Improvement of Fire Blight Resistance in Apple and Pear

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ABSTRACT

Fire blight caused by the bacterium \textit{Erwinia amylovora} is known to incite substantial damage in pomefruit production. The disease originated in North America, from where it slowly spread around the world. Hosts of \textit{E. amylovora} are members of the \textit{Maloidae}, such as apple, pear and quince, and ornamentals, such as \textit{Cotoneaster}, \textit{Crataegus} and \textit{Mespilus}. The disease can affect all tree parts such as blossoms, shoot tips and rootstock crowns. The name of the disease resembles the main symptom in host plants, i.e. the black necrosis of shoots and browning of leaves. Antibiotics provide effective control, but these are increasingly banned due to ecological considerations and the emergence of antibiotic resistant strains of \textit{E. amylovora}. Alternative control strategies are based on copper ions and the application of antagonists. The use of fire blight resistant cultivars is another approach to prevent fire blight epidemics. Fire blight resistance is present in the cultivated apple, but high levels of resistance generally are found in wild species of apple and pear. The introduction of wild species’ traits into cultivars is a slow process in fruit tree breeding. Understanding the genetics of the disease resistance, biotechnology and genetic engineering can promote and accelerate classical breeding. This review focuses on crop improvement for fire blight resistance. We describe the origin of the disease, its spread and the infection tools of the pathogen, summarize the genetic resources available to breeders and strategies to improve apple and pear for fire blight resistance.

Keywords: breeding, \textit{Erwinia amylovora}, genetic engineering, genetic markers, germplasm, \textit{Malus}, MAS

Abbreviations: ABA, abscissic acid; AI, auxin inducer; \textit{ans}, amylovoran synthesis; AMV, alfalfa mosaic virus; AMP, antimicrobial peptide; \textit{Att}, \textit{Atta} ric; AUDPC, area under disease progress curve; B, Budogovsky; BSA, bulked segregant analysis; CaMV, cauliflower mosaic virus; \textit{dfo}, desferrixamine; DIPM, \textit{DspE}-interacting protein of \textit{Malus}; DNA, deoxyribonucleic acid; DPI, days post inoculation; \textit{dpo}, depolymerase; \textit{dsp}, disease-specific; EPS, exopolysaccharide; EST, expressed sequence tag; F3H, flavanone 3β-hydroxylase; FHT, flavanone 3β-hydroxylase; \textit{fox}, ferrioxamine; \textit{G}, Gene™; \textit{glmS}, glucosamine synthase; H-NS, histone-like nucleoid structuring protein; \textit{HEE}, Hrp effectors and effectors; \textit{HEWL}, hen egg white lysozyme; HIPM, HrpN-interacting protein from \textit{Malus}; \textit{HR}, hypersensitive response; \textit{hrc}, hypersensitive reaction conserved; \textit{hrp}, hypersensitivity reaction and pathogenicity; \textit{hsv}, hsp-associated systemic virulence; IVS, index of varietal susceptibility; \textit{JERE}, jasmonate and ethylene responsive element; \textit{LAR}, leucoanthocyanidin reductase; \textit{LG}, linkage group; \textit{LOD}, logarithm of the odd; \textit{LRR}, leucine-rich repeat; \textit{lec}, levansucrase; \textit{M}, Malling; \textit{manA}, mannose 6-phosphate isomerase; \textit{MAS}, marker-assisted selection; \textit{MDA}, megadalton; \textit{MM}, million metric tonnes; \textit{NaCl}, sodium chloride; \textit{NBS}, nucleotide-binding site; \textit{NPR}, nonexpressor of \textit{PR}; \textit{npt}, neomycin phosphotransferase; \textit{ORF}, open reading frame; \textit{OSMP}, osmotin promoter; \textit{QTL}, quantitative trait locus; \textit{PCR}, polymerase chain reaction; \textit{PFGE}, pulse field gel electrophoresis; \textit{Pgst}, potato glutathione-S-transferase; \textit{pin}, protease inhibitor 2; \textit{PMI}, phosphomannose isomerase; \textit{Ppp}, potato patogen related protein; \textit{PR}, patogenesis related; \textit{rcs}, regulator of capsule synthesis; \textit{PST}, phosphate transport system \textit{S}; \textit{rbs}, regulation of levansucrase; \textit{RGA}, resistance gene analogue; \textit{RNA}, ribonucleic acid; \textit{SCAR}, sequence characterised amplified region; \textit{SGT}, small glutamine-rich protein; \textit{SSR}, simple sequence repeat; \textit{TSS}, type three secretion system; \textit{TFL}, terminal flower; \textit{TIR}, toll and interleukin 1 receptor; \textit{UDP}, urindiphosphate; \textit{USDA}, U.S. Department of Agriculture; \textit{V}, Vineland

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INTRODUCTION

Apple, pear and quince are pomefruit that belong to the Maloideae, a sub-family of 28 genera comprising approximately 1,100 species worldwide within the rose family, Rosaceae. They are major contributors to fruit production in the temperate climate zones. The domesticated apple (Malus x domestica Borkh.) is the most important pomefruit and, with approximately 64 million metric tonnes (MMT) produced on 4.7 million hectares of land, it ranked fourth within the fruit crops in 2006, behind bananas (~71 MMT), grapes (~69 MMT) and oranges (~65 MMT) (http://faostat.fao.org). With about 20 MMT pears are ranked eighth. More than 50 countries produce 10,000 MMT or more of apples. The main producers are China (26 MMT), USA (4.6 MMT), Iran (2.7 MMT), Poland (2.3 MMT), Italy (2.1 MMT) and Turkey (2.0 MMT). China, Italy, USA and Spain are the leading pear producing countries (http://faostat.fao.org).

The majority of the world’s pomefruit production is represented by a handful of cultivars (O’Rourke et al. 2003), which are all more or less susceptible to plant diseases. One of the most important plant diseases in pomefruit production is fire blight, caused by the bacteria Erwinia amylovora (Burrill) Winslow et al. It is an epiphytotic disease that occurs on a regular basis in climates conducive to the disease, but occurs erratically in climates more marginal to the pathogen. Disease expression varies from season to season depending on the growing conditions and the cultivar. Blossom infections lead to a reduction in the current crop yield, while the following year’s yield will be reduced if the fruit spurs are also killed. Twig blight also destroys the annual wood that bears the fruit spurs of the following season. In pears and quinces as well as in certain apple cultivars, blight of the twigs and suckers often progresses into the large limbs or the trunk killing the tree. Some typical symptoms of fire blight are shown in Fig. 1.

The disease was first found in the USA in 1780 (Denning 1794), but has since spread around the world. It has been reported in about 40 countries (Table 1), including most of the leading apple and pear producing countries, such as USA, Iran, Poland, Italy, Turkey and Spain. It can cause dramatic economic losses. In 1999, the US apple industry lost an estimated US$35.6 million due to fire blight (Gianessi et al. 2002). Disease management is possible by the use of streptomycin or copper sprays. US apple growers spend about US$ 2.8 million per year on antibiotic sprays (Gianessi et al. 2002). Streptomycin-based products for fire blight control are not allowed in many countries. In Germany, the application of streptomycin-based products, such as “Plantomycin”, “Strepto” and “Firewall 17 WP”, is strongly regulated and only permitted in exceptional cases. No copper-based plant protection products are licensed for use. Products, such as “Regalis” or “Serenade”, whose active ingredients are prohexadione-Ca and Bacillus subtilis strain QST 713, respectively, do not give effective control (BMELV: Feuerbrand-Strategiepapier 2008 bis 2012).

Planting of resistant cultivars is potentially the most promising disease control strategy. Semi-resistant cultivars are currently available on the market, but their fruit quality is not good enough to replace the current leading cultivars. In spite of the economic importance of fire blight, breeding resistant cultivars with attractive fruit of high quality was not rated as high priority in many international apple breeding programs (Laurens 1999), although breeding activities may have increased since this survey. Classical breeding is time-consuming, due to the long juvenile stage of pomefruit, and an expensive process as resistance genes conferring high levels of resistance are mostly present in wild species with small fruits of low quality. Several backcross generations with high quality cultivars are necessary to eliminate most of the negative fruit traits of these wild species in order to achieve marketable fruit. Biotechnological strategies such as genetic engineering provide exciting tools to overcome these difficulties. Some of the published strategies were really effective, but their acceptance by fruit growers and consumers has been minimal to date.

This review summarizes major historical events in the emergence of fire blight becoming a significant orchard disease. We describe the pathogen, its virulence factors, and the opportunities to control the disease by using antagonistic bacteria or bacteriophages. We will collate the findings of the evaluations of genetic resources that form the basis of...
Coast States. The disease appeared in pear orchards in California between 1901 and 1910 as has seldom been seen in a fruit growing area since. Between 1902 and 1904 fire blight spread northward into Oregon and Washington. Following this outbreak in California, fire blight has emerged as a serious problem in apple production since the 1980s (Thomas and Jones 1992). Fire blight was particularly severe in 1991 in South-western Michigan, where the estimated losses were US$ 3.8 million (van der Zwet and Beer 1991). This can be largely attributed to the planting of more susceptible cultivars such as ‘Bartlett’, ‘Beurre Bosé’ and ‘Anjou’, which replaced the less susceptible cultivar ‘Kiefer’. The first outbreak of fire blight in Canada was reported from Ontario province (Harrison 1904) in 1904, followed by the first report from the West Coast of British Columbia in 1911 (Eastham 1922). A 1972 survey in Southern Ontario province revealed that about one-third of the orchards had economic damage due to fire blight (Dueck and Quamme 1973).

The earliest report of fire blight outside the North American continent came from Japan in 1903, where the disease was found on apple trees, presumably imported from America (Uyeda 1903). A similar disease affecting mainly pear has been connected to Erwinia pyrifoliae (Kim et al. 2001; Geider et al. 2009) (see below). The second report came from New Zealand (Campbell 1920), where the disease is believed to have been imported on nursery stock. Cockayne (1921) reported the initial outbreaks in 1919 on apple, pear, quince and hawthorn around Auckland region of the North Island. Fire blight reached the South Island in 1929 despite quarantine regulations (Reid 1930).

It was assumed that during the 1950s E. amylovora was disseminated via infested budwood and trees or by contaminated fruit boxes from North America to North-western Europe and the Northeast region of Africa. Recent work by Jock et al. (2002) observed identical PFGE patterns of E. amylovora strains from New Zealand and Central Europe (Pt1), and related patterns for strains from Egypt, Greece and Turkey (Pt2). However, American and English strains gave rise to dissimilar patterns (Jock and Geider 2004). Therefore, fire blight may have been introduced to Europe and North America only once or a few times, which could have included plant material from New Zealand. The first outbreak in the UK was reported in 1958 on pear trees near Maidstone (Kent) (Crosse et al. 1958). In an effort to control the disease in Northern England, 27,000 pear trees and 19,000 other host plants (Crataegus spp., Cotoneaster spp., Pyracantha spp.) were eradicated from 1958 to 1967 (Lelliott 1968).

The first fire blight symptoms on the mainland of the European continent were found in two distinct locations: one in The Netherlands (The Netherlands Plant Protection Service, 1966) and one on the Baltic Coast of Poland (Borecki et al. 1967). The pathogen presumably came to Poland through infected plant material from Great Britain (van der Zwet 1970).

In the summer of 1968, fire blight was reported for the first time in Denmark, in pear plantations and Crataegus hedgerows in the island of Falster (Jorgensen 1969). The same host plants were the first found infected in Germany in 1971 in the province of Nordfriesland near the border with Denmark (Bömeke 1972; Fischer and Meyer 1972). In the Eastern part of Germany fire blight was found on the Baltic Coast in 1972. Here, the cost of fire blight control was estimated at about US$ 26.6 million between 1972 and 2000 (Naumann, pers. comm.). About 812 km of hawthorn hedges, 74,000 hawthorn bushes, 191,340 pear trees, 236,880 apple trees, 79,600 quince shrubs and 7,600 ornamental plants were eradicated during this period. Despite the complete eradication of all host plants in the infected areas and intensive inspection of nurseries and orchards to locate new...
outbreaks, the spread of the disease from the cooler northern to the warmer and more humid southern part of Germany could not be prevented. Fire blight is regarded as an economic problem in the fruit growing areas of Baden-Württemberg and Rheinland-Pfalz, where 200 ha of fruit trees have been eradicated in each of the years 1993, 1995 and 1996. In 2007, about 1,000 ha of pome fruits were affected in Baden-Württemberg and the economic loss was about € 3.0 million (Molmert, pers. comm.).

**E. amylovora** spread across all of Germany and reached the Czech Republic in 1987 (Kudela 1988), Switzerland in 1989 (Grimm 1989), and Austria in 1993 (Keck et al. 1996). During the time that fire blight spread across the European continent, the disease was also detected across North Africa. In 1964, El-Helaly et al. (1964) reported fire blight near Alexandria in the Nile delta of Egypt, from where the disease spread to Cyprus (1985), Israel (1985), Turkey (1985), Greece (1985), Lebanon (1988), Jordan (1990), Armenia (1990) and Iran (1995). Fire blight moved northward into the mainland of Greece and from there into Macedonia, Bulgaria and Romania. Soon after Macedonia, fire blight was reported from Serbia, Bosnia, Croatia and Albania. By 1996, fire blight appeared in South-eastern Hungary (Hevesi 1996). In 1992, E. amylovora reached the south of Italy and in 1994 it was detected in the Po River valley in northern Italy, near the city of Bologna (Calzolari et al. 1999). The first outbreak of fire blight in Spain appeared in the north in 1995, a few kilometres south of the French border on cider apples (de la Cruz Blanco 1996).

