *Phytopathology* 78:746–50
39. Jang YS, Sohn SI, Wang MH. 2006. The *hrpN* gene of *Erwinia amylovora* stimulates tobacco growth and enhances resistance to *Botrytis cinerea*. *Planta* 223:449–56
40. Jensen PJ, Halbrendt N, Fazio G, Makalowska I, Altman N, et al. 2012. Rootstock-regulated gene expression patterns associated with fire blight resistance in apple. *BMC Genomics* 9:13
41. Jensen PJ, Makalowska I, Altman N, Fazio G, Praul C, et al. 2010. Rootstock-regulated gene expression patterns in apple tree scions. *Tree Genet. Genomes* 6:57–72
42. Johnson KB, Stockwell VO. 1998. Management of fire blight: a case study in microbial ecology. in *Annu. Rev. Phytopathol.* 36:227–48
43. Kamber T, Lansdell TA, Stockwell VO, Ishimaru CA, Smits THM, Duffy B. 2012. Characterization of the antibacterial peptide herbicolin I biosythetic operon in *Pantoea vagans* biocontrol strain C9-1, and prevalence in *Pantoea* species. *Appl. Environ. Microbiol.* 78:4412–19
44. Kamber T, Smits THM, Rezzonico F, Duffy B. 2012. Genomics and current genetic understanding of *Erwinia amylovora* and the fire blight antagonist *Pantoea vagans*. *Trees Struct. Funct.* 26:227–38
45. Khajanchi BK, Sha J, Kozlova EV, Erova TE, Suarez G, et al. 2009. *N*-acylhomoserine lactones involved in quorum sensing control type VI secretion system, biofilm formation, protease production, and in vivo virulence from a clinical isolate of *Aeromonas hydrophila*. *Microbiology* 155:3518–31
46. Khan MA, Duffy B, Gessler C, Patocchi A. 2006. QTL mapping of fire blight resistance in apple. *Mol. Breed.* 17:299–306
47. Khan MA, Zhao YF, Korban SS. 2012. Molecular mechanisms of pathogenesis and resistance to the bacterial pathogen *Erwinia amylovora*, causal agent of fire blight disease in Rosaceae. *Plant Mol. Biol. Rep.* 30:247–60
48. Khan MA, Zhao YF, Korban SS. 2012. A high-throughput apple SNP genotyping platform using the GoldenGate™ assay. *Gene* 494:196–201
49. Koczan JM, McGrath MJ, Zhao Y, Sundin GW. 2009. Contribution of *Erwinia amylovora* exopolysaccharides amylovoran and levan to biofilm formation: implications in pathogenicity. *Phytopathology* 99:1237–44
50. Kube M, Migdoll AM, Gehring I, Heitmann K, Mayer Y, et al. 2010. Genome comparison of the epiphytic bacteria *Erwinia billingiae* and *E. tasmaniensis* with the pear pathogen *E. pyrifoliae*. *BMC Genomics* 11:393
51. Kube M, Migdoll AM, Müller I, Kuhl H, Beck A, et al. 2008. The genome of *Erwinia tasmaniensis* strain Et1/99, a non-pathogenic bacterium in the genus *Erwinia*. *Environ. Microbiol.* 10:2211–22
52. Le Roux P-M, Flachowsky H, Hanke M-V, Gessler C, Patocchi A. 2011. Use of a transgenic early flowering approach in apple (*Malus* × *domestica* Borkh.) to introgress fire blight resistance from "Evereste." *Mol. Breed*. doi: 10.1007/s11032-011-9669-4
53. Le Roux PMF, Khan MA, Broggini GAL, Duffy B, Gessler C, Patocchi A. 2010. Mapping of quantitative trait loci for fire blight resistance in the apple cultivars "Florina" and "Nova Easygro." *Genome* 53:710–22
54. Llop P, Barbé S, López MM. 2012. Functions and origin of plasmids in *Erwinia* species that are pathogenic to or epiphytically associated with pome fruit trees. *Trees Struct. Funct.* 26:31–46
55. Llop P, Cabrefiga J, Smits THM, Dreo T, Barbé S, et al. 2011. Complete sequence, biogeography, and role in aggressiveness of a novel *Erwinia amylovora* plasmid pEI70. *PLoS ONE* 6(12):e28651
56. Llop P, Donat V, Rodríguez M, Cabrefiga J, Ruz L, et al. 2006. An indigenous virulent strain of *Erwinia amylovora* lacking the ubiquitous plasmid pEA29. *Phytopathology* 96:900–7
