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Improvement of Fire Blight Resistance in Apple and Pear

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ABSTRACT

Fire blight caused by the bacterium *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 *E. amylovora* are members of the *Maloidae*, such as apple, pear and quince, and ornamentals, such as *Cotoneaster*, *Crataegus* and *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 *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, *Erwinia amylovora*, genetic engineering, genetic markers, germplasm, *Malus*, MAS
Abbreviations: **ABA**, abscisic acid; **AI**, autoinducer; ***ams***, *amylovoran synthesis*; **AMV**, alfalfa mosaic virus; **AMP**, antimicrobial peptide; ***Att***, *Attacin*; **AUDPC**, area under disease progress curve; **B**, Budagovsky; **BSA**, bulked segregant analysis; **CaMV**, cauliflower mosaic virus; ***dfo***, *desferrioxamine*; **DIPM**, DspE-interacting protein of *Malus*; **DNA**, deoxyriboncleic acid; **DPI**, days post inoculation; ***dpo***, *depolymerase*; ***dsp***, *disease-specific*; **EPS**, exopolysaccharide; **EST**, expressed sequence tag; **F3H**, flavanone 3β-hydroxylase; **FHT**, flavanone 3β-hydroxylase; ***fox***, *ferrioxamine*; **G**, Geneva™; ***glmS***, *glucosamine synthase*; **H-NS**, histone-like nucleoid structuring protein; **HEE**, Hrp effectors and elicitors; **HEWL**, hen egg white lysozyme; **HIPM**, HrpN-interacting protein from *Malus*; **HR**, hypersensitive response; ***hrc***, *hypersensitive reaction conserved*; ***hrp***, *hypersensitvity reaction and pathogenicity*; ***hsv***, *hrp*-associated systemic virulence; **IVS**, index of varietal susceptibility; **JERE**, jasmonate and ethylene responsive element; **LAR**, leucoanthocyanidine reductase; **LG**, linkage group; **LOD**, logaritjm of the odd; **LRR**, leucine-rich repeat; ***lsc***, *levansucrase*; **M**, Malling; ***manA***, *mannose 6-phospate isomerase*; **MAS**, marker-assisted selection; **MDA**, megadalton; *Mf*821, *Malus floribunda* 821; **MM**, Malling-Merton; **MMT**, million metric tonnes; **NaCl**, sodium chloride; **NBS**, nucleotide-binding site; ***NPR***, *nonexpressor of PR*; ***npt***, *neomycin phosphotransferase*; **ORF**, open reading frame; ***OSMp***, *osmotin promoter*; **QTL**, quantitative trait locus; **PCR**, polymerase chain reaction; **PFGE**, pulse field gel electrophoresis; ***Pgst***, *potato glutathione-S-transferase*; ***pin***, *proteinase inhibitor 2*; **PMI**, phosphomannose isomerase; ***Pprp***, *potato patogen related protein*; **PR**, patogenesis related; ***rcs***, *regulator of capsule synthesis*; ***pstS***, *phosphate transport system S*; ***rls***, *regulation of levansucrase*; **RGA**, resistance gene analogue; **RNA**, ribonucleic acid; **SCAR**, sequence characterised amplified region; **SGT**, small glutamine-rich protein; **SSR**, simple sequence repeat; **T3SS**, type three secretion system; ***TFL***, *terminal flower*; **TIR**, toll and interleukin 1 receptor; **UDP**, uridindiphosphate; **USDA**, U.S. Department of Agriculture; **V**, Vineland

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Invited Review

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 a "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).

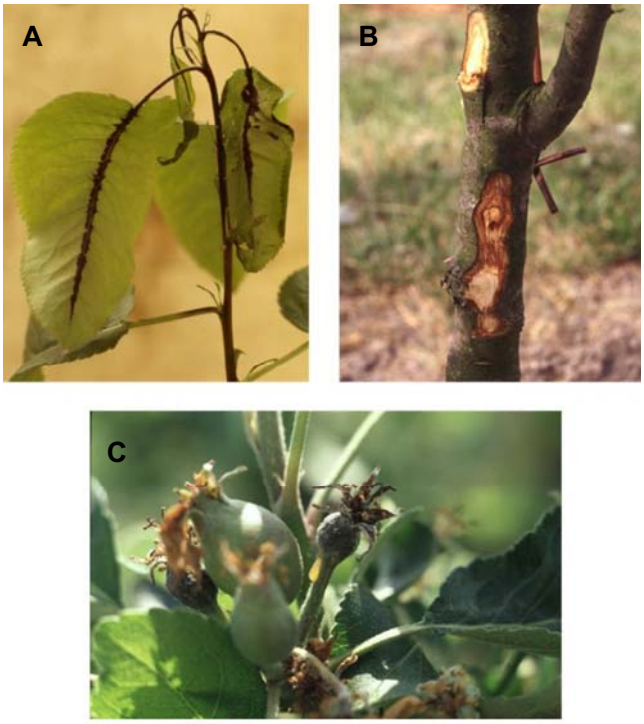

Fig. 1 Symptoms of fire blight. (**A**) Necrotic pear shoot. (**B**) Blighted pear stem. (**C**) Blighted apple fruits with ooze.

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

Table 1 Distribution of fire blight disease worldwide.

Europe	Mediterranean Area
Albania	Armenia
Austria	Cyprus
Belgium	Egypt
Bosnia	Iran
Bulgaria	Israel
Croatia	Jordan
Czech Republic	Lebanon
Denmark	Morocco
England	Turkey
France	**Pacific Rim**
Germany	New Zealand
Greece	**North, Central and South America**
Hungary	Bermuda
Ireland	Canada
Italy	Guatemala
Lichtenstein	Mexico
Luxembourg	USA
Macedonia	
Netherlands	
Norway	
Poland	
Romania	
Serbia	
Slovenia	
Spain	
Sweden	
Switzerland	

for resistance breeding programs. We describe the development of molecular genetics as a breeding tool and the use of biotechnological strategies to improve fire blight resistance of apple and pear cultivars, and discuss their future perspectives.

HISTORY AND IMPORTANCE OF FIRE BLIGHT

The incidence and spread of fire blight throughout the world, has been reviewed previously (Zeller 1974; van der Zwet and Keil 1979; Bonn and van der Zwet 2000), hence we will merely summarise the key events here.

Symptoms of fire blight were first detected in the Hudson valley of New York State as early as 1780 (Denning 1794). With the planting of fruit orchards by settlers, it spread south- and westward and became the major problem of pomefruit production causing huge losses. In 1844, one of the most widespread and destructive epidemics occurred and many Midwestern pear orchards were completely destroyed. The disease also moved into the Southern and Gulf Coast States. The Western plains and the Rocky Mountains could not prevent the expansion of fire blight into the West Coast States. The disease appeared in pear orchards in California in 1888 and *E. amylovora* was identified as the causal agent of fire blight (Pierce 1902). Soon after it had reached the west coast, the disease wrought such havoc in California between 1901 and 1910 as has seldom been seen in a fruit growing area since. Between 1902 and 1904 fire blight reduced the number of pear trees in Fresno, California from 125,000 to 1,500 (Wilson 1907). An estimated two-thirds of the pear trees of the cultivar 'Bartlett' were eliminated during the period 1903-1908 at a cost of US$ 5 million (Woods 1909), while in 1930 alone fire blight losses were estimated at US$ 2,268,260 (Milbraith 1930). This estimate included costs for crop losses, fire blight control, inspection and autumn blight control (Bonn and van der Zwet 2000). However, as there are many factors to be considered, it is often difficult to assess economic losses caused by fire blight. Following this outbreak in California, fire blight spread northward into Oregon and Washington State. Since the first discovery in the Hudson valley of New York in 1780, fire blight has moved into every region of the USA within about 135 years at a time when the movement of humans and goods advanced from horse and wagon to rail and car (van der Zwet and Keil 1979). Human activity has been a very important factor in the spread of the disease in addition to host susceptibility and conducive weather conditions. Because of the favourable weather conditions for *E. amylovora* in the USA, pear culture is now mostly restricted to the drier regions of the west coast. Apple production has not been as seriously affected as apple cultivars are generally more resistant or tolerant to the disease. 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', 'Beurré Bosc' 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 Africa 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 Southern England, 20,000 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 distant 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* hedges on 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

Table 2 Biochemical properties of two antagonistic bacterial species, the pathogens *E. amylovora* and *E. pyrifoliae* and their interactions with plants.

Species	Capsular EPS	Levan formation	Sorbitol utilization	Sucrose metabolism	Virulence on apple/pear	HR on tobacco
E. tasmaniensis	no	yes	no	yes	no	yes
E. billingiae	yes	no	yes	no	no	no
E. amylovora	yes	yes	yes	yes	yes	yes
E. pyrifoliae	yes	no	yes	yes	no/yes	yes

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 (Moltmann, 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 rootstocks 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 *Enterobactereaceae*. 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 actively distributed by insects visiting flowers. The fast multiplication on the stigma and migration to the nectarthodes (Spinelli *et al.* 2005a) is followed by invasion of the flower cluster, surrounding petioles 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, *Erwinia pyrifoliae* (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). HrpL 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 *dspE*/*A* has a dominant role and causes cell death by interacting with plant cell proteins, such as NbSGT1 (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"

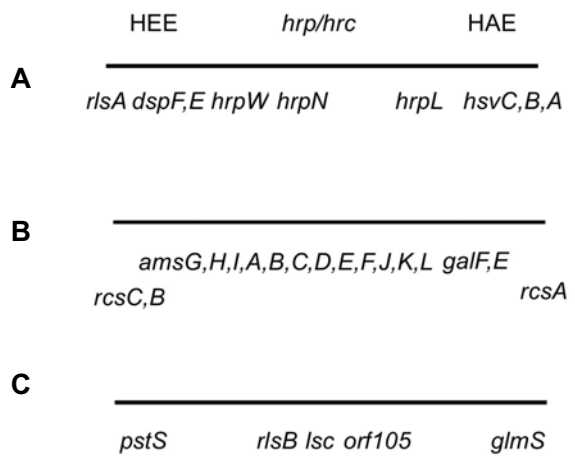

Fig. 2 Arrangement of important genes in virulence regions of *E. amylovora*. (**A**) *hrp*-region; HEE, Hrp effectors and elicitors; RlsA affects levan synthesis. (**B**) *ams* region. The gene order is reversed in respect to the genomic sequence. The regulatory genes *rcsA*, *B*, *C* are located in a distance to the *ams* region. (**C**) *lsc* region. The borders are *pstS*, belonging to the *pst* operon, and *glmS*, involved in glucosamine synthesis.

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* (for *hrp*-associated systemic virulence) increase systemic infection of apple (Oh *et al*. 2005). In blast searches, the gene products resemble proteins involved in phaseolotoxin and nikkomycin biosynthesis, although these compounds have not been shown for *E. amylovora*. A schematic map for location of *hrp*-associated genes is presented in **Fig. 2**.

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 galactoses 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 *galF* and *galE*, involved in the formation of UDP-galactose, the main precursor of the repeating unit. GalE is a 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 phosphorylase 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 (Geier and Geider 1993). Levansucrase expression is affected by the regulatory genes *rlsA*,*B*,*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* (Dellagi *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 (Johnson 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 Gouk 2003; Pusey and Curry 2004). Additional epiphytic bacteria have been investigated as fire blight antagonists. Genomic sequences of *Erwinia tasmaniensis* Et1/99 (Kube *et al*. 2008), *E. pyrifoliae* Ep1/96 and *E. billingiae* Eb661 have been completed. In comparison to *E. amylovora* and *E. pyrifoliae*, the antagonistic Erwinias 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 pathogenic bacteria 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. 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 φEa1h 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-Lebley *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 ‘Kirchensaller 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 'Golden 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 (*Mf*821) into the domesticcated apple, with the first cross made in 1914 (Crandall 1926). Since the 1970s, many *Vf* resistant cultivars 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 *Vf* scab resistance facilitated introgression as phenotyping is relatively easy. However, scab resistance of *Mf*821 is overcome now by the development of pathogen strains virulent to *Mf*821, confirming the vulnerability of single gene resistances. 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 trees, which will be discussed later in the outlook. 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 Tóth *et al*. (2006) found for progeny of 'Prima'.

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 *Se* allele for sensitivity to fire blight (Thompson *et al*. 1975). A number of *P. communis* accessions were identified as being 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; Bagnara *et al*. 1996; Fischer 1996; Lespinasse and Aldwinckle 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 accessions 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; Çitir 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 (*Chaenomeles* spp.) are 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*, *Crateagus*, *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 1987, 1990; Cadic 1987; Cadic *et al*. 1990).

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. A standard 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 Zwet *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.

Genetic resources – apple

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. Lespinasse and Aldwinckle (2000) reported that of the 197 cultivars released 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 paper is 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.