In Australia, 45 symptomatic plant parts were sampled in the Royal Botanic Gardens of Melbourne (RBGM) and the Adelaide Botanic Gardens in 1997, but only two woody samples from RBGM were positive (Jock et al. 2000) and eradicated. Extensive surveys since the original outbreak have not detected the disease or the pathogen. The most recent report of first occurrence of fire blight in a country has come from Morocco (Fatmi et al. 2008).

In general, the dissemination of fire blight happened mainly by the long-distance shipment of contaminated budwood or trees. The occurrences in most European and African countries most likely resulted from the outbreak in England and Egypt in the 1950s and 1960s (Jock et al. 2002). After many years of short-distance spread from there, nearly every country in Europe and the Middle East has become affected.

Fire blight is more severe in warm, humid areas than in cooler and dry regions. Being a much more susceptible host, pear trees are generally more affected than apple trees. The worldwide trend towards planting high-density orchards of susceptible cultivars and rootstockst has resulted in fire blight becoming a major disease of apple.

### ERWINIA AMYLOVORA AND ERWINIA PYRIFOLIAE, PLANT PATHOGENIC BACTERIA CAUSING FIRE BLIGHT AND ASIAN PEAR BLIGHT

*E. amylovora* belongs to the family of Enterobacteriaceae. It is thus related to *Escherichia coli*, which facilitates application of *E. coli* genetics. On the other hand, the relation can cause immunological cross-reactions, which interfere in serological diagnosis. Several PCR detection assays have been described including real time PCR with primers from the chromosome and plasmid pEA29 (Mohammadi et al. 2009).

A major difference between *E. amylovora* and *E. coli* is the restriction in growth of *E. amylovora* to temperatures below 30°C, whereas *E. coli* prefers 37°C. *E. amylovora* can tolerate temperatures above 30°C, spanning the potential range of fire blight to all countries with production of apples and pears from regions of moderate to cold climates such as Scandinavia to hot climates such as Egypt. *E. amylovora* can survive dry conditions (Jock et al. 2005) and is widely distributed by everyday plant visits by pollinators. The fast multiplication on the stigma and migration to the nectar-theses (Spinelli et al. 2005a) is followed by invasion of the flower cluster, surrounding petals and migration into the stem down to the roots (Bogs et al. 1998).

In the past years, reports have been published about a similar disease in Korea (Rhim et al. 1999) and Japan (Kim et al. 2001) affecting Nashi pears. The causative agent, *E. amylovora* (Kim et al. 1999), will also be discussed in this review for some microbiological and molecular properties. Many features of *E. amylovora* may apply to *E. pyrifoliae* (Table 2), but the latter completely lacks levan formation and needs specific primer sets for PCR detection. The genome of *E. amylovora* is currently being analyzed (http://www.sanger.ac.uk/Projects/E_amylovora/).

### Virulence factors of the fire blight pathogen

Mutagenesis revealed two major and several "minor" virulence factors of *E. amylovora* for multiplication in plant tissue to cause disease symptoms. Transposon insertions were screened on immature pears, apple seedlings or flowers and hypersensitive response on tobacco (Steinberger and Beer 1988; Barny et al. 1990; Bellemann and Geider 1992). The sets of integrations were subsequently mapped and gene functions deduced (see Vanneste 2000).

#### Hypersensitive response (HR)

The HR is induced by most plant pathogens and is a resistance reaction as a way for the plant to confine infection. HR is not only induced in non-host plants such as tobacco, but also in host plants that are susceptible to fire blight. The resistance response is accompanied by tissue damage, possibly initiated by oxidative stress (Venisse et al. 2001), that provides access for the pathogen to nutrients from leakage of plant cells. The cluster of genes required for the induction of HR was gradually identified and characterized (see Oh and Beer 2005). HpL is a sigma-like subunit of the bacterial RNA polymerase controlling the expression of many *hrp*-genes. A central role is associated with *hrpN*, encoding for an elicitor of plant defence. Another gene, *hrpW*, interferes with *hrpN* by modifying its action. Several genes of the *hrp* region are involved in the translocation of Hrp-proteins, including pilus formation. Many of them are common to both plant and other pathogenic bacteria and have been named *hrc*-genes. Two genes adjacent to the *hrp* gene cluster also participate in the induction of plant defence, although mutants still cause HR. The large protein encoded by *dpsE/A* has a dominant role and causes cell death by interacting with plant cell proteins, such as NSG1 (Oh et al. 2007). *DspE/A* is most likely transported through the *hrp*-pili of the bacteria to the plant cell and finally transferred into the nucleus, where it seems to affect gene expression. It is related to avirulence genes of other plant pathogenic bacteria and processed by DspF/B as a chaperon. The HrpN-protein, called harpin, was expressed in *E. coli* and crude preparations were applied to plants in order to increase their resistance against pathogens. A commercial preparation has been marketed under the name "Messenger"
for disease protection including fire blight (Eden Bioscience, Bothell, Washington, USA). The data associated with control of fire blight has been ambiguous. This may be due to the difficulty of protein uptake by plant tissue and the associated effect of DspE. Expression of hrpN in plant cells has not provided good plant protection either. A new approach is the studying of harpin with proteins in apple cells, called HIPM (HrpN-interacting protein from Malus) (Oh and Beer 2007). At the opposite side of the hrp cluster, open reading frames (ORFs) with disease enhancing effects were located. Three of them, hsvA, B, C are located in a distance to the ams region. (C) lsc region. The borders are pstS, belonging to the pst operon, and glmS, involved in glucosamine synthesis.

### Synthesis of capsular exopolysaccharide (EPS)

Another set of non-pathogenic E. amylovora mutants was deficient in synthesis of the EPS amylovoran (Bellemann and Geider 1992). This polymer forms a loose capsule around the bacteria protecting them against plant defence recognition mechanisms. Amylovoran is also involved in the protection of cells against desiccation (Jock et al. 2005) and may bind nutrients and cations. The EPS is synthesized by expression of 12 genes of the ams cluster (Bugert and Geider 1995) (Fig. 2). Most of them encode glycosyl transferases synthesizing the repeating unit of three galactosyl residues in the backbone and glucuronic acid and a decorated galactose in the side chain. Half of the repeating units bear a glucose residue as a second side chain. Additional ams genes are involved in the transport, polymerization of the repeating units, and the recycling of the lipid carrier (see Geider 2000).

Mutants in any of these ams genes are non-pathogenic as are mutants of the adjacent genes galP and galE, involved in the formation of UDP-galactose/UDP-glucose epimerase, whereas GalF has no direct enzymatic function and may act as a subunit of GalE or GalU (H. Ullrich, M. Schollmeyer and K. Geider, unpublished).

Regulation of the ams gene cluster is mediated by rcsA, B and C. RcsC is the sensor of a two-component system with kinase and phosphatase activities, activating RcsB, which forms a complex with RcsA for the induction of amylovoran synthesis (Kelm et al. 1997). A global regulator that includes regulation of amylovoran synthesis is H-NS (Hildebrand et al. 2006). Sorbitol seems to affect amylovoran production and is a major carbohydrate of rosaceous plants, the hosts of fire blight.

Amylovoran has a high molecular weight with 1 MDa from suspension cultures (Jumel et al. 1997) up to 5 MDa for EPS preparations on agar plates (M. Schollmeyer and K. Geider, unpublished). It can be degraded by a coat protein of bacteriophages into repeating units, which interferes with colonization of host plants by E. amylovora (Kim and Geider 2000). The viral EPS depolymerase gene has been cloned and expressed in bacteria and in pears and apples (see section below).

### Other virulence factors

A striking feature of E. amylovora is the formation of levan in the presence of sucrose. The enzyme levansucrase is secreted from the cells and cleaves the disaccharide into glucose and polymerizes the second sugar fructose into high-molecular-weight levan. Although levan is a neutral polysaccharide and is also produced by other plant-associated bacteria, it seems to provide a fast protective shield against recognition of pathogen invasion. Mutants in the lsc gene still produce symptoms on immature pears, but migrate slower in apple plants than positive strains (Geider and Geider 1993). Levansucrase expression is affected by the regulatory genes rlsAB, C and by orf105, which shows homology to pore forming holin-like proteins of bacteriophages (Du et al. 2004).

Most E. amylovora strains carry the common plasmid pEA29. In a few cases plasmid-free strains have been found in field isolates, but they can also be created by introduction of an engineered plasmid with the replication origin of pEA29 (Falkenstein et al. 1989). Strains without plasmid pEA29 have growth deficiencies in minimal medium without thiamine, where they grow to a lower density than "normal" strains. The nucleotide sequence of plasmid pEA29 revealed genes associated with thiamine synthesis (McGhee and Jones 2000). This vitamin may be limiting growth of E. amylovora that cannot synthesize thiamine when lacking pEA29.

Iron is essential for all living cells and therefore a competitive compound during bacterial colonization of plants. Several siderophores for iron uptake of E. amylovora have been described, and mutants were created in the genes dfoA and foxR (Dellega et al. 1998). These strains were affected to initiate necrotic symptoms on apple flowers, but weakly in colonization of apple seedlings, suggesting a complex iron uptake system of E. amylovora to substitute missing components.

Analysis of various transposon mutants revealed deficiencies in additional pathways of the cellular metabolism. These comprise amino acid auxotrophy (Bellemann and Geider 1992), and guanine metabolism (Eastgate et al. 1997). In some cases, the mutants were leaky, which add to the picture of best availability of nutrients for symptom formation.

### Sugar metabolism

Carbohydrates are the natural nutrients of plant pathogens. E. amylovora possesses metabolic pathways for sucrose (Bogs and Geider 2000) and the sugar alcohol sorbitol (Aldridge et al. 1997). The functions comprise carbohydrate transport and intracellular degradation. When genes in either pathway are mutated, E. amylovora loses virulence in host plants. Consequently, cleavage of sucrose during levan formation cannot substitute for the direct uptake of the sugar. Sorbitol has been associated with amylovoran synthesis without knowledge of links between both pathways. These carbohydrates contribute to colonization of host plants, where E. amylovora activity measured by hrp genes expression is correlated with sorbitol content changes in the host (Blachinsky et al. 2006), and therefore to optimal pathogen performance.
Identification of *Erwinia amylovora*

Levan formation is typical for many *E. amylovora* strains. Color changes of dyes on the agar medium with sucrose and dome-shaped colonies are indicative, although several strains have been described with low levan synthesis (Bereswill et al. 1997). Complementary, amylovoran production and its induction in the presence of copper ions with formation of yellow colonies are typical for *E. amylovora* (Bereswill et al. 1998). On the other hand, copper ions at low amino acid concentrations can be highly toxic for the growth of *E. amylovora* (Geider 1999).

Primers have been designed from DNA of plasmid pEA29 and the *ams* region to identify *E. amylovora* by PCR assays (see Geider 2005).

Bacterial shoot blight of pear in Korea and Japan caused by *Erwinia pyrifoliae*

Necrotic symptoms resembling fire blight have been observed on Nashi pear trees in South Korea and on the island of Hokkaido in Northern Japan. No epidemiologic studies have been published in an international scientific journal, but the causative agent of the disease was described in several reports (Rhim et al. 1999; Mizuno 2000; Kim et al. 2001; Shresta et al. 2003). The limitation of the pathogen to infect mainly pears indicates a difference to *E. amylovora*. Microbiological assays, sequence analysis of 16S rRNA, and DNA/DNA hybridization kinetics have classified the pear pathogen from Korea into the new species *Erwinia pyrifoliae* (Kim et al. 1999). Several reports based on nucleotide sequence analysis of 16S rRNA and of housekeeping genes (Kim et al. 2001; Waleron et al. 2008) have placed the pear pathogen from Japan closer to *E. pyrifoliae* and more distant to *E. amylovora*. In a pending publication with additional taxonomic criteria, the *Erwinia* strains from Japan will be co-classified with the pathogen from Korea as *E. pyrifoliae* (Geider et al. 2009).

Antagonistic bacteria for control of fire blight

Competition with growth of *E. amylovora* on plant surfaces as in flowers is an efficient way to reduce fire blight (John-son and Stockwell 1998). Several commercial products such as Blightban A506 and Blightban C9-1 are based on a *Pseudomonas fluorescens* strain, and *Pantoea agglomerans*, respectively, the success of which is dependent on both host and environmental conditions (Thomson and Guik 2003; Pusey and Curry 2004). Additional epiphytic bacteria have been investigated as fire blight antagonists (van Diezen 2009). Further, genic bacteria lack important features of phytopathogens (Table 2). *E. billingiae* cannot induce HR and lacks genes to metabolize sucrose, and *E. tasmaniensis* may not synthesize capsular EPS and cannot metabolize sorbitol. On the other hand, a gene cluster related to the *ams* region of *E. amylovora* exists in *E. tasmaniensis* (Kube et al. 2008).

Abundance of the bacteria, sprayed on flowers, reduces propagation of *E. amylovora* (Jakovljevic et al. 2008). Several mechanisms can be assumed, such as competition for nutrients or release of toxic compounds. No toxins have been identified for *E. tasmaniensis* (Geider et al. 2006) in contrast to *P. fluorescens* A506 and *P. agglomerans* C9-1.

Other mechanisms for growth interference with plant pathogens could include signal molecules for bacterial communication called autoinducers, such as AI-2 (Mohammadi and Geider 2007).

It can be concluded that high concentrations of bacteria do not only reduce their own growth speed by entering the stationary phase, but also imply growth retardation for other bacteria. Survival in high densities and release of growth regulators may account for their efficiency. Increases in the density of antagonistic bacteria may only occur at a low level, but they may not grow as dense as the pathogen, because they lack the tools for destructive distribution in plant tissue to access abundant amounts of nutrients.