57. Loh J, Pierson EA, Pierson LS III, Stacey G, Chatterjee A. 2002. Quorum sensing in plant-associated bacteria. *Curr. Opin. Plant Biol.* 5:285–90
58. López MM, Roselló M, Llop P, Ferrer S, Christen R, Gardan L. 2011. *Erwinia piriflorinigrans* sp. nov., a novel pathogen that causes necrosis of pear blossoms. *Int. J. Syst. Evol. Microbiol.* 61:561–67
59. Malnoy M, Boresjza-Wysocka EW, Norelli JL, Flaishman M, Gidoni D, Aldwinckle HS. 2010. Genetic transformation of apple (*Malus* × *domestica*) without use of a selectable marker gene. *Tree Genet. Genomes* 6:423–33
60. Malnoy M, Jin Q, Borejsza-Wysocka E, He SY, Aldwinckle HS. 2007. Overexpression of the apple *MpNPR1* gene causes increased disease resistance in *Malus* × *domestica*. *Mol. Plant-Microbe Interact.* 20:1568–80

61. Malnoy M, Venisse JS, Brisset MN, Chevreau E. 2003. Expression of bovine lactoferrin cDNA confers resistance to *Erwinia amylovora* in transgenic pear. *Mol. Breed.* 12:231–44
62. Malnoy M, Venisse JS, Chevreau E. 2005. Expression of a bacterial effector, harpin N, causes increased resistance to fire blight in *Pyrus communis*. *Tree Genet. Genomes* 1:41–49
63. Mann RA, Blom J, Bühlmann A, Plummer KM, Beer SV, et al. 2012. Comparative analysis of the Hrp pathogenicity island of *Rubus*- and Spiraeoideae-infecting *Erwinia amylovora* strains identifies the IT region as a remnant of an integrative conjugative element. *Gene.* 504:6–12
64. Maxson-Stein K, McGhee GC, Smith JJ, Jones AL, Sundin GW. 2003. Genetic analysis of a pathogenic *Erwinia* sp. isolated from pear in Japan. *Phytopathology* 93:1393–99
65. McGhee GC, Jones AL. 2000. Complete nucleotide sequence of ubiquitous plasmid pEA29 from *Erwinia amylovora* strain Ea88: gene organization and intraspecies variation. *Appl. Environ. Microbiol.* 66:4897–907
66. McGhee GC, Schnabel EL, Maxson-Stein K, Jones B, Stromberg VK, et al. 2002. Relatedness of chromosomal and plasmid DNAs of *Erwinia pyrifoliae* and *Erwinia amylovora*. *Appl. Environ. Microbiol.* 68:6182–92
67. McGhee GC, Sundin GW. 2011. Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assesment of kasugamycin resistance potential in *Erwinia amylovora*. *Phytopathology* 101:192–204
68. McManus PS, Stockwell VO, Sundin GW, Jones AL. 2002. Antibiotic use in plant agriculture. *Annu. Rev. Phytopathol.* 40:443–65
69. McNally RR, Toth IK, Cock PJA, Pritchard L, Hedley PE, et al. 2012. Genetic characterization of the HrpL regulon of the fire blight pathogen *Erwinia amylovora* reveals novel virulence factors. *Mol. Plant Pathol.* 13:160–73
70. Meng XD, Bonasera JM, Kim JF, Nissinen RM, Beer SV. 2006. Apple proteins that interact with DspA/E, a pathogenicity effector of *Erwinia amylovora*, the fire blight pathogen. *Mol. Plant-Microbe Interact.* 19:53–61
71. Metzger M, Bellemann P, Bugert P, Geider K. 1994. Genetics of galactose metabolism of *Erwinia amylovora* and its influence on polysaccharide synthesis and virulence of the fire blight pathogen. *J. Bacteriol.* 176:450–59
72. Milecevicova R, Gosch R, Halbwirth H, Stich K, Hanke MV, et al. 2011. *Erwinia amylovora*–induced defense mechanisms of two apple species that differ in susceptibility to fire blight. *Plant Sci.* 179:60–67
73. Momol MT, Norelli JL, Piccioni DE, Momol EA, Gustafson HL, et al. 1998. Internal movement of *Erwinia amylovora* through symptomless apple scion tissues into the rootstock. *Plant Dis.* 70:1017–19
74. Nissinen RM, Ytterberg AJ, Bogdanove AJ, Van Wijk KJ, Beer SV. 2007. Analyses of the secretomes of *Erwinia amylovora* and selected *hrp* mutants reveal novel type III secreted proteins and an effect of HrpJ on extracellular harpin levels. *Mol. Plant Pathol.* 8:55–67