Momol *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. One resistant seedling 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-virginianae*) as well as aesthetic value. Accessions on which no infections were observed in over 200 recordings are *M.* x *adstringens* 'Almey' and 'Sparkler', *M.* 'Branzam', *M.* 'Cascole', *M.* 'Coralcole', *M.* 'Ökonomierat 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. prattii*, *M. sieboldii*, *M. toringoides*, *M. transitoria*, *M. yunannensis*, and *M. zhaojiaoensis* 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. yunannensis* 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. The resistance varied widely among the Asian and North American species, with the highest proportion of resistant accessions found in *M. baccata, M. fusca*, *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.

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 in *M. baccata, M. fusca*, *M. sieboldii*, *M.* x *atrosanguinea*, *M.* x *floribunda*, *M. prunifolia*, and *M.* x *zumi* (Peil and Richter, unpublished data). A general classification of species as resistant or susceptible to fire blight seems to be impossible, because a wide variability can be found among different accessions of a species. However, a sufficient number of accessions have been identified to date to provide breeders with fire blight resistant material assumed to have different resistance mechanisms to develop new apple cultivars with durable resistance.

Many accessions, in the form of old cultivars and advanced selections, of the domesticated apple have been assessed for their fire blight resistance. A study of 69 apple cultivars showed that some scab-resistant cultivars, including 'Florina', 'Liberty' and 'MacFree', are resistant to fire blight (Aldwinckle *et al*. 1999). 'Liberty', together with another scab-resistant cultivar, 'Enterprise', showed the lowest percentage of shoot necrosis of 14 cultivars tested under field conditions after artificial inoculation (Mohan *et al*. 2002). In a French study, the scab-resistant selections 'Priscilla', 'Perpetu-Evereste', 'Golden Gem' and 'Nova Easygro' demonstrated high resistance in both shoots and flowers (Le Lezec *et al*. 1987). The evaluation of 11 old local cultivars from Hungary identified 'Pónyik alma', 'Sikulai' and 'Szemes alma' as more resistant than the reference cultivars 'Liberty' and 'Remo' (Kása *et al*. 2004). In a preliminary screening of 13 old apple cultivars in Switzerland, 'Schneiderapfel' was the most resistant, while six cultivars were more susceptible than the susceptible reference cultivar 'Gala' (Szalatnay *et al*. 2009).

In Germany, several scab-resistant cultivars that are also resistant to fire blight have been bred in Dresden-Pillnitz (Fischer and Fischer 1996, 1999). Cultivars, such as 'Reanda', 'Remo', 'Rene', 'Resi' and 'Rewena', showed low

Table 3 Mean % necrosis of *Malus* species after artificial shoot inoculation with fire blight bacteria.

Wild species	№ of accessions tested	Mean necrosis (%)	Range of necrosis for accessions
M. baccata	35	41.5	0.0 - 97.6
M. bhutanica	2	66.0	49.2 - 82.8
M. coronaria	2	55.9	46.1 - 65.6
M. florentina	2	46.3	28.8 - 63.8
M. fusca	5	9.2	0.5 - 29.6
M. glaucensens	1	49.0	
M. honanensis	1	89.6	
M. ioensis	1	61.6	
M. kansuensis	2	64.6	43.8 - 85.5
M. komarovii	1	87.7	
M. lancifolia	1	41.7	
M. orientalis	4	40.1	
M. platycarpa	3	50.0	40.3 - 64.4
M. prattii	1	59.7	
M. sargentii	3	75.1	65.7 - 88.5
M. sieboldii	1	9.1	
M. sylvestris	30	71.9	30.7 - 95.6
M. transitoria	1	60.8	
M. trilobata	1	95.8	
M. tschonoskii	1	100.4	
Hybrid species			
M. trilobata x M. baccata	1	30.3	
M. x *arnoldiana*	2	86.4	85.9 - 86.8
M. x *arnoldiana x M. spectabilis* 'van Eseltine'	1	62.5	
M. x *atrosanguinea*	2	1.7	0.0 - 3.3
M. x *dawsoniana*	1	19.7	
M. x *domestica*	2	60.3	20.6 - 100.0
M. x *floribunda*	5	5.9	5.0 - 7.4
M. x *halliana*	1	14.3	
M. x *heterophylla*	1	36.5	
M. x *micromalus*	2	70.4	64.0 - 76.8
M. x *niedzwetzkiyana*	1	22.5	
M. x *prunifolia*	6	36.4	2.6 - 80.7
M. x *purpurea*	1	63.9	
M. x *robusta*	6	7.7	2.9 - 10.2
M. x *soulardii*	1	83.9	
M. x *spectabilis*	1	57.9	
M. x *sublobata*	1	91.8	
M. x *zumi*	2	21.0	7.4 - 34.6

susceptibility to repeated artificial shoot inoculations with the disease (Richter and Fischer 2002; Fischer and Richter 2004). Previously, some selections of the Pi series of apples, such as 'Pinova' that were not specifically bred for disease resistance, had performed well in glasshouse and field tests (Fischer and Schäfer 1990). In Spain, four hybrids ('Raxina 8', 'Raxina 12', 'Raxina 16' and 'Raxina 30') have been selected that combine scab and mildew resistance with resistance to fire blight and tolerance to rosy apple aphid for use in cider apple breeding (Dapena and Blázquez 2004). Apple cultivars and clones from the Polish breeding programme were screened by Sobiczewski *et al*. (2006), who found that 'Free Redstar' and selection J-79 are the most resistant ones of the ten cultivars screened. The first results of fire blight resistance screening in the Hungarian breeding programme showed that the hybrids MR-03 and MR-10 were the most resistant ones of 18 scab- and mildew-resistant selections tested (Tóth *et al*. 2006). Evaluation of commercial orchards in Bulgaria, where *E. amylovora* appeared more recently, showed that the major apple cultivars 'Cooper 4' and 'Starkrimson' had remained free of the disease over a three-year period (Bobev and Decker 1999). In France, the field evaluation following shoot inoculation of current commercial cultivars and advanced selections showed that the most resistant accessions predominantly were breeding selections, e.g. 'Delearly', 'Hacnine', 'Quemoni', 'Florina', 'Delvale', 'Elise', X3189, 'Baujade', 'Delorgue' and 'Gradigold', that are only moderately resistant, on par with 'Golden Delcious' and 'Fuji' (Le Lezec *et al*. 1997a). Further breeding will be required before new commercial cultivars with enhanced resistance will be available.

Genetic resources – pear

The genus *Pyrus*, originating from Caucasus and Central Asia, comprises about 23 species (Chevreau and Skirvin 1992). Three groups of domesticated pears descend from the wild species: the European pear *P. communis* L., the Chinese pear *P. bretschneideri*, and the Japanese or Asian pear *P. pyrifolia* (Burm) Nakai, also known as nashi.

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 *Pyrus* species *P. ussuriensis*, *P. calleryana*, *P. betulaefolia*, *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. elaeagrifolia*, *P. fauriei*, *P. koehnei*, *P. nivalis*, *P. pyrifolia*, *P. regelii*, *P. salicifolia*, 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. betulaefolia* hybrid were highly resistant, showing less than four percent shoot necrosis. Accessions of *P. elaeagrifolia*, *P. fauriei*, *P. koehnei*, *P. nivalis*, *P. pyrifolia*, and *P. salicifolia*, were described as highly susceptible. A natural fire blight infestation 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. betulaefolia* 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. betulaefolia* x *P. ussuriensis*) out of 28 combinations produced progeny with improved levels of fire blight resistance.

In an extensive evaluation of local germplasm comprising 133 *Pyrus*, mostly *P. communis*, accessions collected in Central Europe, 17 accessions remained free of fire blight during a 5-year period of heavy disease pressure 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', 'Smokvarka', 'Tiramka', 'Tursija', and 'Vodenjak' from Yugoslavia; 'Pere Gutui' and 'Rosii Untoase' from Romania; Q21404 and Q21419 from Poland; and 'Arabitka', 'Legkorabi' and 'Bohus' from Hungary. This number of accessions was about one third of the 50 accessions that had remained free in an earlier screening of all of the 384 accessions collected (van der Zwet and Bell 1990). Further south in Europe, severe infections since the introduction of fire blight in the Central Black Sea region in Turkey in the 1980s has led to the replacement of susceptible cultivars with cultivars that have remained relatively unaffected over the years, such as 'Gyaver' ('Kieffer'), and the local varieties 'Keklik', 'Ankara', 'Taş Armudu', and 'Çiçek' (Çitir and Mirik 1999). 'Keklik' and 'Taş', together with 'Ovalı', 'Ekşi Gökdulu' and 'Kara Çıbık', were identified as less susceptible in a second Turkish study performed on 35 local accessions from Western Anatolia (Saygılı *et al*. 1999). In Bulgaria, the cultivars 'Bella di Giugno' and 'Beurré Giffard' were the most resistant commercial cultivars (Bobev

and Decker 1999). 'Beurré Giffard' previously had demonstrated high resistance, but was not as consistent as other old cultivars, such as 'Beurré 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 'Wahlsche Schnapsbirne' as being highly resistant (Zeller and Zeller 1998).

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 'Beurré Alexandre Lucas' (Paprštein *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 'Magness', 'Moonglow', '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', 'Magness', 'Maxine' and 'Moonglow' 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 programmes 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 including 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 dwarfing 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, seven clones of the Geneva™ (G) series have been designated and released for commercialization. The resistant rootstocks G.11 (tolerant), G.30, 'Geneva® 3041' (CG.3041, previously G.41), G.202 and G.935 are descendants of *M.* x *robusta* 5; G.16 of *M.* x *floribunda*; and G.65 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 G.3041 is derived from *M.* x *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 'Budagovsky 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. G.11, G.65, G.16, G.30, 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.118, B.490, 'Bemali', G.11, G.16, G.30, G.65, G.210, CG.3041, CG.4202, 'Supporter 1', 'Novole', OAR1, V.1, and V.3 as tolerant/resistant. CG.3041 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, V.2, V.3, V.6 and V.7) out 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 Webster (2003) reported 'Supporter 1' 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. comm.). 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 breeding with *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 (Paprštein *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 2005) observations will not be sufficient. Many factors, such as host (e.g. vigour, nutrional 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 1991; 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 pollenizer and the rootstock M.9 for a period of three years in Idaho (Mohan *et al*. 2002). In France, 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*. 1984) or cut-leaf (Maas Geesteranus and Heyting 1981; Lespinasse and Paulin 1990) assays, both relying on variable but more than ample inoculum having been introduced into the hosts. Resistance evaluation under controlled conditions have been shown to correlate well with field resistance (Quamme *et al*. 1976), provided there is sufficient replication, even upto 20 has been recommended (Bell *et al*. 1990), to improve resistance assessments as relying on glasshouse inoculation of an individual seedling can be a weak predictor of its field resistance (Quamme *et al*. 1990). Nevertheless, even with inoculation conditions and techniques being as uniform as possible, the findings of fire blight resistance studies have proved to be variable to some extent. Moreover, among replicates of a susceptible accession within an experiment there usually are some that do not become infected. Therefore, the index of varietal susceptibility (IVS) was developed, which integrates both the frequency and severity of the infections into one score to get a more representative assessment of fire blight resistance of an accession (Thibault *et al*. 1987a). The uptake of this method has been limited (e.g. Dondini *et al*. 2004) as breeders have favoured methods based on the mean necrosis (e.g. Peil *et al*. 2007a) or the area under disease progress curve (AUDPC) (e.g. Khan *et al*. 2006) measured as the percenttage necrosis length of the total shoot length. These methods are more commonly used for the identification of QTL 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*. 1987) to fair (e.g. Maroofi and Mostfavi 1996) for apple 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 of grafted scions in the greenhouse for many of the scab-resistant Re-cultivars that are resistant to fire blight, but almost no correlation for the Pillnitzer 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

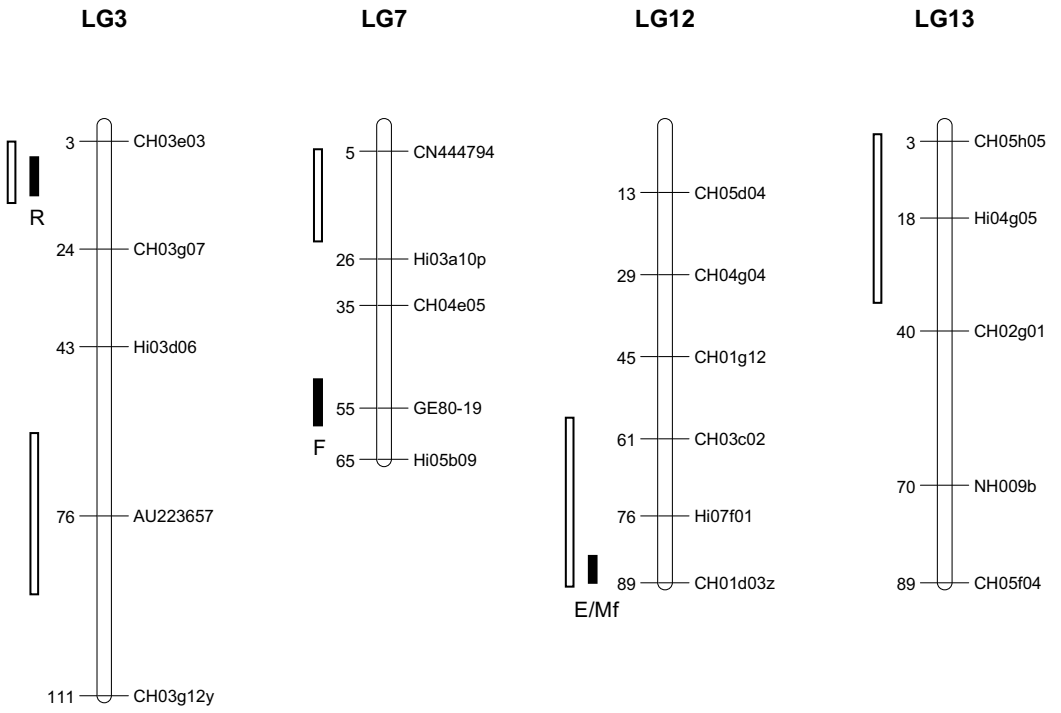

Fig. 3 The global position of quantitative trait loci (QTL) for fire blight resistance on the apple genome based on the skeleton map developed by Silfverberg-Dilworth *et al.* (2006). Only linkage groups that have been shown to carry QTL are presented. ▬ major QTL (R = 'Robusta 5', F = 'Fiesta', E/Mf = 'Evereste'/*Malus floribunda* 821); ▭ minor QTL.