Bacteriophages to control fire blight

Bacteriophages are designed to live at the expense of their bacterial host cells. They attach to cell receptors and inject their nucleic acids into the cell to replicate genome of *amylovora* (Bereswill et al. 1998). At the end of their growth cycle, they assemble new phage particles and usually express a lytic principle for cell lysis. Their application for control of fire blight is still limited to an experimental stage. A protein complex from a Serratia prophage (Jabrane et al. 2002), called Serratin P, has been applied to destruct *E. amylovora*. Viral lysozymes have been used to damage bacteria. Their effect against Gram-negative bacteria is low, because lysozymes do not find targets from the outside of cells (Geider 2006).

As mentioned before, *E. amylovora* cells are encapsulated by the polysaccharide amylovoran. Bacteriophages have to penetrate this layer in order to reach the receptors on the cell surface. For this purpose, they carry a coat protein which can cleave the EPS capsules. The gene encoding the EPS depolymerase gene of *E. amylovora* phage øEt1 has been cloned and expressed in *E. coli* cells (Kim and Geider 2000). The enzyme finally cleaves amylovoran in the galactose backbone into repeating units. Subsequently, the bacteria are recognized by plant defence mechanisms and inactivated.

CLASSICAL BREEDING, GENETIC RESOURCES AND RESISTANCE PHENOTYPING

Breeding strategy and genetics of resistance

With the increased importance as reflected by the losses caused by the disease, the preference to breed for fire blight resistance as a means to control it in pomefruit is increasing in countries where *E. amylovora* has established. Major breeding programmes that have the development of fire blight resistant apple and pear cultivars as an objective are based in the USA, New Zealand, Canada, Turkey, Poland, Germany, Italy, Switzerland, France, and Czechia. The main focus of these programmes is on improving in-plant resistance, but this strategy can be complemented by selection for fire blight avoidance aimed at reducing the opportunities for infection by the pathogen. Preventing trees from producing secondary flowering, which often can be achieved by regular production, has been used as a selection criterion in the pear breeding programme at East Malling, UK (Alston 1994). Also, spur-type trees tend to show lower susceptibility than trees with normal vegetative characteristics, which has been attributed to a higher lignification ratio (Abdollahi and Majidi 2005).

Excellent summaries of apple and pear breeding have been given by Bell et al. (1996) and Janick et al. (1996). Here we provide a short overview of specific aspects of apple and pear breeding for fire blight resistance. Apple and pear trees in general are a combination of a rootstock and a scion grafted onto it. As fire blight is known to infect rootstocks as well as scions, fire blight resistance breeding is relevant to both. The term “classical breeding” used in this paper means the generation of genetic variability by sexual crossing of two selected parents and the selection of progeny fitting breeding aims best. The generation of genetic variability by mutagenesis or somaclonal variation will not be discussed here, although these methods have been applied to improve fire blight resistance in apple and pear (Pinet-Lebly et al. 1992; Donovan et al. 1994). The use of genetic engineering will be reviewed below.

Apple and pear are both highly heterozygous and are propagated clonally in general, but some rootstocks are propagated sexually by seeds from apple cultivars like ‘Bittenfelder Sämling’ and ‘Graham’, and from pear cultivars, such as ‘Kirchenasser Mostbirne’ and ‘Augustbirne’. The high
heterozygosity leads to a very diverse progeny to select from. Both apple and pear have a gametophytic self-incompatibility system limiting inbreeding, but self-compatible cultivars can be found in apple (Matsumoto et al. 1999) as well as in pear (Sato et al. 1988). Although inbreeding causes growth depression and an accumulation of lethal genes, Karnatz (1988) selected some promising inbreds from ‘Goldene Delicious’.

While the low genetic correlation between fire blight resistance and fruit quality traits indicate there are no genetic impediments to combining them in commercial cultivars (Bell et al. 1976), classical breeding in pomefruit has several limitations. The self-incompatibility and the high degree of heterozygosity of apple and pear prevent the introduction of single traits into a given cultivar by repeated backcrossing. Introduction of traits in pomefruit in general is done by pseudo-backcrossing and recurrent selection in the progeny. The use of wild species with small fruit of very low fruit quality and other undesirable traits as donors for fire blight resistance requires repeated pseudo-backcrossing, and therefore takes many years. An example of this strategy is the introgression of scab resistance from the wild species accession M. x floribunda 821 (M/821) into the domesticated apple, with the first cross made in 1914 (Crandall 1916). In the 1970s, many hybrids have been released, but to date none of these varieties have been able to compete with the major apple cultivars based on fruit quality. The single gene dominant nature of the F1 scab resistance facilitated introgression as phenotyping is relatively easy. However, scab resistance of M/821 is overcome now by the development of pathogen strains virulent to M/821, confirming the vulnerability of single gene resistance. In contrast, resistance to fire blight generally is quantitatively inherited (Gardner et al. 1980b; Korban et al. 1988; Fischer 1996; Fischer and Fischer 1996), although there are some reports that suggest the presence of major genes in some resistance sources (Gardner et al. 1980b; Peil et al. 2007a). This is supported by differential interactions, which usually are associated with major genes, shown by some E. amylovora strains (Norelli et al. 1984, 1986; Fazio et al. 2006). Therefore, it is necessary to pyramid several resistance quantitative trait loci (QTL) that condition different mechanisms of resistance in order to achieve durable resistance. Wild species offer a large pool for fire blight resistance breeding, but since this, as mentioned above, is a slow and expensive process, it would be facilitated by breeders cooperating and exchanging pre-breeding material. Another pathway available to the breeders to speed up the process of backcrossing is reducing the long juvenile period of pomefruit, which will be discussed later in this review. This strategy may prove to be a faster way to resistant cultivars than pyramiding QTL that often confer low levels of partial resistance from commercial cultivars and advanced selections. But even in crosses between cultivars susceptible to fire blight, progeny with some resistance, on average 10% in one study (Fischer and Richter 1999), can be detected. Transgression can also be observed as Töth et al. (2006) found in progeny of P. pyrifolia by partial resistance in Poland.

In pear, initial genetic studies suggested the presence of dominant resistance genes in germplasm derived from Pyrus serotina (Drain 1943) and in P. ussuriensis selection 76 (Thompson et al. 1962). However, this theory was later discarded in favour of the resistance of selection 76 being recessive to the S allele for sensitivity to fire blight (Thompson et al. 1975). A number of P. communis accessions were identified by Ueno et al. (2000) found for heterozygous and a few as homozygous for the allele, a situation ideally suited for marker assisted selection (MAS) to identify suitable breeding parents and resistant progeny from breeding populations. Although there was some suggestion for such a gene in another study (Bell et al. 1977), there have been no further confirmations of this hypothesis as segregations in later studies were more in agreement with resistance predominantly being additive (Layne et al. 1968; Bell et al. 1977; Quamme 1981; Bagliano et al. 1996; Fischer 1996; Lespinasse and Aldwinek 2000). This is also more consistent with the finding that some progenies produced an excess of apparently sensitive seedlings (Thompson et al. 1975), which suggests that the parental combinations were not useful for resistance breeding. Another explanation may be that they simply were the result of segregation distortions.

Genetic resources

Knowledge of the fire blight resistance status of cultivars is essential to breeders. A valuable source of information about fire blight resistance of apple and pear cultivars is the review by van der Zwet and Keil (1979). They made a comprehensive literature study and collected data describing fire blight resistance of apple and pear species and cultivars. Of the 400 pear cultivars listed, 17% were predominantly reported as resistant, 33.5% as moderately resistant, 38% as susceptible, while the resistance status of 11.5% of the accesses varied in different reports. Of the 390 apple cultivars listed, 35% were reported to be resistant, 26% moderately resistant, 22% susceptible, and the classification varied for 17% of the cultivars. Van der Zwet and Keil (1979) regarded 0-6% of a tree blighted as resistant, 7-25% as moderately resistant and more than 25% of a tree blighted as susceptible. Since this extensive review, many more reports on fire blight resistance of pomefruit have been published and we will provide a short overview here with a focus on more recent findings in the two major crops, apple and pear. New research activity has developed in this area, often on local germplasm, following the recent incursions of E. amylovora into countries that previously were free of the disease. The sourcing of resistance to fire blight is expected to continue in future, sometimes in the form of international collaboration, which reflects the importance of the disease. For example, in 2007, a ring test to evaluate apple and pear cultivars and selections from European breeding programmes was started in the context of the European COST-Action 864 ‘Pome Fruit Health’.

Reports on germplasm evaluations of loquat (Eriobotrya japonica) (Tsiantos and Psallidas 2004) and quince (Cydonia oblonga) (Maroofi and Mostavafi 1996; Citir and Mirik 1999; Bobev and Decker 1999) are few, which reflects the lesser economic importance of these fruit species on a worldwide scale. Quince, however, is important as a major rootstock in pear production, even though all the current quince rootstocks are (highly) susceptible to fire blight (Lombard and Westwood 1987; Le Lezec et al. 1997b). Pear rootstocks will be discussed below. In contrast to Cydonia, some accessions of the flowering quince (Cheno melos spp.) will be moderately resistant, while the cultivar ‘Contorta’ is highly resistant to fire blight (Bell et al. 2005). Germplasm evaluations for fire blight resistance have been performed in Europe on several other ornamental species in the Rosaceae, such as Cotoneaster, Crataegus, Pyracantha and Sorbus (van der Scheer 1984; Richter 1989; Wilson et al. 1990; Lecomte and Cadic 1993), while most of the breeding activity has been directed at Pyracantha (Bouma 1988, 1990; Cadic 1987; Cadic et al. 1997).

Many germplasm screening studies have been published in recent years, but to date no standard procedure for the evaluation of fire blight resistance exists. The collected data are from trials that vary in bacterial strains used, location and conditions of screening, inoculum concentration, tissue of inoculation, inoculation procedure, and disease measurement and rating. Thus, the comparability of results is often very low. Heterodendron procedure could help to confer reliable and reproducible assessment of fire blight for breeders to select material for breeding. Accepting the scoring system developed for rating fire blight severity on pear trees in the orchard (van der Zweit et al. 1970), but is also well-suited to apple (e.g. Luby et al. 2002), would be a major step on the way to standardization.
Sources for fire blight resistance can be found among wild species and domesticated cultivars. Whereas resistant cultivars may provide breeding success in the first generation, the introgression of resistance from wild species requires several backcross generations to reduce the proportion of unwanted genome of the wild species. Lepinsasse and Aldwinckle (1999) reported that of those sampled during the period between 1920 and 1978, 41% were resistant to fire blight. This appears to be a high proportion, but one has to consider that only a few cultivars, such as ‘Golden Delicious’ and ‘Fuji’, account for the major proportion of the apple production worldwide, and that most of the worldwide important cultivars are susceptible to fire blight. Nevertheless, the breeding progress is promising, which raises the expectation of fire blight resistant cultivars that are able to compete with the current best varieties in the market in the near future.

The genus Malus consists of different species, but the number of different species and the classification into species remains a point of discussion. The number of species recognized by taxonomists ranges from eight to about 122 (Korban and Chen 1992; Robinson et al. 2001; Harris et al. 2002). The taxonomy used in this study was according to Forsline et al. (2003), who reported 27 species in the genus Malus. The domesticated apple is commonly referred to as M. x domestica, although M. pumila might be the correct nomination (Korban and Skirvin 1984). M. x domestica is likely to be an interspecific hybrid. M. sieversii, a wild species native to Central Asia, is assumed to be a major progenitor of the cultivated apple (Morgan and Richards 1993; Juniper et al. 1999), and this region is regarded the center of origin of the domesticated apple (Vavilov 1951; Janick et al. 1996). Several collection trips have been made to Central Asia and the seedlings raised were evaluated for fruit quality and susceptibility/resistance to diseases, including fire blight (Luby et al. 2001). The material from the centres of origin appears to hold promise for resistance to fire blight.

Momot et al. (1999a) inoculated tips of vigorously grown shoots of 1,335 M. sieversii seedlings, raised from seed collected in 1989 and 1993 from 79 mother plants at six sites in Kazakhstan, Kyrgyzstan, Tajikistan and Uzbekistan, with the American E. amylovora reference strain Ea273. Resistance, defined as <20% shoot necrosis, differed among populations and between sites. Some populations had 80% resistant progeny, whereas others had no resistant progeny. Populations from two sites in Kazakhstan and one habitat in Tajikistan showed significantly less disease severity than accessions from the other sites. M. x sieversii seedlings from Uzbekistan with red fruit and 56 mm in diameter is valuable for breeding purposes. Later, Forsline and Aldwinckle (2002) described the natural occurrence of fire blight on 1,151 seedlings of M. sieversii from eight ecosystems in Kazakhstan that were grown in the USDA Apple Germplasm Collection at Geneva, New York State (USA). About 25% of the seedlings showed infections over a four year period and 110 of them were removed because of severe fire blight. In a field evaluation of additional M. sieversii germplasm in New York and Minnesota states, a high proportion (45%) of the New York seedlings were rated moderately to very resistant (Forsline et al. 2008). Follow-up assessment in the glasshouse on about half of the progeny confirmed the resistance in nearly 60% of the seedlings. The level of field resistance of M. sieversii was confirmed as being higher compared with M. x domestica germplasm in a study in New Zealand (Luby et al. 2002). Although natural fire blight occurrence cannot prove resistance, it is a good indicator for resistance and M. sieversii is regarded as a valuable source for breeding based on its performance in the field and its moderate to large fruit size.