75. Norelli JL, Aldwinckle HS, Beer SV. 1986. Differential susceptibility of *Malus* spp. cultivars robusta 5, Novole, and Ottawa 523 to *Erwinia amylovora*. *Plant Dis.* 70:1017–19
76. Norelli JL, Farrell RE, Bassett CL, Baldo AM, Lalli DA, et al. 2009. Rapid transcriptional response of apple to fire blight disease revealed by cDNA suppression subtractive hybridization analysis. *Tree Genet. Genomes* 5:27–40
77. Norelli JL, Jones AL, Aldwinckle HS. 2003. Fire blight management in the twenty-first century: using new technologies that enhance host resistance in apple. *Plant Dis.* 87:756–65
78. Oh C-S, Beer SV. 2005. Molecular genetics of *Erwinia amylovora* involved in the development of fire blight. *FEMS Microbiol. Lett.* 253:185–92
79. Oh C-S, Beer SV. 2007. AtHIPM, an ortholog of the apple HrpN-interacting protein, is a negative regulator of plant growth and mediates the growth-enhancing effect of HrpN in *Arabidopsis*. *Plant Physiol.* 145:426–36
80. Oh C-S, Carpenter CD, Hayes ML, Beer SV. 2010. Secretion and translocation signals and DspB/F-binding domains in the type III effector DspA/E of *Erwinia amylovora*. *Microbiology* 156:1211–20
81. Oh C-S, Martin GB, Beer SV. 2007. DspA/E, a type III effector of *Erwinia amylovora*, is required for early rapid growth in *Nicotiana benthamiana* and causes NbSGT1-dependent cell death. *Mol. Plant Pathol.* 8:255–65
82. Park DH, Thapa SP, Choi B-S, Kim W-S, Hur JH, et al. 2011. Complete genome sequence of Japanese *Erwinia* strain Ejp617, a bacterial shoot blight pathogen of pear. *J. Bacteriol.* 193:586–87

83. Parravicini G, Gessler C, Denancé C, Lasserre-Zuber P, Vergne E, et al. 2011. Identification of serine/threonine kinase and nucleotide-binding site–leucine-rich repeat (NBS-LRR) genes in the fire blight resistance quantitative trait locus of apple cultivar "Evereste." *Mol. Plant Pathol.* 12:493–505
84. Parsot C, Hamiaux C, Page AL. 2003. The various and varying roles of specific chaperones in type III secretion systems. *Curr. Opin. Microbiol.* 6:7–14
85. Paternoster T, Défago G, Duffy B, Gessler C, Pertot I. 2010. Selection of a biocontrol agent based on a potential mechanism of action: degradation of nicotinic acid, a growth factor essential for *Erwinia amylovora*. *Int. Microbiol.* 13:195–206
86. Peace C, Norelli JL. 2009. Genomics approaches to crop improvement in the Rosaceae. In *Genetics and Genomics of Rosaceae*, ed. K Folta, S Gardiner, pp. 55–70. New York: Springer
87. Peil A, Flachowsky H, Hanke M-V, Richter K, Rode J. 2011. Inoculation of *Malus* × *robusta* 5 progeny with a strain breaking resistance to fire blight reveals a minor QTL on LG5. *Acta Hortic.* 986:357–62
88. Peil A, Garcia-Libreros T, Richter K, Trognitz FC, Trognitz B, et al. 2007. Strong evidence for a fire blight resistance gene of *Malus robusta* located on linkage group 3. *Plant Breed.* 126:470–75
89. Perino C, Gaudriault S, Vian B, Barny MA. 1999. Visualization of harpin secretion in planta during infection of apple seedlings by *Erwinia amylovora*. *Cell. Microbiol.* 1:131–41
90. Powney R, Smits THM, Sawbridge T, Frey B, Blom J, et al. 2011. Genome sequence of an *Erwinia amylovora* strain with restricted pathogenicity to *Rubus* plants. *J. Bacteriol.* 193:785–86
91. Pusey PL, Curry EA. 2004. Temperature and pomaceous flower age related to colonization by *Erwinia amylovora* and antagonists. *Phytopathology* 94:901–11
92. Pusey PL, Stockwell VO, Reardon C, Smits THM, Duffy B. 2011. Antibiotic production by *Pantoea agglomerans* biocontrol strain E325 and activity against *Erwinia amylovora* on apple flower stigmas. *Phytopathology* 101:1234–41