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 Fazio, pers. comm.). 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 elaborative 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*. 2005a) 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).

'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 LG13 (**Fig. 3**) of 'Discovery', which explained 5.4% and 7.9%, respectively of the phenotypic variation (Calenge *et al*. 2005a). These minor QTLs 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 another explanation for the lack of QTL identified in 'Prima'. Four genomic regions other than the LG3 region were involved in the digenic interactions between both homologous and homeologous chromosome segments of the three cultivars (Calenge *et al*. 2005a).

'Robusta 5'

A major QTL was identified in two families of 'Robusta 5' 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 ($P(\chi^2 < 0.06) = 0.80$). Treating the resistance 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 5' (Gardner *et al*. 1980b), but have not been demonstrated to date. This may be due 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 5' 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 'Summerred' family (Naik *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 candidates, some of which are expressed in the earlier 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 5' 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 5' QTL on LG3 (Malnoy *et al*. 2008a) using the bin-mapping approach with selected progeny of the M.9 x 'Robusta 5' family (Celton *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). Protein analysis of differential expression in incompatible interactions as applied to a compatible interaction (Heyens *et al*. 2006) may provide further insight into the genes involved in plant resistance to fire blight.

'Evereste' and *Malus floribunda* 821

Recently, two new QTL loci for fire blight resistance were reported. A major QTL from the ornamental crab apple 'Evereste' 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 Hi04c05. Although 'Evereste' 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 'Evereste' suggests that it inherited its fire blight resistance from the unknown pollen parent. The QTL from *M. floribunda* 821 explained over 40% 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 *Rvi6* (*Vf*) 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.* x *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%) (Kellerhals *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 prelimanarily 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,

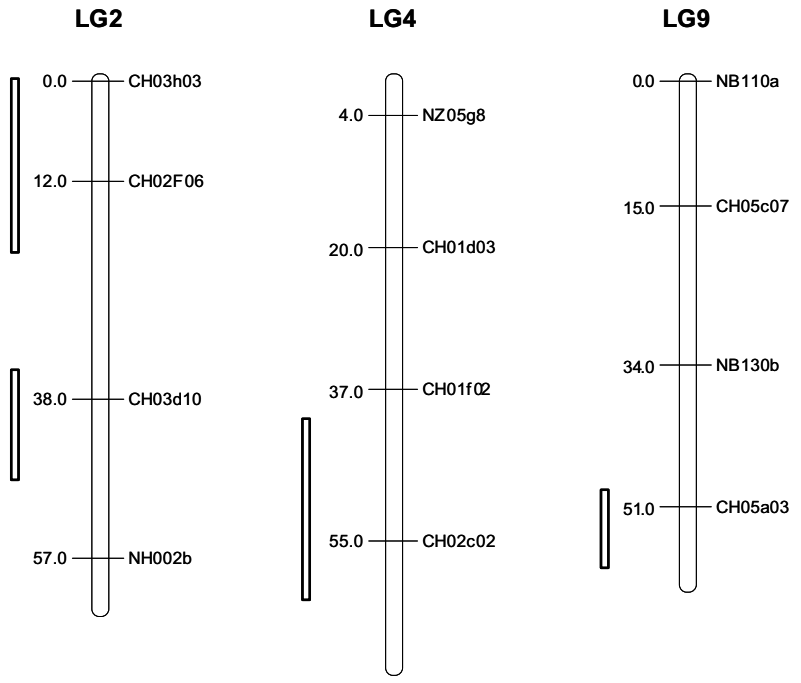

Fig. 4 The global position of quantitative trait loci (QTLs) for fire blight resistance from 'Harrow Sweet' on the pear genome based on a skeleton map compiled from Dondini *et al.* (2004) and Yamamoto *et al.* (2009). Only the linkage groups that have been shown to carry the minor QTL from 'Harrow Sweet' are presented.

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, preferably much stronger QTL 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 RGAs has had limited success to date. While about 35 RGAs with NBS-LRR motifs were identified in the resistant European pear cultivars 'Harrow Sweet', US 309, '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; Afunian *et al*. 2006). As demonstrated above, the QTL approach reflecting the polygenic nature of fire blight resistance in pear has been more successful.

Additional aspects of QTL identification

With the identification of useful QTL particularly in apple, a good start has been made with molecular breeding in pomefruit. In apple, the combined QTL from 'Fiesta' and 'Robusta 5' may provide a strong and durable resistance. However, additional sources with different resistance mechanisms have to be utilized in breeding in order to achieve durable resistances through gene pyramiding. Tightly linked genetic markers will have to be developed for each to ensure the efficient and effective transfer of QTL from germplasm to competitive new cultivars. The high synteny of the apple and pear genomes (Yamamoto *et al*. 2004; Celton *et al*. 2009) will be of mutual benefit in the mapping of additional QTL for fire blight resistance.

As all of the QTL research is based on artificial shoot inoculation in the glasshouse, the QTL identified to date need to be confirmed in artificial flower inoculation and also validated in the field under natural infection conditions, as flowers are the primary site of infection. As mentioned above, the correlation between flower and shoot resistance generally is not high (e.g. Le Lezec *et al*. 1987).

Molecular studies, such as micro-arrays, are generating basic information on the host-pathogen relationships in the *E. amylovora*/apple and pear pathosystems, which is expected to result in the identification of new genetic markers that are linked to genes involved in preventing infection by the pathogen. Once proof of function of these genes has been provided, they can be transferred into new cultivars both via traditional breeding and cisgenesis (Schouten *et al*. 2006a). With the availability of genomes of both apple and *E. amylovora*, host-pathogen interaction research will greatly facilitate the identification of new resistances for use in the breeding of new pomefruit cultivars with durable resistances.

GENETICALLY MODIFIED PLANTS

Genetic engineering became a useful tool to overcome natural hurdles in conventional breeding and selection. Resistance genes can be obtained from many sources and transferred to fruit crops, while preserving the desirable qualities of the transformed cultivars (Norelli *et al*. 2003a). David James pioneered genetic engineering in apple and later in strawberry at East Malling, UK in the late 1980s (James *et al*. 1989). His research was followed by other teams, who developed efficient *Agrobacterium*-mediated transformation protocols that were adapted to specific conditions and various cultivars in apple and pear (Lambert and Tepfer 1992; Welander and Maheswaran 1992; Norelli and Aldwinckle 1993; de Bondt *et al*. 1994; Schaart *et al*. 1995; Yao *et al*. 1995; Mourgues *et al*. 1996; Dolgov *et al*. 2000). Recently, progress in DNA technology in apple was reviewed by Gessler and Patocchi (2007) and by Bulley *et al*. (2007). Gessler and Patocchi (2007) stated that one of the first and most important targets for transgenic apple was fire blight resistance, which was pioneered by the Cornell University group led by Aldwinckle *et al*. (2003).

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 intercellular 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' (Reynoird *et al*. 1999).

In the case of cecropin SB-37, several apple lines have been identified that are 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 secretory 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 several German apple cultivars (Hanke *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 the non-transgenic parent. However, plants containing both 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-transgenics. 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 Crassane'. 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 transgene 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).

Because of the bacterial and animal origin of most of the antimicrobial genes used, their acceptance by growers and consumers was 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 into 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 blossom, it was hypothesized that *hrp*N-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 pear plants of 'Passe Crassane' produced by Chevreau *et al*. (2006) 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 transgene *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 essential for the development 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*. 2008c).

Another strategy to improve fire blight resistance in apple by genetic engineering is using the *dpo* gene of the *E. amylovora* bacteriophage phi-Ea1h. 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 'Pinova' being more resistant to *E. amylovora* in an *in vitro* test (Hanke *et al*. 2002). These experiments were recently 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 enhance resistance to 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* (Malnoy *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 susceptiple under *in vitro* conditions. Three lines 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 *MpNPR1* and *HIPM*, resulting in enhanced resistance (Borejsza-Wysocka *et al*. 2007c).

The *embarras de richesses* – 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*. 2000; Chen and Chen 2002; Faize *et al*. 2003), showed symptoms in an uninfected stage (Fitzgerald *et al*. 2004, for review see Mittler and Rizhsky 2000), and/or had an abnormal habitus (Li *et al*. 2004). Especially the formation of transgene-induced lesion mimics 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 unlike 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 *Pgst1* gene (formerly *Pprp-1*), which encodes a glutathione-*S*-transferase (Hahn and Strittmatter 1994). In potato itself it was found that the *gst1* promoter is not 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 (Malnoy *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 (Malnoy *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, wounding, viral infection, and ethylene (LaRosa *et al*. 1992; Nelson *et al*. 1992). Similar results were obtained in transgenic potato, in which it could be shown that the promoter regions of two osmotin-like proteins are inducible 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; Keionen-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 *pin2Att* transgenic apple plants and the mean value was comparable with that of *CaMV35SAtt* 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 pathogen-inducible, were removed from their native promoters and combined (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 *PR2* 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

blight and research to demonstrate this is in progress. For some strategies that depend on the expression of multiple transgenes, the use of bi-directional promoters (naturally occurring or synthetic) could be advantageous. Bi-directional promoters enable the expression of two different genes simultaneously (for review see Venter 2007).

Secretion of antibacterial proteins into the intercellular space

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 Roemmelt *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.

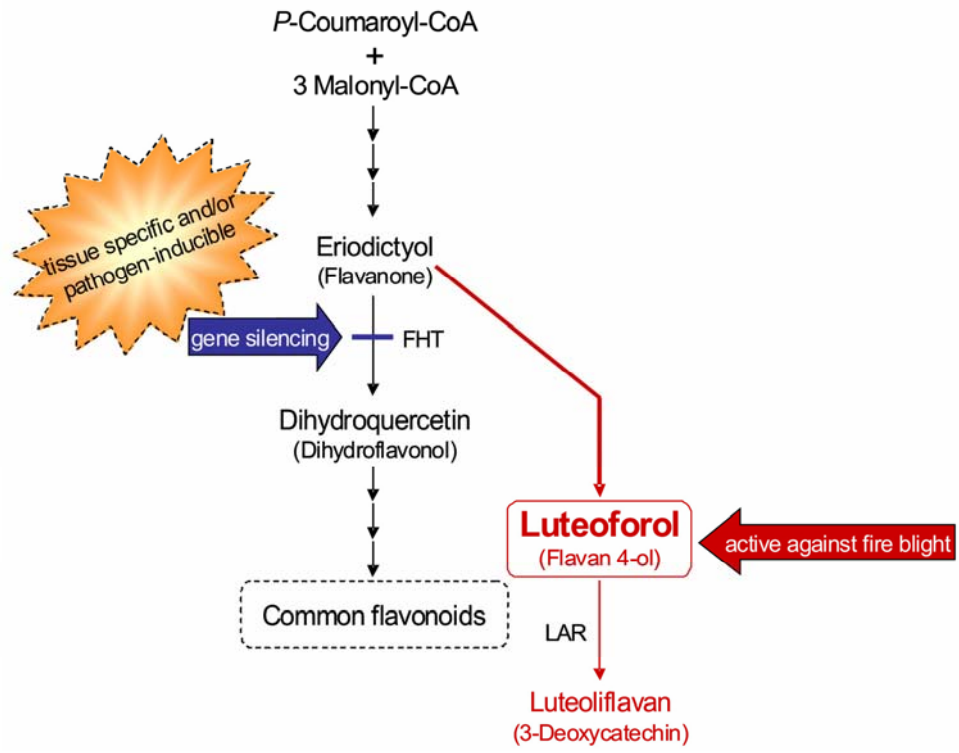

Fig. 5 Section of the flavonoid biosynthesis in apple and pear according to Halbwirth *et al.* (2003, 2006). FHT flavanone-3β-hydroxylase, LAR leucoanthocyanidine reductase, black - common compounds, red - induced compounds, blue – site to take a hand in the pathway.