A range of other wild species has been characterized for their reaction to fire blight. The most extensive study ever performed has been the one on ornamental apples over multiple years and sites across the USA, the data of which has been collated since 1964 by Drs Nichols and Green (Green and den Boer 1995). In the period 1988-1993, over 50,000 observations were made on 646 accessions in the National Crabapple Evaluation Program, not only for fire blight, but also for apple scab (Venturia inaequalis), powdery mildew (Podosphaera leucotricha), and cedar-apple rust (Gymnosporangium juniperi-virginiana) as well as aesthetic value. Accessions on which no infections were observed in over 100 field tests were designated as resistant. Only M. sieversii, ‘Branzam’, ‘M. Cascole’, M ‘Coralcole’, M. ‘Ökonomerat Echtermeyer’, and M. ‘Pink Perfection’ (Green and den Boer 1995). Aldwinckle et al. (2002) evaluated seedlings raised from seed of M. hupehensis, M. kansuensis, M. pratii, M. sieboldii, M. toringoides, M. transitoria, M. yunnanensis, and M. zhaojiaoiensis from Sichuan, China; M. orientalis from Russian Caucasus and Turkey; and M. sylvestris from Germany. The seedlings were inoculated by bisecting the two youngest leaves with scissors dipped in a suspension of strain Ea273. Resistant seedlings were found in all species, but only a few in M. yunnanensis and M. sylvestris, while the proportion of resistant seedlings ranged from 0 to 100% among M. hupehensis accessions. This study complemented a previous one on 139 clones of 46 Malus species, subspecies and hybrids with the same E. amylovora strain used widely in Europe and ‘Spang’, the reference strain. In most cases different accessions of a species showed consistency in disease severity, but, for example, accessions of M. x micromalus showed a large variation in infection.

Table 3 shows the results from recent artificial fire blight inoculations of wild species and hybrid accessions performed on accessions of the German Dresden-Pillnitz apple germplasm collection. Inoculations were performed on grafted shoots over several years with a mixture of three virulent E. amylovora strains by bisecting the two youngest leaves. The results indicate a great variability in resistance between and within the species, with the highest proportion of resistant accessions found in M. baccata, M. fusa, M. prunifolia, and M. yunnanensis, and in the hybrid species M. x dawsoniana, M. x floribunda, M. x robusta, and M. x zumi (Aldwinckle et al. 1999). In most cases different accessions of a species showed consistency in disease severity, but, for example, accessions of M. x micromalus showed a large variation in infection.
susceptibility to repeated artificial shoot inoculations with the disease (Richter and Fischer 2004). Previously, some selections of the Pi series of apples, such as 'Pinova' that were not specifically bred for disease resistance, have appeared in the European market at the USDA site in Kearneysville, even though the flowers of all accessions were shown to be highly susceptible following artificial inoculation (van der Zwet and Bell 1995). These accessions were 'Istambulsko Ahce', 'Karamanka', 'Koreljaci', 'Lubenicarka', 'Maslinka', 'Sijerak', 'Smokvarenica', 'Sytovina', and 'Zdravka'.

In the years, such as 'Gyaver' ('Kieffer'), and the local varieties 'Ek/g250i Gökdulu' and 'Kara Ç/g213b/g213k', were identified as less susceptible in a second Turkish study performed on 35 local accessions of the five most important Prunus species (Peil et al. 2004). Fischer (2005) determined the resistance to fire blight on progenies of crosses between pear species and of open-pollinated progeny in wild species. Only two (P. canescens x P. serrulata and P. betulifolia x P. ussuriensis) of 28 combinations produced progeny with improved levels of fire blight resistance.

A broad overview of resistance of wild pear species is given by van der Zwet et al. (1974). They analysed 107 selections of 17 species, 85 selections from controlled interspecific crosses, and a large number of pear species hybrids. Van der Zwet and Keil (1979) reported the relative resistance to fire blight of the five most important Prunus species P. ussuriensis, P. calleryana, P. betulifolia, P. pyrifolia and P. communis (in descending order of resistance), but a certain degree of resistance could not be assigned to a species, because of the range of resistance present in each species. The high degree of resistance of P. ussuriensis was confirmed by Bell et al. (2005), who determined the resistance of 27 pear taxa, including cultivars, clonal selections, and hybrids of P. amygdaliformis, P. betulifolia, P. calleryana, P. elaeagrinifolia, P. fauriei, P. koehnei, P. nivalis, P. pyrifolia, P. regelii, P. salicaria, and P. ussuriensis, by inoculating actively growing shoots with the cut-leaf method. P. ussuriensis 'Prairie Gem' and a clone derived from open pollination of a P. calleryana x P. betulifolia hybrid were highly resistant, showing less than four percent shoot necrosis. Accessions of P. elaeagrinifolia, P. fauriei, P. koehnei, P. nivalis, P. pyrifolia, and P. salicaria, were described as highly susceptible. A natural fire blight infection of the orchard at the Institute of Fruit Breeding in Dresden-Pillnitz badly affected the pear wild species germplasm collection in 2003. All accessions in stock of P. aromatica, P. austriaca, P. orthocarpa, P. pyrifolia, P. sinensis, and one accession of P. betulifolia succumbed to the disease (Peil et al. 2004). Fischer (2005) determined the resistance to fire blight on progenies of crosses between pear species and of open-pollinated progeny in wild species. Only two (P. canescens x P. serrulata and P. betulifolia x P. ussuriensis) out of 28 combinations produced progeny with improved levels of fire blight resistance.
and Decker 1999). ‘Beuré Giffard’ previously had demonstrated high resistance, but was not as consistent as other old cultivars, such as ‘Beuré Alexandre Lucas’ and ‘Richard Peters’ over four years of evaluation (Thibault et al. 1987b, 1989). Field inoculation of shoots of 20 perry pear cultivars in Germany showed identified the cultivar ‘Wahlische Schnapsbirne’ as being highly resistant (Zeller and Zeller 1994).

A number of advanced selections and new cultivars of the domesticated pear with fire blight resistance have been developed. The most resistant selections, P448-9, P384-39, P384-52 and P384-49, from the East Malling breeding programme have accession 13B83 as a parent, which is an F2 derivative of ‘Farmingdale’ (Alston 1994). In Italy, nine breeding lines showed resistance comparable to ‘Harrow Sweet’, two of which have good horticultural characteristics and have been released as ‘Aida’ and ‘Bohème’ (Bergamaschi et al. 2006). In the Czech Republic, the new cultivars ‘Bohemica’ and ‘Jana’, and selection US-62563-004 were identified as more resistant than ‘Beuré Alexandre Lucas’ (Papprête et al. 2006). The Canadian pear breeding programme by Agriculture and Agri-Food Canada has long been active in breeding for fire blight resistance in pear and has released a number of cultivars in the Harrow series. ‘Harrow Delight’ and ‘Harvest Queen’, released in the early 1980s (Quamme and Spearman 1983), are the parents of a number of new breeding lines being considered for commercialisation with resistance that is stronger than that of ‘Kieffer’ and, in some cases, ‘Old Home’ (Hunter and Bonn 1999). The high resistance of ‘Kieffer’ and ‘Harrow Delight’ was confirmed in tests in Hungary (Honty et al. 2006), and of ‘Harrow Delight’, together with ‘Maggness’, ‘Moon-glow’, ‘Peral Magallon’ and the Italian breeding line 805172, in Greece (Tsiantos and Psallidas 2004). The Asian pear ‘Hosui’ also showed good resistance in shoots and flowers (Honty et al. 2006), which for the latter agrees with earlier findings, but it is generally regarded susceptible to shoot blight (Lecomte 1993). In this study, ‘Shinko’ showed the highest resistance to *E. amylovora* in both shoots and flowers, followed by ‘Jing Bai Li’ and ‘Xue Hua Li’ (Lecomte 1993). ‘Shinko’, ‘Maggness’, ‘Maxine’ and ‘Moon-glow’ also remained free of disease in the field following a major infection period coinciding with the full bloom period of most cultivars in a germplasm collection in Oregon (Spotts and Mielke 1999) and following artificial inoculation in a French study (Le Lezec et al. 1997b). In the latter study, the 11 accessions that were equally or more resistant than ‘Harrow Sweet’ comprised six selections from the Canadian Harrow, and three from the USDA breeding programme as well as ‘Old Home’; these new breeding lines are usually characterized by their high resistance in both flowers and shoots (Thibault et al. 1989). In the Oregon study, ‘Doyenne du Comice’, which is regarded highly susceptible to fire blight (Thibault et al. 1989; Le Lezec et al. 1997b), was not infected when on ‘Bartlett’ and OHxF333 rootstocks, in spite of being in full bloom during the four day infection period (Spotts and Mielke 1999).

A source of fire blight resistance is the USDA genebank in Corvallis, Oregon maintaining more than 160 fire blight resistant Asian cultivars, European and hybrid cultivars and rootstock or species clones (Postman 2008).

**Genetic resources – apple rootstocks**

Rootstock blight is the most fatal form of fire blight in an orchard. Besides directly infecting the rootstock through suckers, *E. amylovora* can be transmitted from the point of infection in the scion to the rootstock through asymptomatic tissue (Momol et al. 1998). Resistant rootstocks cannot prevent fire blight infection of susceptible scion cultivars, but prevent tree losses due to rootstock blight (Norelli et al. 2003a; Aldwinckle et al. 2004). Of the traditional rootstocks of the Malling (M) and Malling-Merton (MM) series, M.7 is regarded resistant, followed by M.2, M.4 and M.111, which are moderately resistant (Ferree and Carlson 1987; Berger and Zeller 1994). The most common rootstocks used for their dwarifying traits, M.9 and M.26, are susceptible.

The most advanced and successful program for breeding fire blight resistant rootstocks is the Geneva rootstock breeding program. The phenotyping strategy for selecting resistant rootstocks consists of inoculating with a mixture of different *E. amylovora* strains in order to select rootstock clones resistant to strains of differing virulence (Norelli et al. 1987). Since 1991, several clones of the Geneva™ (G) series have been designated and released for commercialization. The resistant rootstocks G.11 (tolerant), G.30, ‘Geneva™ 3041’ (CG3041, previously G41), G202 and G935 are descendants of *M. robusta* 5; G.16 of *M. floribunda*; and G65 from a cross of M.27 x ‘Beauty Crab’ (Robinson et al. 2003; Russo et al. 2006). Orchard performance of Geneva™ rootstocks was discussed by Robinson et al. (1999, 2003). Resistance to fire blight was determined in several trials by artificial inoculation of ungrafted liners in the greenhouse or spray-inoculation of blooming orchard trees with ‘Royal Gala’ as scion grafted on the respective rootstocks (Gardner et al. 1980a; Norelli et al. 2002). Differential susceptibility to *E. amylovora* strains was observed for some of the Geneva™ rootstocks (Norelli et al. 2003b; Fazio et al. 2006). Although CG3041 is derived from *M. robusta* 5, it is virtually fully resistant to all strains tested, including the strains reported to overcome *M. x robusta* 5 resistance (Norelli et al. 1986), which suggests that it has inherited other resistance genes.

Interesting results have been presented for the Russian rootstock ‘Budovsky 9’ (B.9), selected from a M.8 x ‘Red Standard’ family. Inoculated rootstock liners of B.9 showed very severe disease symptoms, whereas orchard trees grafted on B.9 displayed high levels of resistance to rootstock blight in several field trials (Norelli et al. 2003b; LoGiudice et al. 2006). Nevertheless, because of the high planting density in the stoolbed, vegetatively propagated rootstocks are highly amenable to infection with fire blight transmitted by wind, insects, or rain, or mechanically (Fischer 2001), which poses a high risk on propagating rootstocks susceptible to fire blight.

Several other breeding programs selected rootstock clones resistant/tolerant to *E. amylovora*. Norelli et al. (2003b) tested many different rootstocks in greenhouse trials with *E. amylovora* strains differing in their pathogenicity. G11, G65, G16, G30, Pillnitzer AU 51-11, M.7, and several breeding clones were regarded as the most resistant, whereas B.9, ‘Ottawa 3’, M.9, and M.26 were the most susceptible. Webster (2003) in his review on breeding and selection of apple rootstocks mentioned M.2, M.4, M.7, M.25, B.11, M.45, B.490, ‘Beurre’, G11, G16, G.30, M.75, G.165, CG3041, CG4202, ‘Supporter 1’, ‘Novole’, OAR1, V1, and V.3 as tolerant/resistant. CG3041 and CG.11, as well as CG.007, the Japanese rootstock ‘Morioka 10’, and the species *M. prunifolia* and *M. sieboldii*, which are commonly used in rootstock breeding in Japan, were also found highly resistant by Bessho et al. (2001). Combining the findings from three years of field inoculation experiments, five (V.1, V2, V3, V6 and V7) of the seven Vineland rootstocks from Canada consistently showed less or similar shoot necrosis compared with M.7 (Cline et al. 2001), the minimum standard of the resistant reference cultivars for the Geneva breeding programme (Cummins and Aldwinckle 1983). As commonly encountered with fire blight (see below), results of screenings are not always in accordance with each other. Whereas Norelli et al. (2003b) determined Pi-AU 51-11 as resistant and Webber et al. (2003) as tolerant/resistant, both rootstocks were medium susceptible in screenings performed in Germany using an inoculum mix of three virulent strains (Fischer 2001, Richter pers. commun.). This might be due to different inoculum techniques, environmental conditions in the greenhouse, strains used or other reasons. A common screening method is necessary to allow comparability and reliability of results.
Genetic resources – pear rootstocks

Next to Pyrus seedlings, quince rootstocks, such as Quince A (QA), Quince C (QC), Quince Adams and Q BA29, are the most common rootstocks in pear production (Lombard and Westwood 1987; Le Lezec et al. 1997b). They however also are most susceptible to fire blight and there appears to be little opportunity for breeding resistant rootstocks from Cydonia germplasm. In contrast, resistance bred into Pyrus has been very successful and is mostly based on the highly resistant OHxF rootstocks (Lombard and Westwood 1987). The parents of these rootstocks, ‘Old Home’ and ‘Farmingdale’, are generally regarded as highly and moderately resistant, respectively (van der Zwet and Keil 1979).