93. Reboutier D, Frankart C, Briand J, Biligui B, Laroche S, et al. 2007. The $HrpN_{ea}$ harpin from *Erwinia amylovora* triggers differential responses on the nonhost *Arabidopsis thaliana* cells and on the host apple cells. *Mol. Plant-Microbe Interact.* 20:94–100
94. Rezzonico F, Duffy B. 2007. The role of *luxS* in the fire blight pathogen *Erwinia amylovora* is limited to metabolism and does not involve quorum sensing. *Mol. Plant-Microbe Interact.* 20:1284–97
95. Rezzonico F, Duffy B. 2008. Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for *luxS* in most bacteria. *BMC Microbiol.* 8:154
96. Rezzonico F, Smits THM, Duffy B. 2011. Diversity, evolution, and functionality of clustered regularly interspaced short palindromic repeat (CRISPR) regions in the fire blight pathogen *Erwinia amylovora*. *Appl. Environ. Microbiol.* 77:3819–29
97. Rezzonico F, Smits THM, Montesinos E, Frey JE, Duffy B. 2009. Genotypic comparison of *Pantoea agglomerans* plant and clinical strains. *BMC Microbiol.* 9:204
98. Rezzonico F, Vogel G, Duffy B, Tonolla M. 2010. Application of whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification and clustering analysis of *Pantoea* species. *Appl. Environ. Microbiol.* 76:4497–509
99. Sarowar S, Zhao Y, Soria-Guerra RE, Ali S, Zheng D, et al. 2011. Expression profiles of differentially regulated genes during the early stages of apple flower infection with *Erwinia amylovora*. *J. Exp. Bot.* 62:4851–61
100. Sebaihia M, Bocsanczy AM, Biehl BS, Quail MA, Perna NT, et al. 2010. Complete genome sequence of the plant pathogen *Erwinia amylovora* strain ATCC 49946. *J. Bacteriol.* 192:2020–21
101. Singh DK, Maximova SN, Jensen PJ, Lehman BL, Ngugi HK, McNellis TW. 2010. *FIBRILLIN4* is required for plastoglobule development and stress resistance in apple and *Arabidopsis*. *Plant Physiol.* 154:1281–93
102. Smits THM, Duffy B. 2011. Genomics of iron acquisition in the plant pathogen *Erwinia amylovora*: insights in the biosynthetic pathway of the siderophore desferrioxamine E. *Arch. Microbiol.* 193:693–99
103. Smits THM, Jaenicke S, Rezzonico F, Kamber T, Goesmann A, et al. 2010. Complete genome sequence of the fire blight pathogen *Erwinia pyrifoliae* DSM 12163^T and comparative genomic insights into plant pathogenicity. *BMC Genomics* 11:2

104. Smits THM, Rezzonico F, Blom J, Frey JE, López MM, Duffy B. 2012. Comparative analysis of the genome of *Erwinia piriflorinigrans* CFBP 5888^T reveals its pathogenic potential and its phylogenetic position within the *Erwinia* species pathogenic to Spiraeoideae host plants. *BMC Genomics*. Submitted
105. Smits THM, Rezzonico F, Duffy B. 2011. Evolutionary insights from *Erwinia amylovora* genomics. *J. Biotechnol.* 155:34–39
106. Smits THM, Rezzonico F, Kamber T, Blom J, Goesmann A, et al. 2010. Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Mol. Plant-Microbe Interact.* 23:384–93
107. Smits THM, Rezzonico F, Kamber T, Goesmann A, Ishimaru CA, et al. 2011. Metabolic versatility and antibacterial metabolite biosynthesis are distinguishing genomic features of the fire blight antagonist *Pantoea vagans* C9-1. *PLoS ONE* 6:e22247
108. Smits THM, Rezzonico F, Kamber T, Goesmann A, Ishimaru CA, et al. 2010. The genome sequence of the biocontrol agent *Pantoea vagans* strain C9-1. *J. Bacteriol.* 192:6486–87
109. Smits THM, Rezzonico F, Pelludat C, Goesmann A, Frey JE, Duffy B. 2010. Genomic and phenotypic characterization of a non-pigmented variant of *Pantoea vagans* biocontrol strain C9-1 lacking the 530 kb megaplasmid pPag3. *FEMS Microbiol. Lett.* 308:48–54