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 paramomycin. 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 Skryabin 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 phosphomannose-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 (Krens *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*-/*cis*genic 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 fire blight resistant apple and pear cultivars, which 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 are a prerequisite for marker development 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-

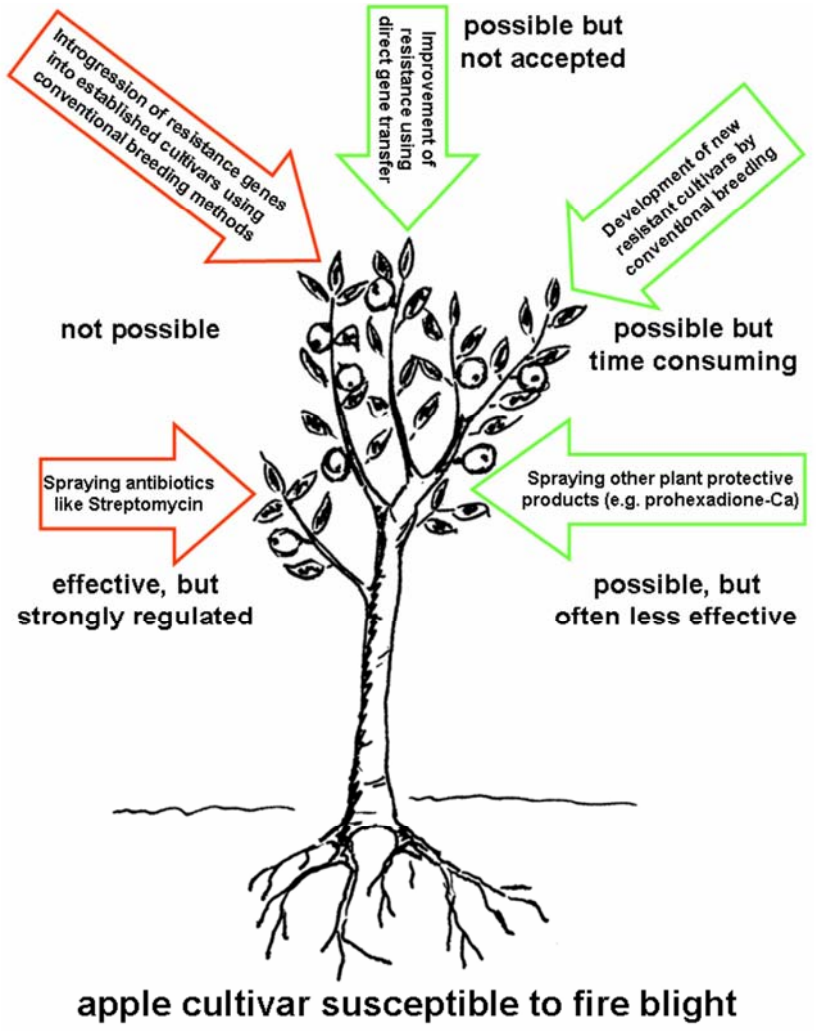

Fig. 6 Possible methods to control fire blight.

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).

REFERENCES

Abdeev RM, Goldenkova IV, Musiychuk KA, Piruzian ES (2003) Expression of a thermostable bacterial cellulase in transgenic tobacco plants. *Russian Journal of Genetics* **39**, 300-305

Abdollahi H, Majidi E (2005) Relation between fire blight resistance and different charactersitics of apple (*Malus domestica* Borkh.) cultivars. *Journal of the National Academy of Bilorussia* **17**, 90-94

Afunian MR, Goodwin PH, Hunter DM (2006) Search for molecular markers linked to fire blight resistance in pear (*Pyrus communis*). *Acta Horticulturae* **704**, 557-565

Aldridge P, Metzger M, Geider K (1997) Genetics of sorbitol metabolism by *Erwinia amylovora* and its influence on bacterial virulence. *Molecular and General Genetics* **256**, 611-619

Aldwinckle HS, Borejsza-Wysocka EE, Malnoy M, Brown SK, Beer SV,

Meng X, Norelli JL, He SY, Jin Q-L (2003) Development of fire blight resistant apple cultivars by genetic engineering. *Acta Horticulturae* **622**, 105-111

Aldwinckle HS, Gustafson HL, Forsline PL, Bhaskara Reddy MV (2002) Fire blight resistance of *Malus* species from Sichuan (China), Russian Caucasus, Turkey and Germany. *Acta Horticulturae* **590**, 369-372

Aldwinckle HS, Gustafson HL, Forsline PL (1999) Evaluation of the core subset of USDA apple germplasm collection for resistance to fire blight. *Acta Horticulturae* **489**, 269-272

Aldwinckle HS, LoGiudice N, Robinson TL, Holleran HT, Fazio G, Johnson WC, Norelli JL (2004) Resistance of apple rootstocks to fire blight infection caused by internal movement of *Erwinia amylovora* from scions infections. *Acta Horticulturae* **663**, 229-233

Alston FH (1994) The position of fire blight (*Erwinia amylovora*) avoidance and resistance amongst pear breeding priorities. *Norwegian Journal of Agricultural Sciences* **Suppl. 17**, 331-336

Bagnara GL, Rivalta L, Laghi M, Quarta R (1996) Evaluation of fire blight resistance in pear: combining ability and breeding strategy. *Acta Horticulturae* **411**, 383-393

Baldi P, Patocchi A, Zini E, Toller C, Velasco R, Komjanc M (2004) Cloning and linkage mapping of resistance gene homologues in apple. *Theoretical and Applied Genetics* **109**, 231-23

Barny MA, Guinebretiere MH, Marcais B, Coissac E, Paulin J-P, Laurent J (1990) Cloning of a large gene cluster involved in *Erwinia amylovora* CFBP 1430 virulence. *Molecular Microbiology* **4**, 777-786

Bauer DW, Garr ER, Kim JF, Norelli JL, Aldwinckle HS, Beer SV (1999) New transgenic approaches for possible increased resistance to fire blight. *Acta Horticulturae* **489**, 301-304

Bell RL, Janick J, Zimmerman RH, van der Zwet T (1976) Relationship between fire blight resistance and fruit quality in pear. *HortScience* **11**, 500-502

Bell RL, Janick J, Zimmerman RH, van der Zwet T (1977) Estimation of heritability and combining ability for fire blight resistance in pear. *Journal of the American Society for Horticultural Science* **102**, 133-138

Bell AC, TG Ranney, TA Eaker, Sutton TB (2005) Resistance to fire blight among flowering pears and quince. *HortScience* **40**, 413-415

Bell RL, van der Zwet T, Bonn WG, Thibault B, Lecomte P (1990) Environmental and strain effects on screening for fire blight resistance. *Acta Horticulturae* **273**, 343-349

Bell RL, Quamme HA, Layne REC, Skirvin RM (1996) Pears. In: Janick J, Moore JN (Eds) *Fruit Breeding, Tree and Tropical Fruits* (Vol I), John Wiley & Sons, Inc., New York, pp 441-514

Bellemann P, Geider K (1992) Localization of transposon insertions in pathogenicity mutants of *Erwinia amylovora* and their biochemical characterization. *Journal of General Microbiology* **138**, 931-940

Bereswill S, Jock S, Aldridge P, Janse JD, Geider K (1997) Molecular characterization of natural *Erwinia amylovora* strains deficient in levan synthesis. *Physiological and Molecular Plant Pathology* **51**, 215-225

Bereswill S, Jock S, Bellemann P, Geider K (1998) Identification of *Erwinia amylovora* by growth morphology on agar containing copper sulfate and by capsule staining with lectin. *Plant Disease* **82**, 158-164

Bergamaschi M, Rivalta L, Sirri S, Biondi E, Ramilli F, Bazzi C (2006) Reactivity to fire blight of new promising pear selections. *Acta Horticulturae* **704**, 571-576

Berger F, Zeller W (1994) Untersuchungen zur Resistenz von Apfel- und Birnenunterlagen gegenüber dem Feuerbrand (*Erwinia amylovora*). *Erwerbs-Obstbau* **36**, 15-17

Bessho H, Brown SK, Norelli JL, Aldwinckle HS, Cummins JN (2001) Observations on the susceptibility of Japanese apple cultivars and rootstock selections to fire blight. *Journal American Pomological Society* **55**, 120-124

Blachinsky D, Shtienberg D, Zamski E, Weinthal D, Manulis S (2006) Effects of pear tree physiology on fire blight progression in perennial branches and on expression of pathogenicity genes in *Erwinia amylovora*. *European Journal of Plant Pathology* **116**, 315-324

BMELV (2008) Feuerbrand-Strategiepapier 2008 bis 2012. Strategie zur Bekämpfung des Feuerbranderregers im Obstbau ohne Antibiotika 2008 bis 2012. Available online: http://www.bmelv.de/nn_751176/SharedDocs/downloads/04-Landwirtschaft/Pflanzenschutz/Feuerbrand-Strate-giepapier.html

Bobev S, Deckers T (1999) Field susceptibility to fire blight of pome fruits in Bulgaria. *Acta Horticulturae* **489**, 221-224

Bogs J, Bruchmuller I, Erbar C, Geider K (1998) Colonization of host plants by the fire blight pathogen *Erwinia amylovora* marked with genes for bioluminescence and fluorescence. *Phytopathology* **88**, 416-421

Bogs J, Geider K (2000) Molecular analysis of sucrose metabolism of *Erwinia amylovora* and influence on bacterial virulence. *Journal of Bacteriology* **182**, 5351-5358

Bogs J, Richter K, Kim WS, Jock S, Geider K (2004) Alternative methods to describe virulence of *Erwinia amylovora* and host-plant resistance against fireblight. *Plant Pathology* **53**, 80-89

Bolar JP, Norelli JL, Wong KW, Hayes CK, Harman GE, Aldwinckle HS (2000) Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scab and reduces vigor. *Phytopathology* **90**, 72-77

Bömeke H (1972) Noch einmal Feuerbrand. *Gesunde Pflanzen* **24**, 184-190

Bonn WG, van der Zwet T (2000) Distribution and economic importance of fire blight. In: Vanneste JL (Ed) *Fire Blight - The Disease and its Causal Agent, Erwinia amylovora*, CABI Publishing, New York, pp 37-53

Borecki Z, Basak W, Zawadzka B, Millikan D (1967) Fire blight in central Europe. *Plant Disease Reporter* **51**, 3

Borejsza-Wysocka EE, Malnoy M, Kim W-S, Geider K, Beer SV, Aldwinckle HS (2007a) Expression of the Phi-EA1h phage depolymerase gene with constitutive and inducible promoters, translation enhancer and signal sequence in transgenic apple increases resistance to fire blight. *Acta Horticulturae* **738**, 273-276

Borejsza-Wysocka EE, Malnoy M, Kim W-S, Geider K, Beer SV, Aldwinckle HS (2007b) Expression of phi-Ea 1h phage depolymerase gene with constitutive and inducible promoters, translation enhancer and signal sequence in transgenic apple plants increases resistance to fire blight. *Acta Horticulturae* **738**, 273-276

Borejsza-Wysocka EE, Malnoy M, Meng X, Bonasera JM, Nissinen RM, Kim JF, Beer SV, Aldwinckle HS (2004) Silencing of apple proteins that interact with *DspE*, a pathogenicity effector from *Erwinia amylovora*, as a strategy to increase resistance to fire blight. *Acta Horticulturae* **663**, 469-474

Borejsza-Wysocka EE, Malnoy M, Meng X, Bonasera JM, Nissinen RM, Kim JF, Beer SV, Aldwinckle HS (2006) The fire blight resistance of apple clones in which DspE-interacting proteins are silenced. *Acta Horticulturae* **704**, 509-513

Borejsza-Wysocka EE, Malnoy M, Norelli JL, Beer SV, He SH, Aldwinckle HS (2007c) Strategies for obtaining fire blight resistance in apple by rDNA. *Acta Horticulturae* **738**, 283-285

Borejsza-Wysocka EE, Norelli JL, Aldwinckle HS, Ko K (1999) Transformation on authentic M.26 apple rootstock for enhanced resistance to fire blight. *Acta Horticulturae* **489**, 259-266

Bouma AS (1987) Breeding woody ornamentals for fire blight resistance. *Acta Horticulturae* **217**, 293-298

Bouma AS (1990) Breeding *Pyracantha* for fire blight resistance. *Acta Horticulturae* **273**, 327-334

Brisset MN, Paulin J-P (2006) Plant mechanisms interfering with fire blight infection. *Acta Horticulturae* **704**, 483-487

Bubán T, Orosz-Kovács Zs, Farkas Á (2003) The nectary as the primary site of infection by *Erwinia amylovora* (Burr.) Winslow *et al*.: a mini review. *Plant Systematics and Evolution* **238**, 183-194

Bugert P, Geider K (1995) Molecular analysis of the *ams* operon required for exopolysaccharide synthesis of *Erwinia amylovora*. *Molecular Microbiology* **15**, 917-33