The high resistance of the OHxF rootstocks, which were selected from a range of crosses in the extensive Oregon breeding programme (Reimer 1950), was confirmed in France (Le Lezec et al. 1997b), while OHxF333 remained unaffected in a test in Germany (Berger and Zeller 1994). In the Czech Republic, OHxF87 was the preferred out of four fire blight resistant OHxF rootstocks for its horticultural characteristics (Papstein et al. 2006). At East Malling, the rootstock selection QR517-9, which is an open-pollinated progeny of ‘Ankara’ pear, was shown to be highly resistant, while the open-pollinated progeny QR193-2 derived from Q51 was marginally more resistant than ‘Conference’ (Alston 1994).

Resistance phenotyping

One of the most important points in breeding is a reliable determination of phenotypic traits. The breeder has to look in his sources for crossings for the respective trait and in the progeny to verify that the trait was inherited. An early phenotyping in progeny is desirable but for fire blight not always possible. In some countries such as Germany fire blight is a quarantine disease and planting of inoculated seedlings into the field is not allowed. Depending on the purpose and feasibility, different screening methods are required.

The common strategies used by breeders for the sourcing of resistances basically are artificial inoculation of grafted scions in the glasshouse or field, and screening of germplasm in the field by relying on natural infection. The inoculation of immature fruit is not a useful measure of resistance (Paulin et al. 1990), nor excised leaf bioassays with leaves harvested in the field (Donovan 1991), but the use of in vitro techniques holds some promise (Duron et al. 1987; Viseur and Tapia y Figueroa 1987; Viseur 1990; Donovan 1991). Applying the different phenotyping techniques to the same germplasm will readily lead to different findings on the genetics of resistance (Thompson et al. 1975). Field evaluation of flowering trees (e.g. Spotts and Mielke 1999), Forsline and Aldwinckle (2002) or Peil et al. (2004) provides a more true picture of the resistance, but also will more readily lead to escapes, hence overestimation of resistance, since the success of this approach is highly dependent on the occurrence of susceptible flower tissues and the right environmental conditions for infection. Without sufficient disease pressure, one year (Chartier et al. 1992) and even multiple year (Abdollahi and Majidi 1997) observations will not be sufficient. Many factors, such as host (e.g. vigour, nutritional state, cell turgor, tissue age) and environmental (both soil and weather) conditions (van der Zwet and Keil 1979; van der Zwet et al. 1981; Lemaire et al. 1990; van der Zwet and Beer 1981; Suleman and Steiner 1994; Pusey 2000; Shwartz et al. 2003; Pusey and Curry 2004; Blachinsky et al. 2006; Brisset and Paulin 2006; Pusey et al. 2008); the rootstock used (Spotts and Mielke 1999); and the presence of non-hosts species of the Rosaceae that can support epiphytic growth of E. amylovora (Johnson et al. 2006), affect disease establishment and development. Luby et al. (2002) accommodated the variable conditions by taking into account both the flowering time and infection periods in the evaluation of diverse germplasm with varying flowering times in the field. In some cases, artificial inoculation of trees in the field is possible under environmental conditions conducive to the disease, or natural fire blight incidences in orchards have been applied to describe phenotypic reactions of cultivars, breeding clones or wild species accessions. For example, field inoculation was applied to 14 commercial varieties, one crabapple pollinizer and the rootstock M.9 for a period of six years (Mielke et al. 1999). This approach was used to evaluate apple, pear and rootstock germplasm and breeding lines (Le Lezec et al. 1997a, 1997b) and in Germany to test if results from greenhouse screenings and natural inoculations are correlated (Peil et al. 2004).

Artificial inoculation under optimal conditions will improve the chances of establishing infection (Thompson et al. 1962; Thibault et al. 1987a, 1989; Le Lezec et al. 1997a, 1997b), but will not necessarily make the findings from different experiments comparable. For fire blight, different phenotyping procedures have been reported varying in inoculated tissue, inoculation procedure, concentration of inoculum, site of inoculation (orchard or greenhouse), screening procedure and scoring scale. Today, most experiments involve shoot inoculation by hypodermic needle (Norelli et al. 1961) or cut-leaves (Geesteranus and Heyting 1981) and scoring as lesion number, lesion length (LNL) and severity with a modified version of the area under disease progress curve (AUDPC) (e.g. Khan et al. 2006) measured as the percentage necrosis length of the total shoot length. These methods are more commonly and Mosthain (1996) favours the QTIL in spite of the call for developing an internationally standardised assay technique for genetic studies (van der Zwet and Keil 1979).

In the orchard, trees are primarily infected through blossoms, while greenhouse tests are mainly performed by artificial shoot inoculation. The correlation between susceptibility of shoots and flowers is weak (e.g. Le Lezec et al. 1997a). To fair (e.g. Mielke et al. 1999) and moderate for pear (Thibault et al. 1989). In one study on apple, the largest discrepancies were shown for ‘Reinette Clochard’, which showed high susceptibility for the shoots and low for the flowers, while the reverse was the case for ‘Royal Gala’, ‘Mutsu’ and ‘Blushing Golden’ (Le Lezec et al. 1987). Peil et al. (2004) found a good correlation between susceptibility of trees in the field under natural conditions and scorings of artificial inoculations in the greenhouse for many of the seed-resistant Re-cultivars that are resistant to fire blight, but almost no correlation for the Pillnitizer Pi-cultivars, which are more or less susceptible to fire blight. Glasshouse screening is most efficient in identify-ing highly susceptible phenotypes (Maas Geesteranus and Heyting 1981; van der Zwet et al. 1981; Lespinasse and Aldwinckle 2000), and possibly the highly resistant ones, therefore is a great improvement over observations of natural infections in the field. For intermediate conditions and scorings of artificial inoculations in the greenhouse for many of the seed-resistant Re-cultivars that are resistant to fire blight, but almost no correlation for the Pillnitizer Pi-cultivars, which are more or less susceptible to fire blight. Glasshouse screening is most efficient in identify-ing highly susceptible phenotypes (Maas Geesteranus and Heyting 1981; van der Zwet et al. 1981; Lespinasse and Aldwinckle 2000), and possibly the highly resistant ones, therefore is a great improvement over observations of natural infections in the field. For intermediate
resistant accessions, the success rate of glasshouse screening is variable and needs to be followed up with field evaluations (Fischer and Schäfer 1990).

A major factor in comparing phenotyping results is the strain of *E. amylovora* used as differential host-pathogen interaction have been shown to exist in the *E. amylovora-Malus* pathosystem and often is associated with the host from which the strain was isolated (Quamme and Bonn 1981; Norelli et al. 1984, 1986, 1987; Paulin and Lespinasse 1987, 1990; Korban et al. 1988; Bell et al. 1990; Taylor et al. 2002; Fazio et al. 2006; Pulawska et al. 2006). Also, isolates may behave differently when inoculated individually or in a mixture, with mixtures being common for the screening of breeding populations (Paulin and Lespinasse 1990). Different isolates may therefore identify different QTL for fire blight resistance in the same accession, as was recently demonstrated for ‘Robusta 5’ (Gennaro et al. 2005) and the locus E/Mf = ‘Fiesta’/‘Evereste’ (Malus floribunda 821). The existence of such differential interactions raises the question whether the individual components of polygenic resistances show gene-for-gene relationships (Flor 1956) and therefore are exposed to the same risk as major genes are to changes at avirulence loci in the pathogen. The high susceptibility of the normally highly resistant accession ‘Robusta 5’ to isolate Ea 266 (Norelli et al. 1986) lends strong support to this hypothesis.

**MOLECULAR BREEDING FOR FIRE BLIGHT RESISTANCE IN POMEFRUIT**

Genetic marker development and mapping of resistances to fire blight in pomefruit has been slow compared to that of resistances to other pests and disease in apple and pear. As discussed above, while extensive germplasm evaluations have resulted in the identification of many sources of resistance to this disease, to date only two QTL that are of interest for cultivar breeding have been mapped on the apple genome (Gardiner et al. 2007). As discussed above, the disease being notoriously difficult to phenotype and the quantitative genetic nature of fire blight resistance are factors in the slow progress made to date. A good understanding of its heritability based on robust, but elaborate phenotyping techniques is required for the efficient breeding of new resistant cultivars (Lespinasse and Aldwinckle 2000) as well as genome mapping of QTL. Here, we present an overview of the QTL mapped in pomefruit to date. The approach to genetic mapping differs from the one applied to major genes, since a well saturated linkage map is required. A bulked segregant analysis (BSA) between extremely resistant and susceptible progeny may still enable the identification of major QTL, but generally will not allow the resistance complex to be fully determined (Collard et al. 2005). Until recently, well-saturated genetic maps were relatively expensive to develop and therefore formed a bottleneck for the mapping of resistance factors. However, with molecular techniques developing fast, map development costs become less of an obstacle, and more so if the map is made for a breeding population that is segregating for a range of traits of interest. Genetic maps have been developed for up to 20 pomefruit accessions to date (Gardiner et al. 2007; Itai 2007). The few used for fire blight resistance QTL identification in apple and pear will be discussed in more detail below.

**Fire blight resistance QTL in apple**

‘Fiesta’

A major QTL for fire blight resistance was first identified in the apple cultivar ‘Fiesta’ (F) in crosses with ‘Prima’ (P) and ‘Discovery’ (D) in France (Calenge et al. 2005) and was confirmed in a second family of ‘Fiesta’ x ‘Discovery’ (FxD) in Switzerland (Khan et al. 2006). The QTL explained about 35–40% of the phenotypic variation from 7 to 27 days post inoculation (DPI) across all experiments with a very high correlation between the observation dates. It maps towards the lower end of linkage group 7 (LG7) near marker GE80-19 (Calenge et al. 2005a) (Fig. 3) and the locus has been named FBF7 (Khan et al. 2007). A second, very minor QTL was identified at the top end of LG3, but only in the cross with ‘Discovery’. It was significant (LOD score over 3) for the necrosis at 7 dpi, where it explained only 4.4% of the phenotypic variation, but not at 14 dpi (Calenge et al. 2005a).

![Fig. 3 The global position of quantitative trait loci (QTL) for fire blight resistance on the apple genome based on the skeleton map developed by Silfverberg-Dilworth et al. (2006). Only linkage groups that have been shown to carry QTL are presented.](image-url)
‘Discovery’ and ‘Prima’

A further three minor QTL were identified in the ‘Fiesta’ families, but they were not stable. Two were identified only at 7 dpi in the FxD family: one at the distal end of LG12 and one at the proximal end of LG3 (Fig. 3) of ‘Discovery’, which explained 5.4% and 7.9%, respectively, of the phenotypic variation (Calenge et al. 2005a). These minor QTL were not identified in the Swiss FxD family (Khan et al. 2006), which confirms the low stability of the QTL, or they can be explained by differential interactions of the isolate used. The third QTL was mapped at the distal end of LG3 of ‘Prima’, and was significant at 14 dpi, but not at 7 dpi. The absence of a major QTL in ‘Prima’ was somewhat unexpected since this cultivar is considered to be partially resistant in the field. This may indicate that its flowers, which are the primary point of infection, play an important role in the expression of field resistance. Another explanation again may be that the E. amylovora isolate used in the experiments can overcome the ‘Prima’ resistance (Calenge et al. 2005a).

A further 12 digenic interactions that also involved loci at other linkage groups were identified, which indicates that epistatic effects are present and need further investigation as they may be an explanation for the incomplete expression of resistance in ‘Prima’. Four genomic regions other than the LG3 region were involved in the digenic interactions between both homologous and homologous chromosome segments of the three cultivars (Calenge et al. 2005a).

‘Robusta’

A major QTL was identified in two families of ‘Robusta’ crossed with the susceptible accessions ‘Idared’ in Germany (Peil et al. 2007a, 2007b) and ‘Malling 9’ in New Zealand (Peil et al. 2008a). It maps in between the simple sequence repeat (SSR) markers CH03e03 and CH03g07 at the proximal end of LG3 (Fig. 3). The QTL is very strong as it explains a very high 67-83% of the phenotypic variation, while in each family about 20-25% of the seedlings remained disease-free (Peil et al. 2008a). Of the 31 seedlings of the ‘Idared’ family not showing any symptoms in the first year, 28 showed the allele of marker CH03e03 linked to the resistance (Peil et al. 2007a). Also, the mean disease severity of the plants carrying the allele was 11% shoot blight, but 60% for the progeny without the allele. Regarding the plants with <30% shoot blight as resistant and those with >30% blight as susceptible yielded a resistant to susceptible ratio of 75:72 that was not significantly different from 1:1 (P(x² < 0.05) = 0.80). Treating the plants as a dominant single locus gene resulted in the gene mapping right at the top of LG3 at about 7 cM (‘Idared’) and 11 cM (M.9) above marker CH03e03 (Peil et al. 2008a). However, the resistance distributions of the progenies support the hypothesis that additional minor resistance QTL are present in ‘Robusta’ (Gardner et al. 1980b), but have not been demonstrated to date. This may be due to the small size of the families studied and/or differential interactions of E. amylovora isolates with these minor QTL (Gennaro Fazio, pers. comm.). Research is in progress to investigate this further.