110. Song X, Fan H, Wei Z-M. 2002. Receptors for hypersensitive response elicitors and uses thereof. *U.S. Patent No. 20020007501*
111. Soria-Guerra RE, Rosales-Mendoza SR, Gasic K, Wisniewski ME, Band M, Korban SS. 2011. Gene expression is highly regulated in early developing fruit of apple. *Plant Mol. Biol. Rep.* 29:885–97
112. Thomson SV. 1986. The role of the stigma in fire blight infections. *Phytopathology* 76:476–82
113. Treutter D. 2006. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biol.* 7:581–91
114. Triplett LR, Melotto M, Sundin GW. 2009. Functional analysis of the N terminus of the *Erwinia amylovora* secreted effector DspA/E reveals features required for secretion, translocation, and binding to the chaperone DspB/F. *Mol. Plant-Microbe Interact.* 22:1282–92
115. Triplett LR, Wedemeyer WJ, Sundin GW. 2010. Homology-based modeling of the *Erwinia amylovora* type III secretion chaperone DspF used to identify amino acids required for virulence and interaction with the effector DspE. *Res. Microbiol.* 161:613–18
116. Truman W, de Zabala MT, Grant M. 2006. Type III effectors orchestrate a complex interplay between transcriptional networks to modify basal defence responses during pathogenesis and resistance. *Plant J.* 46:14–33
117. van der Zwet T, Orolaza-Halbrendt N, Zeller W. 2011. *Fire Blight: History, Biology and Management*. St Paul, MN: APS Press. 420 pp.
118. Vanneste JL. 2000. What is fire blight? Who is *Erwinia amylovora*? How to control it? In *Fire Blight*, ed. J. Vanneste, pp. 1–7. New York: CABI
119. Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, et al. 2010. The genome emergence of the domesticated apple (*Malus* × *domestica* Borkh.). *Nat. Genet.* 42:833–41
120. Venisse JS, Barny MA, Paulin JP, Brisset MN. 2003. Involvement of three pathogenicity factors of *Erwinia amylovora* in the oxidative stress associated with compatible interaction in pear. *FEBS Lett.* 537:198–202
121. Venisse JS, Gullner G, Brisset MN. 2001. Evidence for the involvement of an oxidative stress in the initiation of infection of pear by *Erwinia amylovora*. *Plant Physiol.* 125:2164–72
122. Venisse JS, Malnoy M, Faize M, Brisset MN. 2002. Modulation of plant defense responses of *Malus* spp. during compatible and incompatible interaction with *Erwinia amylovora*. *Mol. Plant-Microbe Interact.* 15:1204–12
123. Wang D, Qi M, Calla B, Korban SS, Clough SJ, et al. 2012. Genome-wide identification of genes regulated by the Rcs phosphorelay system in *Erwinia amylovora*. *Mol. Plant-Microbe Interact.* 25:6–17
124. Yoder KS, Miller SS, Byers RE. 1999. Suppression of fireblight in apple shoots by prohexadione-calcium following experimental and natural inoculation. *HortScience* 34:1202–4
125. Zhang W, Xu S, Li J, Shen X, Wang Y, Yuan Z. 2011. Modulation of a thermoregulated type VI secretion system by AHL-dependent quorum sensing in *Yersinia pseudotuberculosis*. *Arch. Microbiol.* 193:351–63
126. Zhao Y, Sundin GW, Wang D. 2009. Construction and analysis of pathogenicity island deletion mutants of *Erwinia amylovora*. *Can. J. Microbiol.* 55:457–64

127. Zhao YF, Blumer SE, Sundin GW. 2005. Identification of *Erwinia amylovora* genes induced during infection of immature pear tissue. *J. Bacteriol.* 187:8088–103
128. Zhao YF, He SY, Sundin GW. 2006. The *Erwinia amylovora avrRpt2$_{EA}$* gene contributes to virulence on pear and AvrRpt2$_{EA}$ is recognized by *Arabidopsis* RPS2 when expressed in *Pseudomonas syringae*. *Mol. Plant-Microbe Interact.* 19:644–54

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Contents

Errata

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