Bulley SM, Malnoy M, Atkinson RG, Aldwinckle HS (2007) Transformed apples: traits of significance to growers and consumers. *Transgenic Plant Journal* **1**, 267-279

Bus V, van de Weg, Durel C-E, Gessler C, Parisi L, Rikkerink E, Gardiner S, Meulenbroek B, Calenge F, Patocchi A, Laurens F (2004) Delineation of a scab resistance gene cluster on linkage group 2 of apple. *Acta Horticulturae* **663**, 57-62

Cadic A (1987) Breeding *Pyracantha* for fire blight resistance. *Acta Horticulturae* **217**, 299-303

Cadic A, Paulin J-P, Belin J (1990) New *Pyracantha* resistant to scab (*Spilocaea pyracanthae* (Otth.) Rostrup) and to fire blight (*Erwinia amylovora* (Burr.) Winsl. *et al*.). *Acta Horticulturae* **273**, 303-309

Calenge F, Drouet D, Denancé C, van de Weg WE, Brisset MN, Paulin J-P, Durel C-E (2005a) Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. *Theoretical and Applied Genetics* **111**, 128-135

Calenge F, van der Linden G, van de Weg E, Schouten HJ, van Arkel G, Denancé C, Durel C-E (2005b) Resistance gene analogues identified through the NBS-profiling method map close to major genes and QTL for disease resistance in apple. *Theoretical and Applied Genetics* **110**, 660-668

Calzolari A, Finelli F, Mazzoli GL (1999) A severe unforeseen outbreak of fire blight in the Emilia-Romagna region. *Acta Horticulturae* **489**, 171-176

Campbell JA (1920) The orchard: the outbreak of fire blight. *New Zealand Journal of Agriculture* **20**, 181-182

Carlsson A, Engstrom P, Palva ET, Bennich H (1991) Attacin, an antibacterial protein from *Hyalophora cecropia*, inhibits synthesis of outer membrane proteins in *Escherichia coli* by interfering with *omp* gene transcription. *Infection and Immunity* **59**, 3040-3045

Celton J-M, Tustin DS, Chagné D, Gardiner SE (2009) Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequences. *Tree Genetics and Genomes* **5**, 93-107

Chartier R, Bore J-M, Paulin J-P (1992) Sensibilité des pommiers a cidre au feu bactérien. *Phytoma* **440**, 24-31

Chen C, Chen Z (2002) Potentiation of developmentally regulated plant defense response by AtWRKY18, a pathogen-induced *Arabidopsis* transcription factor. *Plant Physiology* **129**, 706-771

Chevreau E, Malnoy M, Boccara M (2006) Constitutive expression of *Erwinia amylovora* hrpN in transgenic pears reduces fire blight susceptibility. *Acta Horticulturae* **704**, 535-540

Chevreau E, Malnoy M, Mourgues F, Brisset MN, Reynoird JP (2000)

Genetic engineering of pear for increased fire blight resistance. *Acta Horticulturae* **538**, 639-643

Chevreau E, Skirvin RM (1992) Biotechnology of perennial fruit crops. In: Hammerschlag FA, Litz RE (Eds) *Biotechnology of Perennial Fruit Crops*, CAB International, Cambridge, pp 263-276

Çitir A, Mirik M (1999) Fire blight of pome fruits and search for resistant or tolerant cultivars in Amasya and Tokat regions in Turkey. *Acta Horticulturae* **489**, 215-219

Cline JA, Hunter DM, Bonn WG, Bijl M (2001) Resistance of the Vineland series of apple rootstocks to fire blight caused by *Erwinia amylovora*. *Journal American Pomological Society* **55**, 218-221

Cockayne AH (1921) Fire blight and its control: the hawthorn question. *New Zealand Journal of Agriculture* **23**, 30-36

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* **142**, 169-196

Costa G, Andreotti C, Bucchi F, Sabatini E, Bazzi C, Malaguti S, Rademacher W (2001) Prohexadione-Ca (Apogee): growth regulation and reduced fire blight incidence in pear. *HortScience* **36**, 931-933

Crandall CS (1926) Apple breeding at the University of Illinois. *Illinois Agriculture Experimental Station Bulletin* **275**, 341-600

Crosse JE, Bennett M, Garrett CME (1958) Fire blight of pear in England. *Nature* **182**, 1530

Cummins JN, Aldwinckle HS (1983) Breeding apple rootstocks. *Plant Breeding Reviews* **1**, 294-394

Dai ZY, Hooker BS, Anderson DB, Thomas SR (2000) Improved plant-based production of E1 endoglucanase using potato: expression optimization and tissue targeting. *Molecular Breeding* **6**, 277-285

Dai ZY, Hooker BS, Quesenberry RD, Thomas SR (2005) Optimization of *Acidothermus cellulolyticus* endoglucanase (E1) production in transgenic tobacco plants by transcriptional, post-transcription and post-translational modification. *Transgenic Research* **14**, 627-643

Dapena E, Blázquez MD (2004) Improvement of the resistance to scab, rosy apple aphid and fire blight in a breeding programme of cider apple cultivars. *Acta Horticulturae* **663**, 725-727

de la Cruz Blanco J (1996) Lucidencias climaticas y fytosanitarias en los cultivos espanoles durante 1995. *Phytoma-Espana* **78**, 22-27

de Bondt A, Eggermont K, Druart P, Devil M, Goderis I, Vanderleyden J, Broekaert WE (1994) *Agrobacterium*-mediated transformation of apple (*Malus* x *domestica* Borkh) - an assessment of factors affecting gene-transfer efficiency during early transformation steps. *Plant Cell Reports* **13**, 587-593

de Bondt A, Eggermont K, Penninckx I, Goderis I, Broekaert WF (1996) *Agrobacterium*-mediated transformation of apple (*Malus* x *domestica* Borkh): an assessment of factors affecting regeneration of transgenic plants. *Plant Cell Reports* **15**, 549-554

Degenhardt J, Poppe A, Montag J, Szankowski I (2006) The use of the phosphomannose-isomerase/mannose selection system to recover transgenic apple plants. *Plant Cell Reports* **25**, 1149-1156

Degenhardt J, Poppe A, Rösner L, Szankowski I (2007) Alternative selection systems in apple transformation. *Acta Horticulturae* **738**, 287-292

Dellagi A, Brisset M-N, Paulin J-P, Expert D (1998) Dual role of desferrioxamine in *Erwinia amylovora* pathogenicity. *Molecular Plant-Microbe Interactions* **11**, 734-742

Denning W (1794) On the decay of apple trees. *New York Society for the Promotion of Agricultural Arts and Manufacturers Transaction* **2**, 219-222

Dolgov SV, Hanke M-V (2006) Transgenic temperate fruit tree rootstocks. In: Fladung M, Ewald D (Eds) *Tree Transgenesis: Recent Developments*, Springer-Verlag Berlin, Heidelberg, pp 335-350

Dolgov SV, Miroschnichenko DN, Schestibratov KA (2000) Agrobacterial transformation of apple cultivar and rootstock. *Acta Horticulturae* **538**, 619-624

Dolgov SV, Skryabin KG (2004) Transgenic apple clonal rootstock resistant to Basta herbicide. *Acta Horticulturae* **663**, 499-502

Dondini L, Gaiotti F, Pierantoni L, Chiodini R, Tartarini S, Sansavini S, Bazzi C (2006) QTLs related to fire blight resistance in pear. *Acta Horticulturae* **704**, 567-570

Dondini L, Pierantoni L, Gaiotti F, Chiodini R, Tartarini S, Bazzi C, Sansavini S (2004) Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. *Molecular Breeding* **14**, 407-418

Dondini L, Tartarini S, Norelli E, Sansavini S (2002) Cloning and characterization of resistance gene analogs (RGA) in European pear (*Pyrus communis*). *Acta Horticulturae* **596**, 557-565

Donovan A (1991) Screening for fire blight resistance in apple (*Malus pumila*) using excised leaf assays from *in vitro* and *in vivo* grown material. *Annals of Applied Biology* **119**, 59-68

Donovan AM, Morgan A, Valobra-Piagnani C, Ridout MS, James DJ, Garrett CME (1994) Assessment of somaclonal variation in apple. I. Resistance to the fire blight pathogen, *Erwinia amylovora*. *Journal of Horticultural Science* **69**, 105-113

Drain BD (1943) Southern pear breeding. *Journal of the American Society of Horticultural Science* **42**, 301-304

Du Z, Jakovljevic V, Salm H, Geider K (2004) Creation and genetic restoration of *Erwinia amylovora* strains with low levan synthesis. *Physiological and Molecular Plant Pathology* **65**, 115-122

Dueck J, Quamme HA (1973) Fire blight in southern Ontario in 1972. *Canadian Plant Disease Survey* **53**, 101-104

Durel C-E, Denancé C, Brisset M-N (2009) Two distinct major QTL for resistance to fire blight co-localize on linkage group 12 in apple genotypes 'Evereste' and *Malus floribunda* clone 821. *Genome* **52**, 139-147

Düring K (1996) Genetic engineering for resistance to bacteria in transgenic plants by introduction of foreign genes. *Molecular Breeding* **2**, 297-305

Düring K, Porsh P, Fladung M, Lörz H (1993) Transgenic potato plants resistant to the phytopathogenic bacterium *Erwinia carotovora*. *The Plant Journal* **3**, 587-598

Duron M, Paulin JP, Brisset MN (1987) Use of *in vitro* propagated plant material for rating fire blight susceptibility. *Acta Horticulturae* **217**, 317-324

Eastgate JA, Thompson L, Milner J, Cooper RM, Ollitt CE, Roberts IS (1997) Identification of a nonpathogenic *Erwinia amylovora guaB* mutant. *Plant Pathology* **46**, 594-599

Eastham JW (1922) Report of Provincial Plant Pathologist, Vancouver. *British Columbia Department Agriculture Annual Report* **16**, 64-69

El-Helaly AF, Abo-El-Dahab MK, El-Goorani MA (1964) The occurrence of the fire blight disease of pear in Egypt. *Phytopathologia Mediterranea* **3**, 156-163

Faize M, Malnoy M, Dupuis F, Chevalier M, Parisi L, Chevreau E (2003) Chitinases of *Trichoderma atroviride* induce scab resistance and some metabolic changes in two cultivars of apple. *Phytopathology* **93**, 1496-1504

Falkenstein H, Zeller W, Geider K (1989) The 29 kb plasmid, common in strains of *Erwinia amylovora*, modulates development of fireblight symptoms. *Journal of General Microbiology* **135**, 2643-2650

Fatmi M, Bougsiba M, Saoud H (2008) First report of fire blight caused by *Erwinia amylovora* on pear, apple, and quince in Morocco. *Plant Disease* **92**, 314

Fazio G, Aldwinckle HS, McQuin RP, Robinson T (2006) Differential susceptibility to fire blight in commercial and experimental apple rootstock cultivars. *Acta Horticulturae* **704**, 527-530

Fernando WGD, Jones AL (1999) Prohexadione calcium - a tool for reducing secondary fire blight infection. *Acta Horticulturae* **489**, 597-600

Ferree DC, Carlson RF (1987) Apple rootstocks. In: Rom RC, Carlson RF (Eds) *Rootstocks for Fruit Crops*, John Wiley & Sons, New York, USA, pp 107-143

Fischer C, Fischer M (1996) Results in apple breeding at Dresden-Pillnitz - review. *Garten-bauwissenschaft* **61**, 139-146

Fischer C, Schäfer HJ (1990) Vergleichende Untersuchungen der Resistenz von Apfelsorten gegenüber Feuerbrand im Gewächshaus und im Freiland. *Obstbau* **37**, 299-300

Fischer C, Richter K (2004) Fire blight resistant apple cultivars produced by conventional breeding. *Acta Horticulturae* **663**, 721-724

Fischer C, Richter K (1999) Results on fire blight resistance in the Pillnitzer apple breeding programme. *Acta Horticulturae* **489**, 279-263

Fischer H, Meyer J (1972) Praktische Erfahrungen bei der Bekämpfung der Feuerbrandkrankheit (Erwinia amylovora) 1971. *Gesunde Pflanzen* **24**, 62-71

Fischer M (1996) Ergebnisse der Resistenzprüfungen gegenüber *Erwinia amylovora* (Burrill) Winslow *et al*. an *Malus*- und *Pyrus*-Nachkommen im Rahmen der Unterlagenzüchtung. *Erwebsobstbau* **38**, 77-80

Fischer M (2001) New dwarfing and semi-dwarfing Pillnitz apple and pear rootstocks. *Acta Horticulturae* **557**, 55-61

Fischer M (2005) Ergebnisse des Naumburger-Pillnitzer Birnenunterlagenzuchtprogrammes. *Erwerbs-Obstbau* **47**, 6-11

Fischer M, Fischer C (1999) Evaluation of *Malus* species and cultivars at the Fruit Genebank Dresden-Pillnitz and its use for apple resistance breeding. *Genetic Resources and Crop Evolution* **46**, 235-241