Since the ‘Robusta’ fire blight resistance QTL maps to the same region as the minor QTL from ‘Fiesta’ and most likely are different, they are linked and possibly allelic. This relationship is enhanced by the mapping of a resistance gene analogue (RGA) of the nucleotide-binding site leucine-rich repeat (NBS-LRR) class ARGH32 to the same genomic region of apple. It mapped at a distance of 4.4 cM from SSR marker CH03e03 near the top of LG3 of ‘Fiesta’, while a further two RGAs were mapped to the same region in ‘Discovery’ (Baldi et al. 2004), suggesting that this genomic region is of importance for resistance traits. Several RGAs were mapped to the distal end of LG12 of an ‘Antonovka Debnicka’ x ‘Summered’ family (Naïk et al. 2006) in the region equivalent to the minor QTL from ‘Discovery’ (Fig. 3). The mapping of additional RGAs showed further linkages with apple scab and powdery mildew genes and QTL, but none co-segregated with fire blight resistance QTL identified to date (Calenge et al. 2005b). Recently, studies have been initiated to search for candidate genes among expressed sequence tags (ESTs) that are involved in responses to fire blight infection and can be used as markers for resistance. E. amylovora challenge of the susceptible cultivar ‘Royal Gala’ resulted in the identification of 468 expressed sequence tags (ESTs) that were differentially expressed in the early phase (the first 1-2 hours) of the infection process, others in the later phase (1 day and over) (Norelli et al. 2009). A further 190 candidate genes were differentially expressed between the fire blight-resistant rootstock G.3041 derived from ‘Robusta’ and the susceptible rootstock M.26 (Malnoy et al. 2008a). To date, one candidate gene, a serine threonine protein kinase, has been mapped to the ‘Robusta’ QTL on LG3 (Malnoy et al. 2008b) using the bin-mapping approach with selected progeny of the M.9 x ‘Robusta’ family (Colton et al. 2009). Some QTL may be shown to form part of the genes that control the phenylpropanoid pathway as it may be involved in fire blight resistance (Venisse et al. 2002; Pontais et al. 2006), whereas reactive oxygen species in the host do not inhibit E. amylovora, but rather help infection by damaging plant tissues (Brisset and Paulin 2006).

‘Eve’erest’ and Malus floribunda 821

Recently, two new QTL loci for fire blight resistance were reported. A major QTL from the ornamental crab apple ‘Eve’erest’ explaining 50%-53%, or up to 70% at 14 DP following log transformation of the length of necrosis, of the phenotypic variation in a cross with the susceptible rootstock ‘MM.106’ was mapped to the very distal end of LG12 below marker HI23d11y (Durel et al. 2009) (Fig. 3). A very small QTL explaining about 6% of the variation was mapped on linkage group 15 near marker HI04e05. Although ‘Eve’erest’ is a selection from an open-pollinated F4 derivative of M. floribunda 821, it appears to carry a different resistance allele at, or near the same locus on LG12 (Durel et al. 2009) (Fig. 3). Marker HI23d11y was non-informative in this respect, but an allele of marker HI07f01 about 10 cM above the QTL that is unique to ‘Eve’erest’ suggests that it inherited its fire blight resistance from the unknown pollen parent. The QTL from M. floribunda 821 explained over 60% of the phenotypic variation in a cross with ‘Golden Delicious’ and up to 48% of the variation at 14 DPI following log transformation. M. floribunda 821 is the progenitor of many cultivars that carry its Rv6 (Ef) scab resistance gene (Hough et al. 1953), very few if any are expected to carry its fire blight resistance QTL since often over eight generations separate these selections from M. floribunda 821.

Marker-assisted selection in apple

Of the additive QTL identified in M. × domestica accessions, the main LG7 QTL from ‘Fiesta’ was the only one that was stable across the different families and experiments and therefore has value for breeding purposes. As its broad-sense heritability at about 0.90 is high (Calenge et al. 2005a; Khan et al. 2006) and the resistance is present in a large-fruited breeding parent with acceptable fruit quality, it can be readily transferred into new apple cultivars. Several genetic markers are available to aid breeders with marker-assisted introgression of the resistance. While marker E37M40 would be the most exact predictor of resistance as it maps to the peak of the QTL, the sequence characterised amplified region (SCAR) markers AE10 and GE80-19 are preferred for their ease to score them (Khan et al. 2007). These markers flank E37M40 at 4 and 6 cM, respectively, and using them together would ensure that the whole QTL
region is covered and the marker pair would therefore be highly effective in MAS (Gimelfarb and Lande 1995).

With the aid of a set of four markers linked to the major LG7 QTL from ‘Fiesta’, it was traced back to its original source ‘Ribston Pippin’ via ‘Cox’s Orange Pippin’ (Khan et al. 2007). However, since the marker information is incomplete, the possibility that the resistance is derived from the unknown pollen parent of ‘Cox’s Orange Pippin’, cannot be excluded. While GE80-19 appears to be specific to the QTL, AE10 and the SSR marker CH-F7-Fb1 are much less so, since the specific alleles linked to FBF7 were present in 11 accessions that do not have ‘Cox’s Orange Pippin’ in their pedigree. In spite of these limitations, the amplification of both markers AE10 and GE80-19 generally proved to be good predictors of the presence of fire blight resistance and therefore for the selection of resistant breeding parents. The 11 accessions evaluated carrying both markers on average were more resistant than the 20 accessions not assumed to carry the ‘Fiesta’ LG7 QTL (Khan et al. 2007). The accessions ‘Reanda’, ‘Remo’ and ‘Kidd’s Orange Red’, which all are derived from ‘Cox’s Orange Pippin’, were the most resistant to fire blight out of the 31 accessions tested. Validation of MAS in a ‘Milwa’ x 1217 (resistant) family, where the progeny carrying both markers showed significantly more resistance than the progeny not carrying the markers, confirmed the usefulness of the markers in breeding. Both markers AE10 and GE80-19 have also been applied to a FAW 9991 x ‘Enterprise’ family, with the resistant parent showing both markers but the susceptible parent the AE10 marker, too, to preferentially select for fruit quality from the progeny carrying both markers (49.7%) (Keller-hals et al. 2009).

The QTL from ‘Fiesta’ and ‘Robusta 5’ exemplify the differences between sourcing resistances from commercial cultivars versus those from crabapples. The advantage of the ‘Fiesta’ cultivar is that it is present in a large-fruited cultivar with acceptable fruit quality useful for breeding, but confers only a moderate, partial resistance (Calenge et al. 2005a; Khan et al. 2006, 2007). In contrast, ‘Robusta 5’ confers (near) immunity, but is a typical small-fruited crabapple with low fruit quality and therefore would require several generations of backcrossing before it can be bred into a new cultivar. As the resistance appears to be of a monogenic nature, this could be readily achieved, but at the same time increases the risk of the resistance being overcome by races of the pathogen. Single gene resistances generally involve gene-for-gene relationships that have a higher risk of being overcome by the pathogen, but may also apply to partial resistances, such as the FBF7 resistance. This is further supported by the identification of differential resistances in apple rootstocks, although several of those derived from ‘Robusta 5’, such as ‘Geneva’ 3041 and ‘Geneva’ 5179, have demonstrated full resistance to the four strains of E. amylovora tested (Fazio et al. 2006). Therefore, research is in progress to identify other sources of fire blight resistance that will provide additional QTL for pyramiding in order to achieve durable resistances. Recently, a new QTL explaining 26% of the phenotypic variation was preliminarily mapped to LG10 of ‘Florina’ in a cross with Nova Easygro’, while a further eight minor QTL distributed over 6 linkage groups that together only explained less than 10% of the phenotypic variation were identified (Khan et al. 2008). Also, a number of fire blight resistant accessions that do not carry the ‘Cox’s Orange Pippin’ resistance, such as ‘Priscilla’ and ‘Starking Delicious’, have been identified (Khan et al. 2007). Research on additional resistance sources is in progress to identify QTL in ‘Rewena’ and M. fusca populations (Peil, unpublished data).

**Fire blight resistance QTL in pear**

Four QTL for fire blight resistance were identified on the genome of ‘Harrow Sweet’ in a cross with the highly susceptible cultivar ‘Passe Crassane’. The strongest QTL mapped to the proximal end of LG2 (Fig. 4) and explained 24.6%, i.e. about half of the total phenotypic variation explained by all the QTL identified based on incidence alone (Dondini et al. 2004). At about 16.5%, the phenotypic variation explained by this QTL was lower when based on severity and IVS, while the other QTL ranged from 6.9% (incidence) to 12.0 (IVS), which confirms the polygenetic nature of the resistance (Dondini et al. 2004). Both QTL on LG2 map to the same genomic regions where a number of scab resistance genes have been mapped in apple (Bus et al. 2004). Preliminary findings showed that the proximal region of LG2 also harbours an epistatic QTL for fire blight resistance from ‘Prima’ (Calenge et al. 2005). A further two epistatic QTL from both ‘Prima’ and ‘Fiesta’ were mapped near SSR marker CH01h01 to the distal end of LG9. This marker is closely linked to another SSR marker, CH05a03,
which is linked to the QTL on LG9 of ‘Harrow Sweet’ (Dondini et al. 2004).

Eight out of the 10 progeny of the family carrying all the marker alleles linked to resistance QTL were grouped in the IVS classes 1 and 2 for highest resistance, while all five progeny without any of the marker alleles were grouped in the most susceptible class (Dondini et al. 2004, 2006). This suggests that MAS for these QTL will be useful in breeding, but additional QTL with greater penetrance will be required to increase the efficiency of breeding new pear cultivars with durable resistance to fire blight.

As in apple, the approach to identify genetic markers linked to fire blight resistance by screening RAGs has had limited success to date. While about 35 RAGs with NBS-LRR motifs were identified in the resistant European pear cultivars ‘Harrow Sweet’, ‘US 709’, ‘Old Home’ and ‘Seckel’, no clear linkage with fire blight resistance could be established to differentiate them from susceptible cultivars, such as ‘Passe Crassane’ and ‘Bartlett’. The limited success of this approach has been attributed to the incorrect assumption that fire blight resistance is simply inherited (Dondini et al. 2002; Afnian et al. 2006). As demonstrated above, the QTL approach reflecting the polygenic nature of fire blight resistance in pear has been more successful.

**Expression of antimicrobial proteins in plants**

Initially, genetic engineering for fire blight resistance was focused on transferring genes for antimicrobial proteins with low toxicity to eucaryotic cells. Antimicrobial peptides (AMPs) have been the object of attention in past years as candidates for plant protection products. Sequences coding for AMPs have been expressed in model or crop plants providing different degrees of protection against plant pathogens (Montesinos 2007). In apple and pear the AMPs attacin, lysozymes, and cecropin analogs were used. Attacins are a group of antibacterial proteins produced by *Hyalophora cecropia* pupae (Hultmark et al. 1983). The mechanism of antibacterial activity of this protein is to inhibit the synthesis of the outer membrane protein in Gram negative bacteria (Carlsson et al. 1991).

The apple rootstock M.26 has been transformed by *Agrobacterium*-mediated transformation using a gene encoding the lytic protein attacin E showing increased fire blight resistance both in vitro and in greenhouse tests (Norelli et al. 1994a; Borejsza-Wysocka et al. 1999). Genes encoding the lytic proteins attacin E, hen egg white lysozyme, and the cecropin analogs, SB-37 and Shiva-1, have been transferred to ‘Royal Gala’ apple and 28 transgenic lines out of 64 developed significantly less fire blight than non-transgenic ‘Royal Gala’ controls in greenhouse tests. One transgenic line, TG138, containing the *attacin E* gene under the control of the proteinase inhibitor II promoter, showed only 5% shoot length blight compared with 56% in non-transgenic ‘Royal Gala’ controls (Norelli et al. 2000). Transgenic apple expressing attacin E targeted to the intracellular space, where *E. amylovora* multiplies before infection, using a signal peptide has significantly reduced fire blight, even in apple plants with low attacin E production levels (Ko et al. 2000). Integration of the *attacin E* gene was accomplished also for pear. A significant reduction of symptoms in the *in vitro* test was observed for six lines out of eleven, in comparison with the susceptible control ‘Passe Crassane’ (Reynoard et al. 1999). In the case of cecropin SB-37, several apple lines have been identified that’s significantly more resistant than the ‘Royal Gala’ parent. However, there was a lack of correlation between detectable cecropin and field resistance (Norelli et al. 1999b). Similar results were obtained with hen egg white lysozyme (HEWL)-transgenics, where one of the ‘Royal Gala’ HEWL-transgenic lines was identified as resistant in both field and greenhouse tests (Norelli et al. 1999a). It was also shown by other research groups that MB39, a cecropin B analogue, joined to a secretion coding sequence from barley α-amylase, and placed under the control of a wound-inducible tobacco osmotin promoter was effective against *E. amylovora* in ‘Royal Gala’ apple. Three of the seven transgenics were 2.5 to 3.3-fold more resistant to *E. amylovora* than the non-transformed ‘Royal Gala’ control (Liu et al. 2001). The T4 lysozyme gene from the bacteriophage T4 was also transformed into apple using *Agrobacterium*-mediated transformation (Hank et al. 1999, 2000). There was a large variability among lines and plants of one and the same line in fire blight resistance in greenhouse tests. Ko et al. (1999, 2002) reported on the effect of five different constructs containing attacin E and T4 lysozyme expressed either singly or in combination in the apple cultivar ‘Galaxy’. Generally, transgenic lines containing attacin E under the control of the potato protease inhibitor II promoter had higher attacin E expression than those under the control of the enhanced CaMV35S promoter. The untrans-
lated leader sequence of alfalfa mosaic virus RNA4 increased attacin E expression levels, while a signal peptide sequence resulted in lower attacin E levels in transgenic lines. Attacin E was degraded in intercellular fluid extract, indicating that reduction of attacin E levels could be explained by intercellular degradation. Disease evaluation in controlled environment chambers showed that some transgenic lines had significantly higher disease resistance than their non-transformed counterparts. However, in transgenic pear plants containing Pgst1 genes showed no significant reduction in disease, indicating there was no advantage in combining these genes in the plant (Ko et al. 2002), but may be the combination makes the resistance more durable.