Fischer R, Schumann D, Zimmermann S, Drossard J, Sack M, Schillberg S (1999) Expression and characterization of bispecific single-chain *Fv* fragments produced in transgenic plants. *European Journal of Biochemistry* **262**, 810-816

Fitzgerald HA, Chern MS, Navarre R, Ronald PC (2004) Overexpression of (At)NPR1 in rice leads to a BTH- and environment-induced lesion-mimic/cell death phenotype. *Molecular Plant-Microbe Interactions* **17**, 140-151

Flachowsky H, Birk T, Hanke V (2004) Preliminary results to establish an alternative selection system for apple transformation. *Acta Horticulturae* **663**, 425-430

Flachowsky H, Peil A, Rollins J, Hanke M-V, Richter K, Lee D-H (2008b) Improved fire blight resistance in transgenic apple lines by constitutive overexpression of the *MbR4* gene of *Malus baccata*. *Acta Horticulturae* **793**, 287-291

Flachowsky H, Peil A, Sopanen T, Elo A, Hanke V (2007) Overexpression of *BpMADS4* from silver birch (*Betula pendula* Roth.) induces early-flowering in apple (*Malus* x *domestica* Borkh.). *Plant Breeding* **126**, 137-145

Flachowsky H, Richter K, Kim W-S, Geider K, Hanke M-V (2008a) Transgenic expression of a viral EPS-depolymerase is potential useful to induce fire blight resistance in apple. *Annals of Applied Biology* **153**, 345-355

Flor HH (1956) The complementary genic systems in flax and flax rust. *Advances in Genetics* **8**, 29-54

Forsline PL, Aldwinckle HS (2002) Natural occurrence of fire blight in USDA apple germplasm collection after 10 years of observation. *Acta Horticulturae*

590, 351-357

Forsline PL, Aldwinckle HS, Dickson EE, Luby JL, Hokanson SC (2003) Collection, maintainance, characterization, and utilization of wild apples of Central Asia. *Horticultural Reviews Volume* **29**, 1-61

Forsline PL, Luby JL, Aldwinckle HS (2008) Fire blight incidence on *Malus sieversii* grown in New York and Minnesota. *Acta Horticulturae* **793**, 345-350

Gardiner SE, Bus VGM, Rusholme RL, Chagné D, Rikkerink EHA (2007) Apple. In: Kole C (Ed) *Genome Mapping and Molecular Breeding in Plants*. *Fruits and Nuts*, Springer-Verlag, Berlin Heidelberg, pp 1-62

Gardner RG, Cummins JN, Alswinckle HS (1980a) Fire blight resistance in the Geneva apple rootstock breeding program. *Journal of the American Society of Horticulture Science* **105**, 907-912

Gardner RG, Cummins JN, Aldwinckle HS (1980b) Inheritance of fire blight resistance in *Malus* in relation to rootstock breeding. *Journal of the American Society of Horticultural Science* **105**, 912-916

Geider K (1999) Interference of copper sulphate in growth of *Erwinia amylovora*. *Journal of Phytopathology* **147**, 521-526

Geider K (2000) Exopolysaccharides of *Erwinia amylovora*: structure, biosynthesis, regulation, role in pathogenicity of amylovoran and levan. In: Vanneste JL (Ed) *Fire Blight - The Disease and its Causal Agent, Erwinia amylovora*, CABI Publishing, New York, pp 117-140

Geider K (2005) Molecular detection of fire blight and differentiation of *Erwinia amylovora* strains. *Phytopathologia Polonica* **35**, 57-68

Geider K (2006) Characterization of antagonistic bacteria and a viral lysozyme for control of fire blight. *Phytopathologia Polonica* **39**, 87-92

Geider K, Auling G, Du Z, Jakovljevic V, Jock S, Völksch B (2006) *Erwinia tasmaniensis* sp. nov., a non-phytopathogenic bacterium from apple and pear trees. *International Journal of Systematic and Evolutionary Microbiology* **56**, 2937-2943

Geider K, Auling G, Jakovljevic V, Völksch B (2009) A polyphasic approach assigns the pathogenic *Erwinia* strains from diseased pear trees in Japan to *Erwinia pyrifoliae*. *Letters in Applied Microbiology* **48**, 324-330

Geier G, Geider K (1993) Characterization and influence on virulence of the levansucrase gene from the fire blight pathogen *Erwinia amylovora*. *Physiological and Molecular Plant Pathology* **42**, 387-404

Gessler C, Patocchi A (2007) Recombinant DNA technology in apple. *Advances in Biochemical Engineering/Biotechnology* **107**, 113-132

Gianessi L, Silvers C, Sankula S, Carpenter J (2002) Plant biotechnology: current and potential impact for improving pest management in US agriculture: an analysis of 40 case studies. National Center for Food and Agricultural Policy, June Report. Available online: http://www.ncfap.org/40CaseStudies.htm

Gimelfarb A, Lande R (1995) Marker-assisted selection and marker-QTL associations in hybrid populations. *Theoretical and Applied Genetics* **91**, 522-528

Green TL, den Boer JH (1995) Crabapple disease and aesthetic ratings. *Malus* **9 (2)**, 3-58

Grimm R (1989) Erster Nachweis von Feuerbrand in der Schweiz. *Schweizerische Zeitschrift für Obst und Weinbau* **125**, 514-516

Gurr SJ, Rushton PJ (2005) Engineering plants with increased disease resistance: how we are going to express it? *Trends in Biotechnology* **23**, 283-290

Hahn K, Strittmatter G (1994) Pathogen defence gene *prp1-1* from potato encodes an auxine responsive gluthatione *S*-transferase. *European Journal of Biochemistry* **226**, 619-626

Halbwirth H, Fischer TC, Roemmelt S, Spinelli F, Schlangen K, Peterek S, Sabatini E, Messina C, Speakman JB, Andreotti C, Rademacher W, Bazzi C, Costa G, Treutter D, Forkmann G, Stich K (2003) Induction of antimicrobial 3-deoxyflavonoids in pome fruit trees controls fire blight. *Zeitschrift für Naturforschung C-A Journal of Biosciences* **58**, 765-770

Halbwirth H, Fischer TC, Schlangen K, Rademacher W, Schleifer KJ, Forkmann G, Stich K (2006) Screening for inhibitors of 2-oxoglutarate-dependent dioxygenases: flavanone 3 beta-hydroxylase and flavonol synthase. *Plant Science* **171**, 194-205

Hanke V, Düring K, Norelli JL, Aldwinckle HS (1999) Transformation of apple cultivars with *T4*-lysozyme-gene to increase disease resistance. *Acta Horticulturae* **489**, 253-256

Hanke M-V, Flachowsky H, Peil A, Hättasch C (2007) No flower no fruit - genetic potentials to trigger flowering in fruit trees. *Genes, Genomes and Genomics* **1**, 1-20

Hanke V, Hiller I, Klotzsche G, Richter K, Norelli JL, Aldwinckle HS (2000) Transformation in apple for increased disease resistance. *Acta Horticulturae* **538**, 611-616

Hanke V, Kim W-S, Geider K (2002). Plant transformation for induction of fire blight resistance: transgenic apples expressing viral EPS-Deploymerase. *Acta Horticulturae* **590**, 393-395

Harris SA, Robinson JP, Juniper BE (2002) Genetic clues to the origin of apple. *Trends in Genetics* **18**, 426-430

Harrison FC (1904) Some bacterial disease of plants prevalent in Ontario: fire blight or twig blight. *Ontario Agricultural College Bulletin* **136**, 1-9

Heise A, Lippok B, Kirsch C, Hahlbrock K (2002) Two immediate-early pathogen-responsive members of the *AtCMPG* gene family in *Arabidopsis thaliana* and the W-box-containing elicitor-response element of *AtCMPG1*. *Proceedings of the National Academy of Sciences USA* **99**, 15834-15835

Hevesi M (1996) Az *Erwinia amylovora* (Burrill) Winslow *et al*. hazai meg jelenese alman. *Novehyvedelem* **32**, 225-228

Heyens K, Valcke R, Dumonmt D, Robben J, Noben J-P (2006) Differential expression of proteins in apple following inoculation with *Erwinia amylovora*. *Acta Horticulturae* **704**, 489-494

Hildebrand M, Aldridge P, Geider K (2006) Characterization of *hns* genes from *Erwinia amylovora*. *Molecular Genetics and Genomics* **275**, 310-319

Honty K, Göndör M, Tóth M, Kása K, Hevesi M (2006) Susceptibility of pear cultivars to Fire Blight in Hungary. *Acta Horticulturae* **704**, 583-587

Hough LF, Shay JR, Dayton DF (1953) Apple scab resistance from *Malus floribunda* Sieb. *Proceedings of the American Society for Horticultural Science* **62**, 341-347

Hultmark D, Engström A, Andersson K, Steiner H, Bennich H, Boman HG (1983) Insect immunity. Attacins, a family of antibacterial proteins from *Hyalophora cecropia*. *The EMBO Journal* **2**, 571-576

Hunter DM, Bonn WG (1999) Selection for fire blight resistance in pear seedling populations using field and greenhouse screening with virulent strains of *Erwinia amylovora*. *Acta Horticulturae* **489**, 209-214

Itai A (2007) Pear. In: Kole C (Ed) *Genome Mapping and Molecular Breeding in Plants*. *Fruits and Nuts*, Springer-Verlag, Berlin Heidelberg, pp 157-170

Jabrane A, Sabri A, Compere P, Jacques P, Vandenberghe I, Van Beeumen J, Thonart P (2002) Characterization of Serracin P, a phage-tail-like bacteriocin, and its activity against *Erwinia amylovora*, the fire blight pathogen. *Applied and Environmental Microbiology* **68**, 5704-5710

Jakovljevic V, Jock S, Du Z, Geider K (2008) Hypersensitive response and acyl-homoserine lactone production of the fire blight antagonists *Erwinia tasmaniens*is and *E. billingiae*. *Microbial Biotechnology* **1**, 416-424

James DJ, Passey AJ, Barbara DJ, Bevan M (1989) Genetic transformation of apple (*Malus pumila* Mill) using a disarmed Ti-binary vector. *Plant Cell Reports* **7**, 658-661

Janick J, Cummins JN, Brown SK, Hemmat M (1996) Apples. In: Janick J, Moore JN (Eds) *Fruit Breeding, Tree and Tropical Fruits* (Vol I), John Wiley & Sons, New York, pp 1-77

Jock S and Geider K (2004) Molecular differentiation of *Erwinia amylovora* strains from North America and of two Asian pear pathogens by analyses of PFGE patterns and *hrpN* genes. *Environmental Microbiology* **6**, 480-490

Jock S, Kim W-K, Donat V, Lopez MM, Bazzi C, Geider K (2002) Following spread of fire blight in Western, Central and Southern Europe by molecular differentiation of *Erwinia amylovora* strains with PFGE analysis. *Environmental Microbiology* **4**, 106-114

Jock S, Langlotz C, Geider K (2005) Survival and possible spread of *Erwinia amylovora* and related plant-pathogenic bacteria exposed to environmental stress conditions. *Journal of Phytopathology* **153**, 87-93

Jock S, Rodoni B, Gillings M, Kim W-S, Copes C, Merriman P, Geider K (2000) Screening of ornamental plants from the Botanic Gardens of Melbourne and Adelaide for the occurrence of Erwinia amylovora. *Australasian Plant Pathology* **29**, 120-128

Johnson KB, Sawyer TL, Temple TN (2006) Rates of epiphytic growth of *Erwinia amylovora* on flowers common in the landscape. *Plant Disease* **90**, 1331-1336

Johnson KB, Stockwell VO (1998) Management of fire blight: a case study in microbial ecology. *Annual Review of Phytopathology* **36**, 227-248

Jorgensen HA (1969) Ildsot – en alvorlig trussel for frugtavl og planteskoledrift i Danmark. *Erhvervsfrugtavleren* **36**, 48-51

Jumel K, Geider K, Harding SE (1997) The solution molecular weight and shape of the bacterial exopolysaccharides amylovoran and stewartan. *International Journal of Biological Macromolecules* **20**, 251-258

Juniper BE, Watkins R, Harris SA (1999) The origin of apple. *Acta Horticulturae* **484**, 27-33

Karnatz A (1988) New apple cultivars after self-pollination. *Acta Horticulturae* **224**, 169-175

Kása K, Hevesi M, Tóth M (2004) Evaluation of traditional Hungarian apple cultivars as sources of resistance to fire blight. *Acta Horticulturae* **663**, 225-228

Keck M, Reich H, Chartier R, Paulin J-P (1996) First record of fire blight (*Erwinia amylovora*) in Austria: preliminary experiments on the survival on fruit boxes. *Acta Horticulturae* **411**, 9-11

Keil M, Sanchez-Serrano, Willmitzer L (1989) Both wound-inducible and tuber-specific expression are mediated by the promoter of a single member of the potato proteinase inhibitor II gene family. *The EMBO Journal* **8**, 1323-1330

Keionen-Mettälä K, Pappinen A, Von-Weissenberg K (1998) Comparisons of the efficiency of some promoters in silver birch (*Betula pendula*). *Plant Cell Reports* **17**, 356-361

Kellerhals M, Spuhler M, Duffy B, Patocchi A, Frey JE (2009) Selection efficiency in apple breeding. *Acta Horticulturae* **814**, 177-184

Kelm O, Kieker C, Geider K, Bernhard F (1997) Interaction of the regulator proteins RcsA and RcsB with the promoter region of the operon for amylovoran biosynthesis by *Erwinia amylovora*. *Molecular and General Genetics* **256**, 72-83

Khan MA, Duffy B, Gessler C, Patocchi A (2006) QTL mapping of fire blight resistance in apple. *Molecular Breeding* **17**, 299-306

Khan MA, Durel C-E, Duffy B, Drouet D, Kellerhals M, Gessler C, Pa-

tocchi A (2007) Development of molecular markers linked to the 'Fiesta' linkage group 7 major QTL for fire blight resistance and their application for marker-assisted selection. *Genome* **50**, 568-577

Khan MA, Durel C-E, Duffy B, Drouet D, Kellerhals M, Gessler C, Patocchi A (2009) Identification and validation of QTLs linked to fire blight resistance in *Malu*s and their applicability in marker-assisted selection. *Acta Horticulturae* **814**, 753-758

Khanna HK, Daggard GE (2006) Targeted expression of redesigned and codon optimised synthetic gene leads to recrystallisation inhibition and reduced electrolyte leakage in spring wheat at sub-zero temperatures. *Plant Cell Reports* **25**, 1336-1346

Kim W-K, Gardan L, Rhim S-L, Geider K (1999) *Erwinia pyrifoliae* sp. nov., a novel pathogen affecting Asian pear trees (*Pyrus pyrifolia* Nakai). *International Journal of Systematic Bacteriology* **49**, 899-906

Kim W-S, Geider K (2000) Characterization of a viral EPS-depolymerase as a potential tool for control of fire blight. *Phytopathology* **90**, 1263-1268

Kim W-S, Hildebrand M, Jock S, Geider K (2001) Molecular comparison of pathogenic bacteria from pear trees in Japan and the fire blight pathogen *Erwinia amylovora*. *Microbiology/SGM* **147**, 2951-2959

Kirsch C, Logemann E, Lippok B, Schmelzer E, Hahlbrock K (2001) A highly specific pathogen-responsive promoter element from the immediate-early activated *CMPG1* gene in *Petroselinum crispum*. *The Plant Journal* **26**, 217-227

Ko K, Brown SK, Norelli JL, Boresjza-Wysocka EE, Aldwinckle HS (1999) Effect of multiple transgenes on resistance to fire blight. *Acta Horticulturae* **489**, 257-258

Ko K, Norelli JL, Reynoird J-P, Aldwinckle HS, Brown SK (2002) T4 lysozyme and attacin E genes enhances resistance of transgenic 'Galaxy' apple against *Erwinia amylovora*. *Journal of the American Society of Horticultural Science* **127**, 515-519

Ko K, Norelli JL, Reynoird J-P, Boresjza-Wysocka E, Brown SK, Aldwinckle HS (2000) Effect of untranslated leader sequence of AMV RNA4 and signal peptide of pathogenesis-related protein 1b on attacin gene expression, and resistance to fire blight in transgenic apple. *Biotechnology Letters* **22**, 373-381

Korban SS, Ries SM, Klopmeyer MJ, Morrisey JF, Hattermann DR (1988) Genotypic responses of scab-resistant apple cultivars/selections to two strains of *Erwinia amylovora* and the inheritance of resistance to fire blight. *Annals of Applied Biology* **113**, 101-105

Korban SS, Skirvin RM (1984) Nomenclature of the cultivated apple. *HortScience* **19**, 177-180

Korban SS, Chen H (1992) Apple. In: Hammerschlag FA, Litz RE (Eds) *Biotechnology of Perennial Fruit Crops*. CAB International, Cambridge, pp 203-227

Krens FA, Pelgrom KTB, Schaart JG, den Nijs APM, Rouwendal GJA, (2004) Clean vector technology for marker-free transgenic fruit crops. *Acta Horticulturae* **663**, 431-435

Kube M, Migdoll A, Müller I, Kuhl H, Beck A, Reinhardt R, Geider K (2008) The genome of *Erwinia tasmaniensis* strain Et1/99, a novel non-phytopathogenic bacterium in the genus *Erwinia*. *Environmental Microbiology* **10**, 2211-2222

Kudela V (1988) *Erwinia amylovora*, causal agent of rosaceous plants in Czechoslovakia. *Ochrana Rostlin* **24**, 173-182

Lambert C, Tepfer D (1992) Use of *Agrobacterium rhizogenes* to create transgenic apple trees having an altered organogenic response to hormones. *Theoretical and Applied Genetics* **85**, 105-109

LaRosa PC, Chen Z, Nelson DE, Singh NK, Hasegawa PM, Bressan RA (1992) Osmotin gene expression is posttranscriptionally regulated. *Plant Physiology* **100**, 409-415

Laurens F (1999) Review of the current apple breeding programmes in the world: objectives for scion cultivar improvement. *Acta Horticulturae* **484**, 163-170

Layne REC, Bailey CH, Hough LF (1968) Efficacy of transmission of fire blight resistance in *Pyrus*. *Canadian Journal of Plant Science* **48**, 231-243

Le Lezec M, Lecomte P, Laurens F, Michelesi J-C (1997a) Sensibilité variétale au feu bactérien (1e partie). *L'Arboriculture Fruitière* **503**, 57-62

Le Lezec M, Lecomte P, Laurens F, Michelesi J-C (1997b) Sensibilité variétale au feu bactérien (2e partie). *L'Arboriculture Fruitière* **504**, 33-37

Le Lezec M, Paulin JP, Lecomte P (1987) Shoot and blossom susceptibility to fire blight of apple cultivars. *Acta Horticulturae* **217**, 311-315

Lebedev VG, Skryabin KG, Dolgov SV (2002) Transgenic pear clonal rootstocks resistant to herbicide Basta. *Acta Horticulturae* **596**, 193-197

Lecomte P (1993) Shoot and blossom susceptibility to fire blight (*Erwinia amylovora*) of Asian pear cultivars. *Acta Horticulturae* **338**, 397-406

Lecomte P, Cadic A (1993) Further results on shoot susceptibility of *Cotoneaster* to fire blight. *Acta Horticulturae* **338**, 407-412

Lelliott RA (1968) Fire blight in England – its nature and its attempted eradication. *European Plant Protection Organization Publication Series A* **45**, 10-14

Lemaire F, Couvreur C, Paulin J-P, Cadic A (1990) Influence of calcium and potassium nutrition of fire blight susceptibility. *Acta Horticulturae* **273**, 189-194

Lespinasse Y, Aldwinckle HS (2000) Breeding for resistance to fire blight. In: Vanneste JL (Ed) *Fire Blight: the Disease and Its Causativ Agent, Erwinia amylovora*, CABI Publishing, New York, pp 253-273

Lespinasse Y, Paulin J-P (1990) Apple breeding programme for fire blight resistance: strategy used and first results. *Acta Horticulturae* **273**, 285-291

Li J, Brader G, Palva ET (2004) The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defence. *The Plant Cell* **16**, 319-331

Liu Q, Ingersoll J, Salih S, Meng R, Hammerschlag F (2001) Response of transgenic Royal Gala apple (*Malus* x *domestica* Borkh.) shoots carrying a modified cecropin *MB39* gene, to *Erwinia amylovora*. *Plant Cell Reports* **20**, 306-312

LoGiudice N, Aldwinkle HS, Robinson TL, Fazio G (2006) The nature of resistance of the 'B.9' apple rootstock to fire blight. *Acta Horticulturae* **704**, 515-520

Lombard PB, Westwood MN (1987) Pear rootstocks. In: Rom RC, Carlson RF (Eds) *Rootstocks for Fruit Crops*, John Wiley & Sons, New York, USA, pp 145-183

Luby J, Forsline P, Aldwinckle H, Bus V, Geibel (2001) Silk Road apples – collection, evaluation, and utilization of *Malus sieversii* from Central Asia. *HortScience* **36**, 225-231

Luby JJ, Alspach PA, Bus VGM, Oraguzie NC (2002) Field resistance to fire blight in a diverse apple (*Malus* sp.) germplasm collection. *Journal of the American Society for Horticultural Science* **127**, 245-253

Maas Geesteranus HP, Heyting J (1981) The value of topleaf inoculation to demonstrate genetic resistance in *Pomoidae* species to *Erwinia amylovora* (Burr.) Winslow *et al*. *Acta Horticulturae* **117**, 75-82

Malnoy M, Baldo A, Carlisle C, Celton J-M, Norelli JL, Farrell Jr RE, Bassett CL, Gardiner S, Aldwinckle HS (2008a) Identification and mapping of genes differentially expressed during interaction of resistant and susceptible apple cultivars (*Malus* x *domestica*) with *Erwinia amylovora*. *Acta Horticulturae* **793**, 195-199

Malnoy M, Borejsza-Wysocka EE, Abbott P, Lewis S, Norelli JL, Flaishman M, Gidoni D, Aldwinckle HS (2007b) Genetic transformation of apple without use of a selectable marker. *Acta Horticulturae* **738**, 319-322

Malnoy M, Borejsza-Wysocka EE, Jin Q-L, He SY, Aldwinckle HS (2004) Over-expression of the apple gene *MpNPR1* causes increased disease resistance in *Malus* x *domestica*. *Acta Horticulturae* **663**, 463-467

Malnoy M, Borejsza-Wysocka EE, Jin Q-L, He SY, Aldwinckle HS (2006a) Transgenic apple lines over-expressing the apple gene *MpNPR1* have increased resistance to fire blight. *Acta Horticulturae* **704**, 521-526

Malnoy M, Borejsza-Wysocka EE, Oh CS, Pascal-Omeñaca L, Beer SV, Aldwinckle HS (2008b) Silencing HIPM, the apple protein that interacts with HrpN of *Erwinia amylovora*. *Acta Horticulturae* **793**, 261-264

Malnoy M, Faize M, Venisse JS, Geider K, Chevreau E (2005) Expression of viral EPS-depolymerase reduces fire blight susceptibility in transgenic pear. *Plant Cell Reports* **23**, 632-638

Malnoy M, Jin Q, Borejsza-Wysocka EE, He SY, Aldwinckle HS (2007a) Overexpression of the apple *MpNPR1* gene confers increased disease resistance in *Malus* x *domestica*. *Molecular Plant-Microbe Interactions* **20**, 1568-1580

Malnoy M, Melotto M, Borejsza-Wysocka EE, He SY, Aldwinckle HS (2008c) Expression of the type III secretion chaperone protein DspF of *Erwinia amylovora* in apple increases resistance to fire blight. *Acta Horticulturae* **793**, 237-240

Malnoy M, Reynoird JP, Borejsza-Wysocka EE, Aldwinckle HS (2006b) Activation of the pathogen-inducible *Gst1* promoter of potato after elicitation by *Venturia inaequalis* and *Erwinia amylovora* in transgenic apple (*Malus x domestica*). *Transgenic Research* **15**, 83-93

Malnoy M, Reynoird JP, Chevreau E (2000) Preliminary evaluation of new gene transfer strategies for resistance to fire blight in pear. *Acta Horticulturae* **538**, 635-637

Maroofi A, Mostafavi M (1996) Evaluation of the resistance of apple, pear and quince varieties to fire blight. *Acta Horticulturae* **411**, 395-399

Martini M, Egen M, Rüntz I, Strittmatter G (1993) Promoter sequences of a potato pathogenesis-related gene mediate transcriptional activation selectively upon fungal infection. *Molecular and General Genetics* **236**, 179-186

Matsumoto S, Komori S, Kitahara K, Imazu S, Soejima J (1999) S-genotypes of 15 apple cultivars and self-incompatibility of Megumi. *Journal of the Japanese Society of Horticultural Science* **68**, 236-241

McGhee GC, Jones AL (2000) Complete nucleotide sequence of ubiquitous plasmid pEA29 from *Erwinia amylovora* strain Ea88: gene organization and intraspecies variation. *Applied and Environmental Microbiology* **66**, 4897-4907

Milbraith DG (1930) Blight in pears in 1930. *Blue Anchor* **7 (11)**, 25-26

Mittler R, Rizhsky L (2000) Transgene-induced lesion mimic. *Plant Molecular Biology* **44**, 335-344

Mizuno A, Sato S, Kawai A, Nishiyama K (2000) Taxonomic position of the causal pathogen of bacterial shoot blight of pear. *Journal of General Plant Pathology* **66**, 48-58

Mohammadi M, Geider K (2007) Autoinducer-2 of the fire blight pathogen *Erwinia amylovora* and other plant-associated bacteria. *FEMS Microbiology Letters* **266**, 34-41