Norelli et al. (1999a) summarized the work performed on evaluating the antimicrobial proteins, such as attacin E, cecropins, hen egg white and T4 lysozymes for their effect on fire blight resistance. The best fire blight resistance has been observed with attacin E-transgenic. Many of these transgenic lines of ‘Royal Gala’, ‘Galaxy’ and M.26 have been tested for fire blight resistance in the field for 3-4 years indicating that resistance is stable (Aldwinckle et al. 2003).

In pear, different strategies were considered to enhance resistance to fire blight, including the use of lytic peptide genes, such as attacin E or T4 lysozyme, and of the lactoferrin gene of bovine origin. Lactoferrin is an iron-chelating agent that competes with the siderophore of E. amylovora and reduces the biological availability of iron for the invading bacteria. Malnoy et al. (2000) used these strategies to transform the susceptible pear cultivar ‘Passe Crasanee’.

Fire blight susceptibility tested in vitro was slightly reduced in some lines. Previously, Reynoird et al. (1999) transformed the same pear cultivar using the attacin E gene. A significant reduction of symptoms was observed for six out of eleven lines compared with the susceptible control using in vitro inoculation. Chevreau et al. (2000) summarized the results obtained so far with different constructs expressed in pear from the INRA, Angers genetic engineering programme. Preliminary results indicated a large variability of transgenic expression using antibacterial genes in pear, too. It was possible to detect by in vitro inoculation some clones with up to 50% symptom reduction. Results of the greenhouse inoculation have not been reported to date. Since then, the programme has focused on two directions. In the first one, new antibacterial genes, such as the combination of the T4-lysozyme and attacin genes (Ko et al. 1999), are tested and in the second one, a more specific inhibition of pathogenicity factors using the lactoferrin and depolymerase genes (Chevreau et al. 2000).

Both the bactericidal and animal origin of most of the antimicrobial genes used, their acceptance by growers and consumers has been judged doubtful (Norelli et al. 2003a). In order to avoid the use of heterologous transgenes and unintended effects on non-target bacteria, recent research on genetic transformation in apple and pear, like in other plant species, has emphasized promoting plant defence reactions rather than introducing AMPs.

Promoting plant defence reactions

The pathogen-induced plant resistance approach starts from the theory that the pathogen secretes substances that are recognized by the host and may initiate the defence cascade (Gessler and Patocchi 2007). E. amylovora uses a type three secretion system (T3SS) to deliver effector proteins into plant host cells. Once inside, these effector proteins are thought to be involved in suppressing host defence responses, re-directing normal host metabolism to facilitate pathogen multiplication and initiating cell necrosis. Required for these interactions are the clustered bacterial hrp genes which encode a large set of proteins broadly conserved among plant and animal pathogens. Since the E. amylovora effector protein harpin has given significant protection against fire blight infection, probably by inducing systemic acquired resistance when sprayed on apple blossoms, it was hypothesized that hrpN-transgenic apple plants may have increased resistance to fire blight (Bauer et al. 1999). The hrpN gene driven by a Pgst1 promoter, which previously was shown to be induced in E. amylovora challenged leaves (Malnoy et al. 2006b), was transferred to M.26 apple rootstock. In growth chamber tests, some lines showed an increased resistance to the pathogen, which was confirmed in field trials (Aldwinckle et al. 2003). Transgenic ‘Passe Crasanne' harpin (Bauer et al. 2005) and expressing the hrpN effector gene, showed a significant reduction of susceptibility to fire blight in vitro, which could be related to the degree of expression of the transgenic hrpN.

The protein HIPM encoded by a gene of a cDNA library from apple was found to interact with HrpN. Using RNAi technology, the HIPM gene was silenced in apple. Susceptibility to fire blight in transgenic ‘Galaxy’ apple was found to be reduced by 50% because of reduced HIPM expression (Malnoy et al. 2008b).

Next to the hrp cluster of bacterial genes in E. amylovora is the “disease-specific (dsp)” region that is required for pathogenicity, but not for elicitation of the hypersensitive reaction (HR). The disease specific gene dspE of the bacterium encodes a pathogenicity effector protein, which is the cognate effector of fire blight disease. The DspE protein interacts physically and specifically with four similar leucin-rich-repeat (LRR) receptor-like serine/threonine kinases from apple. The genes encoding the four DspE-interacting proteins of Malus (DIPM genes), are conserved in all hosts of E. amylovora, but not in non-host plants. Interaction between the DIPMs and DspE is thought to be involved in disease development. Sense sequences form non-conserved regions of each gene were used for transformations of the apple cultivar ‘Galaxy’ aimed at silencing the DIPM genes and preventing interactions with DspE. Silencing was obtained in some apple clones and some lines showed increased resistance in the growth chamber using artificial shoot inoculation (Borejsza-Wysocka et al. 2004, 2006).

The secretion of the effector protein DspE encoded by the dsp-cluster into the host cells via T3SS is facilitated by the chaperon protein DspF. Because the function of chaperon proteins is mediated by physical interaction with the cognate effector proteins, it was hypothesized that expression of DspF in apple might interfere with the virulence function of DspE in the host cell, hence reducing fire blight susceptibility. Transgenic DspF apple plants were recovered from these experiments that indicated a 50-80% decreased susceptibility to fire blight shoot infection (Malnoy et al. 2004).

Another strategy to improve fire blight resistance in apple by genetic engineering is using the dpo gene of the E. amylovora bacteriophage phi-Ea11h. This gene encodes a depolymerase that degrades the capsular exopolysaccharide of E. amylovora. Expression of the EPS-dpo gene resulted in 61 out of 83 transgenic lines of the apple cultivar ‘Pinao’ being more resistant to E. amylovora in an in vitro test (Hanke et al. 2002). These experiments were summarized (Flachowsky et al. 2008a). No correlation was obtained for the transgenic lines between the level of dpo gene expression and both the level of DPO activity and the disease resistance in vitro. However, DPO activity did correlate positively with resistance to fire blight in vitro. Seven clones had less disease than the non-transformed genotype when measured in the greenhouse, but no statistically significant differences were found, except for one line. This line showed the highest DPO activity, and the least susceptibility to fire blight in vitro as well as in greenhouse tests (Flachowsky et al. 2008a). The same gene was used in pear and only two out of 15 lines showed a consistent increase of fire blight resistance in vitro and in the greenhouse (Malnoy et al. 2005). Recently, it was shown by Borejsza-Wysocka et al. (2007b) that expression of the dpo gene fused to the alfalfa mosaic virus (AMV) translation enhancer and to the signal sequence of the PR1b gene from potato can reduce
the length of shoot necrosis from 94% in non-transformed plants to 48-51% in transgenic M.26 apple rootstock (Borejsza-Wysocka et al. 2007a). Overexpression of an apple-own gene involved in the pathogen defence mechanism was first described by the Aldwinckle group. The NPR1 protein is pivotal in the systemic acquired resistance defence reaction of Arabidopsis thaliana to pathogen attack. When overexpressed, it appears to encode a cell-to-cell spread of fungal and bacterial pathogens in A. thaliana and to bacterial blight in rice. An NPR1 ortholog, MdNPR1, was cloned from Malus x domestica and overexpressed in the apple accessions ‘Galaxy’ and M.26. The activation of some PR proteins was demonstrated and resistance to fire blight was evaluated in the growth chamber, where MdNPR1-‘Galaxy’ clones showed a reduced shoot necrosis compared with 80.0% in the control plants. However, this approach has a limited effect on the high susceptibility of the apple rootstock M.26 when the gene is under the control of the inducible promoter pin2 (Malnyo et al. 2004, 2006a, 2007a). A second approach using an apple gene was recently published by Flachowsky et al. (2008b). The TIR-NBS LRR gene Mbr4 of Malus baccata was constitutively overexpressed in the apple cultivar ‘Pinova’. Most transgenic clones were less susceptible under in vitro conditions. They showed significantly increased resistance in the greenhouse.

Genes from E. amylovora (hrp, dsp) and E. amylovora phages (dpo) appear also effective at increasing fire blight resistance. They are probably more acceptable than animal genes. However, most acceptable to consumers and growers are likely to be alterations in the expression of native apple genes, such as MtNPR1 and HIPM, resulting in enhanced resistance (Borejsza-Wysocka et al. 2007c).

The embarras de richesse – a choice of strategies to express transgenes

After selecting an appropriate transgene, it is of particular importance where the transgene should be expressed for achieving fire blight resistance after transformation. Whereas constitutive promoters were the magic bullet in former times, it was found that their application to express transgenes has often led to problems (Gurr and Rushton 2005). In some cases it was found that the transgenic plants had an increased resistance, but had a reduced size (Bolar et al. 2004). Especially the formation of symptoms in an uninfected stage (Fitzgerald et al. 2004, for a review see Mittler and Rizhsky 2000), and/or had an abnormal habitus (Li et al. 2004). Especially the formation of transgenic mummies was often described. The spontaneous formation of HR-like lesions in the absence of the pathogen was mainly found after overexpression of genes, which may be classified into four different groups: pathogen-derived genes, signal transduction-inducing genes, general metabolism-perturbing genes, and killer genes (for review see Mittler and Rizhsky 2000). With the contemporary knowledge, spontaneous HR is not surprising because all transgenic cells are pre-programmed into ‘defence mode’ (Gurr and Rushton 2005) and they are wasting resources by being in a constant state of alert. Inducible (chemically-, wound- or pathogen-inducible) promoters are often discussed as a possible tool to prevent such problems. The use of chemically inducible promoters is possible in principle, but their use requires continuous monitoring of the orchard to detect the first visible disease symptoms and to determine the best time point for chemical application. Beyond it, this strategy is unlikely the main idea to reduce the application of chemicals by using genetically modified plants with improved resistance. The best strategy to our opinion seems to be the expression of the transgene only in the presence of the pathogen and in tissue where it is needed. Therefore, a pathogen-inducible promoter has to be preferred, but to discover the most appropriate promoter is not easy to realize, because the promoter has to meet many different requirements depending on the strategy applied. The promoter should be activated rapidly with no or a low level of background expression in the absence of the pathogen. Furthermore, the promoter should not be inducible by the transgene itself (Gurr and Rushton 2005). Only few pathogen inducible promoters (gst1, OSMp and pin2) have been tested in apple to date. The gst1 promoter normally drives the potato Pst1 gene (formerly Prp-1), which encodes a glutathione-S-transferase (Hahn and Strittmatter 1994). In potato itself it is not activated by pathogen infection, but it is inducible by heat shock, light/dark switches, or wounding. Furthermore, the promoter led to a rapid and local transcriptional induction after infection with different pathogens (Martini et al. 1993; Strittmatter et al. 1996). This promoter was also tested in two apple genotypes (‘Gala’ and M.26) and a similar pattern of induction was found (Malnyo et al. 2006b). In comparison to the CaMV35S promoter, the level of expression was obviously lower and ranged between 8 and 15%. It is interesting to note that systemic induction of the gst1 promoter was found in non-treated leaves (Malnyo et al. 2006b). The other two promoters have not been characterized intensively in apple as was done for gst1. The OSMp promoter, which normally drives an osmotin gene of tobacco, was used to express a modified cecropin SB37 gene (MB39) in apple (Liu et al. 2001). In tobacco, it was shown that osmotin gene expression is activated by ABA, NaCl, salicylic acid, wounding, and fungal infection (Zhu et al. 1995). However, several of the OSMp:MB39 transgenic apple plants were more resistant to E. amylovora (Liu et al. 2001). But expression data on MB39 as well as an intensive characterization of the OSMp promoter in apple are missing. The pin2 promoter, which originates from the proteinase inhibitor II gene of potato (Keil et al. 1989), has been used several times in apple (Norelli et al. 1994b; Ko et al. 2000, 2002). This promoter is described as wound-inducible in potato and tobacco (Sanchez-Serrano et al. 1987; Peña-Cortés et al. 1988; Keil et al. 1989). Furthermore, it was reported that the pin2 promoter also leads to a constitutive expression as shown in transgenic tobacco, birch and apple (Thornburg et al. 1987; Keonen-Mettälä et al. 1998; Ko et al. 2000, 2002). In transgenic apple it was found that the pin2-mediated expression of the attacin E (atte) gene of Hyalophora cecropia increased 1 h after wounding and decreased 24 h after wounding (Ko et al. 2002). Based on the fact that atte expression was also detectable without wounding in pin2At transgenic apple plants and the mean value of the gene expression was comparable with the untransformed control, the promoter appears to be active in the absence of a pathogen. It seems that the CaMV35S:Pin2 transgenic plants (Ko et al. 2002), the pin2 promoter does not seem an ideal alternative to CaMV35S.

In contrast to the endless searching for appropriate native pathogen-inducible promoters, the design of novel synthetic promoters, involving the elimination of unwanted background expression, holds promise (Rushton et al. 2002). On this account, different cis-acting elements, known to be involved in wound- and pathogen-inducible, were removed from the CaMV35S promoter (alone, in blocks, or in combination with other elements) with a minimal promoter (Rushton et al. 2002; reviewed by Gurr and Rushton 2005). It was impossible to separate the pathogen-inducibility from other expression patterns (e.g. induction by wounding) for some of these elements (W1, W2, S Box and JERE). However, synthetic promoters containing other elements, such as Box D from the parsley PR5 gene or the W Box containing elements E17 and F, are not inducible by wounding (Kirsch 2001; Heise et al. 2002; Rushton et al. 2002). Synthetic promoters containing Box D are among the best pathogen-inducible promoters tested to date. They showed little background expression and were inducible by several pathogens (Rushton et al. 2002). Because of the fact that most of the published synthetic promoters were only tested on their inducibility by fungal pathogens, one cannot conclude with certainty that these promoters are also inducible by fire
It is common knowledge that *E. amylovora* bacteria travel in plants via the xylem and through intercellular spaces. Bogs et al. (1998) described rapid migration of the bacteria through xylem vessels and a slow colonization of the parenchyma after artificial inoculation. After application to leaf surfaces, the bacteria move through the epidermis into the intercellular space of parenchyma tissues and into the vascular system (Bogs et al. 2004). The transgenic expression of antibacterial proteins in the cytosol of plant cells, as done in the past, therefore does not seem the best strategy. The secretion of such proteins to the apoplastic space seems to be essential and advantageous to improve the resistance effectively (Düring et al. 1993; Düring 1996). Signal peptides, which can transport proteins efficiently to the intercellular space, have been described several times (Fischer et al. 1999; Abdeev et al. 2003; Dai et al. 2000, 2005; Khanna and Daggard 2006). The idea that resistance is improved after secretion is supported by the results of Ko et al. (2000). The authors compared different transgenic apple lines expressing the antimicrobial AttE protein with and without translation enhancer and signal peptide, respectively. They found that the AMV translation enhancer increased the amount of AttE and that lines containing the signal peptide to transport AtTE to the intercellular space showed the highest resistance to fire blight. Similar results were described by Borejsza-Wysocka et al. (2007b). They fused the EPS-depolymerase gene to the AMV translation enhancer and to a signal sequence. In contrast to previous results obtained in other labs on transgenic apple plants expressing the dpo gene without AMV and signal peptide, the authors found that several transgenic M.26 clones containing the fused gene construct were significantly less infected than non-transformed M.26 control plants.

Transgenic regulation of selected flavonoid pathway genes

Recently, it was shown for apple and pear that the resistance to fire blight was improved after application of the plant growth retardant prohexadione-Ca (Fernando and Jones 1999; Momol et al. 1999b; Yoder et al. 1999; Costa et al. 2001; Spinelli et al. 2005b), which does not possess any bactericidal activities by itself (Rademacher et al. 1999; Rademacher 2004). Prohexadione-Ca is a structural analogue to 2-oxoglutarate, which inhibits 2-oxoglutarate-dependent dioxygenases (Rademacher 2000). Of particular interest in view of fire blight resistance is flavanone-3β-hydroxylase (FHT, F3H), which is one of the three 2-oxoglutarate-dependent dioxygenases of the flavonoid pathway in leaves of apple and pear (Halbwirth et al. 2006). The inhibition of FHT by prohexadione-Ca as shown by Roemelt et al. (2003) leads to the accumulation of eriodictyol, which is converted into luteoforol and subsequently to luteoliflavan (Fig. 5). Luteoforol is an intermediate, which shows strong antimicrobial effects (for review see Halbwirth et al. 2003). In contrast to luteoliflavan, which does not possess any antimicrobial activity, it was recently shown that luteoforol is highly active against different strains of *E. amylovora* (Spinelli et al. 2005b). Neither luteoforol nor luteoliflavan are processed naturally in apple. They were only found after inhibition of FHT. Such an inhibition is possible by application of prohexadione-Ca as well as by silencing of the FHT-encoding gene. In contrast to the application of prohexadione-Ca, the application of biotechnological approaches seems to have at least two advantages. Firstly, FHT activity could be inhibited without blocking other 2-oxoglutarate-dependent dioxygenases. Therefore, no undesirable site effects are expected. Secondly, by using tissue specific pathogen-inducible promoters (naturally occurring or synthetic), it is possible to inhibit FHT only in the presence of the pathogen and in tissue where it is needed. Transgenic plants with an FHT antisense have been created recently (Schlangen et al. 2007) and results of the effect on fire blight are expected in the near future.

**Fig. 5** Section of the flavonoid biosynthesis in apple and pear according to Halbwirth et al. (2003, 2006). FHT flavanone-3β-hydroxylase, LAR leucoanthocyanidine reductase, black - common compounds, red - induced compounds, blue – site to take a hand in the pathway.
Alternative selection systems – towards cisgenic plants

The selection of transgenic apple plants is still performed by using the *nptII* selectable marker gene. The *nptII* gene encodes for a neomycin phosphotransferase II, which confers resistance to aminoglycoside antibiotics, such as kanamycin, neomycin, geneticin and paromomycin. The practicality of these four antibiotics was pioneered in apple by Jay Norelli and Herb Aldwinckle (Norelli and Aldwinckle 1993), who have found that three of them (kanamycin, neomycin and paromomycin) are effective.

Since that time, nearly all of the published studies on apple have been performed using the *nptII* gene as selectable marker gene and kanamycin as selectable agent. However, public concerns about the release of genetically engineered plants into the environment demand marker-free transgenic plants or at least selectable markers other than *nptII*. Recently, some studies have been published that focused on the establishment of new selection strategies (for review see Gessler and Patocchi 2007). Herbicide resistance genes like *bar*, which confers resistance to phosphinothricin (BASTA), were used several times in apple transformation (de Bondt et al. 1996; Lebedev et al. 2002; Szankowski et al. 2003; Dolgov and Skyrabin 2004). Such genes have obvious advantages from a practical point of view. Herbicide resistant plants would facilitate the use of herbicides in nurseries and young orchards (Bulley et al. 2007). However, ownership restrictions as well as the fact that the transformation efficiency does not appear to be as good as with *nptII* (de Bondt et al. 1996; Dolgov and Hanke 2006), make it clear that herbicide resistance genes are not real alternatives. A more promising candidate seems to be the *manA* gene of *E. coli*. This gene encodes for a phosphomannomase-isomerase (PMI) that catalyzes the conversion of mannose-6-phosphate to fructose-6-phosphate, and makes it possible for transgenic plant cells to utilize mannose as a carbon source. Several authors tested the PMI system in apple (Flachowsky et al. 2004; Zhu et al. 2004; Degenhardt et al. 2006, 2007), but with variable success. However, the results recently published by Degenhardt and co-workers clearly demonstrate that the PMI system possibly represents a promising alternative to *nptII*.

As wonderful as it is to have alternative selectable marker genes, the ultimate aim is a marker-free transgenic plant (Gessler and Patocchi 2007). This can be achieved by using “clean vector technologies” or the transformation without marker genes. Only one report exists on the “proof of concept” of the use of a “clean vector technology” in apple (Kandel et al. 2004). The transformation without the use of marker genes has been reported twice (Flachowsky et al. 2004; Malnoy et al. 2007b). The results obtained in these studies were promising, but no molecular evidence exists to date whether such a procedure really works in apple. However, such technologies offer the possibility to transfer genes from one (e.g. resistant) to another (e.g. susceptible) apple cultivar without involving DNA from non-crossable organisms. The emerging product will be a plant with an apple gene containing introns and flanking regions such as native promoter and terminator in a sense orientation. Such plants were recently termed “cisgenic” plants (Schouten et al. 2006a, 2006b). With marker-free *trans-cisigenic* systems having progressed to the stage of commercial application, the only limitation for the technology to become commonplace is its acceptance by consumers.

OUTLOOK

The aim of pomefruit growers is to produce fruits with excellent inner and outer quality with as little input of plant protection agents as possible, to increase economic benefits and the ecological value of the products and their production environment. The bacterial disease fire blight provides a major challenge for growers of pomefruit with limited tools to control the disease as well as for breeders, using traditional or modern breeding methods, to supply resistant cultivars (Fig. 6). Currently, effective combat of fire blight is possibly only with antibiotics, which raises both social and ecological issues regarding the acceptance of fruit harvested from trees treated with them. To date, alternative protection agents have proved to be less effective; hence the major means of controlling the disease is by growing resistant cultivars. The actual challenge for breeders is to provide blight resistant apple and pear cultivars that are able to compete with the most important cultivars in the world market. In this paper, we have summarized the present knowledge about the infection process and machinery of the pathogen, and have provided an overview of genetic resources available to breeders and their use in cultivar development. We have reviewed both the development and application of molecular markers as a tool in classical breeding, and genetic engineering, and specifically cisgenesis as an alternative tool to improve fire blight resistance in existing cultivars. The question is whether this knowledge can be applied to develop new strategies in breeding to enlarge the number of options breeders have, and to facilitate breeding of high quality apple and pear cultivars resistant to the pathogen *E. amylovora*.

The historical and continued spread of fire blight showed that quarantine measures, and the eradication of ornamental hosts in the vicinity of pomefruit have been ineffective in confining the disease in the absence of major natural barriers. The increasing knowledge on the interplay between the infection mechanisms of the pathogen and the physiological state of the host, particularly in relation to the flowers as the primary site of infection in the orchard (Bubán et al. 2003), will facilitate the development of both genetic and non-genetic fire blight control strategies. The research will be boosted by the publication of the whole genome sequence of both apple and the pathogen as it offers opportunities for new insights into host-pathogen interactions that will help to develop more effective plant protection agents and to identify new plant genes involved in recognition and defence of the pathogen. The understanding will be extended to interactions with other rosaceous species that are non-hosts for fire blight, even though the pathogen may be able to survive and even multiply on such hosts (Johnson et al. 2006), increasingly aided by comparative and functional genomics studies (Shulaev et al. 2008).

The extensive evaluation of apple and pear germplasm that has taken place to date has resulted in the identification of many genetic sources of resistance, which put resistance breeding in good state for achieving durable resistance. The detection and confirmation of new QTL for resistance to fire blight might be a promising approach for marker assisted selection and offer the opportunity to pyramid different QTL with different mechanisms of resistance to provide cultivars with durable resistance. In the near future, a set of markers will be available to screen seedlings for pyramided fire blight QTL, or for that matter a whole range of genes that allows the selection of designer fruits.

The next step after identification of QTL for resistance is the isolation of the corresponding resistance genes, which is a current aim of some working groups. The identification and isolation of genes conferring resistance to fire blight could solve three problems: the long time needed for backcross generations, the competitiveness of fire blight resistant cultivars, and the objection by many people to “transgenics”. With these resistance genes (including their own regulatory sequences) from apple or pear, prevalent cultivars could be improved in a relatively short time. These “cisgenic” cultivars would contain DNA from the same genus only, making them more acceptable to large parts of the population. The transgenic approach might be useful for research and elucidating physiological processes in the tree, but is strictly limited due to the refusal of transgenic products by a significant proportion of people worldwide.

With technologies for the large-scale genotyping of seedling populations advancing rapidly and becoming more cost effective at the same time, the ability of breeders to res-
Fire blight. Peil et al.

pond rapidly to changes in consumer preferences is predominantly hampered by the long juvenile period of pomefruit trees. Therefore, reducing the juvenile period will be of considerable value and several techniques based on plant physiological principles are available to breeders (Hanke et al. 2007; Volz et al. 2009). However, at least one very promising “semi-transgenic” approach has been developed, with which a dramatic reduction of the generation time in apple was achieved by having seedlings that are only several months old, produce fertile flowers. Overexpression of the \( BpMADS4 \) gene from silver birch (\( Betula pendula \)) (Flachowsky et al. 2007) resulted in some transformants flowering after four to eight months since the introduction of the gene. Most flowers were morphologically normal and developed normally into fruit following successful pollination by non-transformed accessions. Rapid cycling of several generations with “\( BpMADS4 \)-Pinova” combined with whole-genome selection (Volz et al. 2009), with a final selection phase for “non-transgenic” progeny that do not carry the transgene, would enable the introduction of traits from crab apple into the cultivated apple within a period of several years (Peil et al. 2008b). This is a very promising approach to accelerating the breeding for fire blight resistance with e.g. the “Robusta 5” QTL (Peil et al. 2007a), and to pyramid resistances to this as well as other diseases. Provided that such plants indeed are regarded non-transgenic, irrespective of the use of a transgenic plant to speed up the generation cycle, this approach will be favourable to transgenic fruit, which at this stage are not well-received by a large proportion of consumers, and ‘compete’ with cisgenic fruit. There will be a place for the ‘fast-breeding’ approach as breeders still will have to apply classical breeding to meet consumer demands for new high quality pomefruit cultivars, while cisgenesis provides them with the opportunity to ‘retro-fit’ gene cassettes for novel traits and disease resistance into existing cultivars.

FINAL NOTE

The genome of the Korean \( E. pyrifoliae \) strain Ep1/96 has been sequenced and the chromosome as well as 3 plasmids were annotated (accession numbers FP236842 (chromosome), FP236827 (pEP03), FP236828 (pEP05), FP236829 (pEP36)). In addition, the genomic sequence of \( E. billingiae \) strain Eb661 will be available soon (accession numbers FP236843 (chromosome), FP236826 (pEB102) and FP236830 (pEB170)). Genome comparisons for the antagonistic bacteria \( E. billingiae \) and \( E. tasmaniensis \) with the pear pathogen \( E. pyrifoliae \) will be discussed in another manuscript (in preparation).

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