Mohammadi M, Moltmann E, Zeller W, Geider K (2009) Characterization of naturally occurring *Erwinia amylovora* strains lacking the common plasmid

pEA29 and their detection by real-time PCR. *European Journal of Plant Pathology* **124**, 293-302

Mohan SK, Fallahi E, Bijman VP (2002) Evaluation of apple varieties for susceptibility to *Erwinia amylovora* by artificial inoculation under field conditions. *Acta Horticulturae* **90**, 373-375

Momol MT, Forsline PL, Lamboy WF, Aldwinckle HS (1999a) Fire blight resistance and horticultural evaluation of wild *Malus* populations from Central Asia. *Acta Horticulturae* **489**, 229-233

Momol MT, Norelli JL, Piccioni DE, Momol EA Gustafson HL, Cummins JN, Aldwinckle HS (1998) Internal movement of *Erwinia amylovora* through symptomless apple scion tissues into the rootstock. *Plant Disease* **82**, 646-650

Momol MT, Ugine JD, Norelli JL, Aldwinckle HS (1999b) The effect of prohexadione calcium, SAR inducers and calcium on the control of shoot blight caused by *Erwinia amylovora* on apple. *Acta Horticulturae* **489**, 601-605

Montesinos E (2007) Antimicrobial peptides and plant disease control. *FEMS Microbiology Letters* **270**, 1-11

Morgan J, Richards A (1993) *The Book of Apples*, Ebury Press, London, UK, 288 pp

Mourgues F, Chevreau E, Lambert C, de Bondt A (1996) Efficient *Agrobacterium*-mediated transformation and recovery of transgenic plants from pear (*Pyrus communis* L.). *Plant Cell Reports* **16**, 245-249

Naik S, Hampson C, Gasic K, Bakkeren G, Korban SS (2006) Development and linkage mapping of E-STS and RGA markers for functional gene homologues in apple. *Genome* **49**, 959-968

Nelson DE, Raghothama KG, Singh NK, Hasegawa PM, Bressan RA (1992) Analysis of structure and transcriptional activation of an osmotin gene. *Plant Molecular Biology* **19**, 577-588

Netherlands Plant Protection Service (1966) A case of fire blight in The Netherlands. *European and Mediterranean Plant Protection Organization Report* **286**, Wageningen, 1 p

Norelli JL, Aldwinckle HS (1993) The role of aminoglycoside antibiotics in the regeneration and selection of neomycin phosphotransferase-transgenic apple tissue. *Journal of the American Society for Horticultural Science* **118**, 311-316

Norelli J, Aldwinckle HS, Beer SV (1984) Differential host x pathogen interactions among cultivars of apple and strains of *Erwinia amylovora*. *Phytopathology* **74**, 136-139

Norelli JL, Aldwinckle HS, Beer SV (1986) Differential susceptibility of *Malus* spp. cultivars Robusta 5, Novole, and Ottawa 523 to *Erwinia amylovora*. *Plant Disease* **70**, 1017-1019

Norelli JL, Aldwinckle HS, Beer SV, Lamb RC (1987) The effects of virulence of *Erwinia amylovora* on the evaluation of fire blight resistance in *Malus*. *Phytopathology* **77**, 1551-1555

Norelli JL, Aldwinckle HS, Destéfano-Beltrán L, Jaynes JM (1994a) Transgenic apple plants containing lytic proteins have increased resistance to *Erwinia amylovora*. *Journal of Cellular Biochemistry* **18A**, 89

Norelli JL, Aldwinckle HS, Destéfano-Beltrán L, Jaynes JM (1994b) Transgenic 'Malling 26' apple expressing the attacin E gene has increased resistance to *Erwinia amylovora*. *Euphytica* **77**, 123-128

Norelli JL, Aldwinkle HS, Holleran HT, Robinson TL (2002) Resistance of 'Geneva' apple rootstocks to *Erwinia amylovora* when grown as potted plants and orchard trees. *Acta Horticulturae* **590**, 359-362

Norelli JL, Borejsza-Wysocka E, Momol MT, Mills JZ, Grethel A, Aldwinckle HS (1999a) Genetic transformation for fire blight resistance in apple. *Acta Horticulturae* **489**, 295-296

Norelli JL, Borejsza-Wysocka E, Reynoird JP, Aldwinckle HS (2000) Transgenic 'Royal Gala"apple expressing attacin E has increased field resistance to *Erwinia amylovora* (fire blight). *Acta Horticulturae* **538**, 631-633

Norelli JL, Farrell RE, Bassett CL, Baldo AM, Lalli DA, Aldwinckle HS, Wisniewski ME (2009) Rapid transcriptional response of apple to fire blight disease revealed by cDNA suppression subtractive hybridization analysis. *Tree Genomes and Genetics* **5**, 27-40

Norelli JL, Holleran HT, Johnson WC, Robinson TL, Aldwinckle HS (2003b) Resistance of Geneva and other apple rootstocks to *Erwinia amylovora*. *Plant Disease* **87**, *26-32*

Norelli JL, Jones AL, Aldwinckle HS (2003a) Fire blight management in the twenty-first century – using new technologies that enhance host resistance in apple. *Plant Disease* **87**, 756-765

Norelli JL, Mills JA, Momol TM, Aldwinckle HS (1999b) Effect of cecropin-like transgenes on fire blight resistance of apple. *Acta Horticulturae* **489**, 273-278

Oh CS, Beer SV (2005) Molecular genetics of *Erwinia amylovora* involved in the development of fire blight. *FEMS Microbiology Letters* **253**, 185-192

Oh CS, Kim JF, Beer SV (2005) The Hrp pathogenicity island of *Erwinia amylovora* and the identification of three novel genes required for systemic infection. *Molecular Plant Pathology* **6**, 125-138

Oh C-S, Martin G, Beer SV (2007) DspA/E, a type III effector of *Erwinia amylovora*, is required for early rapid growth in *Nicotiana benthamiana* and causes NbSGT1-dependent cell death. *Molecular Plant Pathology* **8**, 255-265

Oh S-C, Beer SV (2007) AtHIPM, an ortholog of the apple HrpN-interacting protein, is a negative regulator of plant growth and mediates the growth-enhancing effect of HrpN in arabidopsis. *Plant Physiology* **145**, 426-436

O'Rourke D, Janick J, Sansavini S (2003) World apple cultivar dynamics. *Chronica Horticulturae* **43**, 10-13

Paprštein F, Kosina J, Korba J, Šillerová (2006) Evaluation of resistance to fire blight in Czech pear cultivars. *Acta Horticulturae* **704**, 577-582

Paulin J-P, Keck M, Chartier R, Zislavsky (1990) Trials on the estimation of the fore blight susceptibility by inoculations of immature pome fruits. *Pflanzenschutzberichte* **51 (2)**, 91-98

Paulin J-P, Lespinasse Y (1987) Evaluation with different isolates of *Erwinia amylovora* of the susceptibility to fire blight of apple cultivars. *Acta Horticulturae* **217**, 253-261

Paulin J-P, Lespinasse Y (1990) Pathogenicity of strains of *Erwinia amylovora* to some apple cultivars in the greenhouse. *Acta Horticulturae* **273**, 319-326

Peil A, Flachowsky H, Hanke M-V (2008b) High speed apple breeding – from vision to reality. *Abstract Book 4th International Rosaceae Genomics Conference*, 16-19 March 2008, Pucón, Chile, p 32

Peil A, Garcia-Libreros T, Richter K, Trognitz FC, Trognitz B, Hanke M-V, Flachowsky H (2007a) Strong evidence for a fire blight resistance gene of Malus robusta located on linkage group 3. *Plant Breeding* **126**, 470-475

Peil A, Hanke M-V, Flachowsky H, Richter K, Garcia T, Trognitz B (2007b) Developing molecular markers for marker assisted selection of fire blight resistant apple seedlings. *Acta Horticulturae* **763**, 117-121

Peil A, Richter K, Garcia-Libreros T, Hanke M-V, Flachowsky H, Celton J-M, Horner M, Gardiner S, Bus V (2008a) Confirmation of the fire blight QTL of *Malus* x *robusta* 5 on linkage group 3. *Acta Horticulturae* **793**, 297-303

Peil A, Richter K, Höfer M, Hanke M-V (2004) Beschreibung des Feuerbrandbefalls am Institut für Obstzüchtung in Dresden-Pillnitz im Jahr 2003. *Erwerbs-Obstbau* **46**, 141-148

Peña-Cortés H, Sanchez-Serrano M, Rocha-Sosa M, Willmitzer L (1988) Systemic induction of proteinase inhibitor-II gene expression in potato plants by wounding. *Planta* **174**, 84-89

Pierce NB (1902) Pear blight in California. *Science* **16**, 193-194

Pinet-Lebley C, Turpin EX, Chevreaux E (1992) Effect of gamma and ultraviolet irradiation on adventitious regeneration from *in vitro* cultured pear leaves. *Euphytica* **62**, 225-233

Pontais I, Paulin J-P, Brisset MN (2006) Involvement of the phenylpropanoid pathway in the resistance of apple to *Erwinia amylovora*. *Acta Horticulturae* **704**, 479-480

Postman JD (2008) The USDA quince and pear genebank in Oregon, a world source of fire blight resistance. *Acta Horticulturae* **793**, 357-362

Pulawska J, Kielak K, Sobiczewski P (2006) Phenotypic and genetic diversity of selected *Erwinia amylovora* strains from Poland. *Acta Horticulturae* **704**, 439-444

Pusey PL (2000) The role of water in epiphytic colonization and infection of pomaceous flowers by *Erwinia amylovaora*. *Phytopathology* **90**, 1352-1357

Pusey PL, Curry EA (2004) Temperature and pomaceous flower age related to colonization by *Erwinia amylovaora* and antagonists. *Phytopathology* **94**, 901-911

Pusey PL, Rudell DR, Curry EA, Mattheis JP (2008) Characterization of stigma exudates in aqueous extracts from apple and pear flowers. *HortScience* **43**, 1471-1478

Quamme HA (1981) Heritability and effectiveness of selection for fire blight resistance in young pear seedlings inoculated in the greenhouse. *Proceedings of the Eucarpia Fruit Section Symposium – Tree Fruit Breeding*, 3-7 September 1979, INRA, Angers, F, pp 73-81

Quamme HA, Bonn WG (1981) Virulence of *Erwinia amylovora* and its influence on the determination of fire blight resistance of pear cultivars and seedlings. *Canadian Journal of Plant Pathology* **3**, 187-282

Quamme HA, Kappel F, Hall JW (1990) Efficacy of early selection for fire blight resistance and the analysis of combining ability for fire blight resistance in several pear progenies. *Canadian Journal of Plant Science* **70**, 905-913

Quamme HA, Spearman GA (1983) 'Harvest Queen' and 'Harrow Delight' pear. *HortScience* **18**, 770-772

Quamme HA, van der Zwet T, Dirks V (1976) Relationship of fire blight resistance of young pear seedlings inoculated in the greenhouse to mature seedling trees naturally infected in the field. *Plant Disease Reporter* **60**, 660-664

Rademacher W (2000) Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 501-531

Rademacher W (2004) Prohexadione-Ca induces resistance to fire blight and other diseases. *EPPO Bulletin* **34**, 383-388

Rademacher W, Stammler G, Creemers G (1999) Prohexadione-Ca: effects against scab in apples. *Phytopathology* **89**, 63-64

Reid WD (1930) The diagnosis of fire blight in New Zealand. *New Zealand Journal of Science and Technology* **12**, 166-172

Reimer FC (1950) Development of blight resistant French pear rootstocks. *Station Bulletin 485*, Agricultural Experiment Station, Oregon State College, Corvallis, USA, 24 pp

Reynoird JP, Mourgues F, Norelli J, Aldwinckle HS, Brisset MN, Chevreau E (1999) First evidence for improved resistance to fire blight in transgenic pear expressing the attacin E gene from *Hyalophora cecropia*. *Plant Science* **149**, 23-31

Rhim S-L, Völksch B, Gardan L, Paulin J-P, Langlotz C, Kim W-S, Geider K (1999) *Erwinia pyrifoliae*, an *Erwinia* species different from *Erwinia amylovora*, causes a necrotic disease of Asian pear trees. *Plant Pathology* **48**, 514-520

Richter K (1989) Ermittlung der Resistenz von Ziergehölzen gegen *Erwinia amylovora* (Burrill) Winslow *et al*. *Archiv für Phytopathologie und Pflanzenschutz* **25**, 339-346

Richter K, Fischer C (2002) Stability of fire blight resistance in apple. *Acta Horticulturae* **590**, 381-384

Robinson JP, Harris SA, Juniper BE (2001) Taxonmy of the genus *Malus* Mill. (*Rosaceae*) with emphasis on the cultivated apple, *Malus domestica* Borkh. *Plant Systematics and Evolution* **226**, 35-38

Robinson T, Aldwinckle HS, Fazio G, Holleran T (2003) The Geneva series of apple rootstocks from Cornell: performance, disease resistance, and commercialization. *Acta Horticulturae* **622**, 513-520

Robinson TL, Hoying SA, Cummins JN, Johnson WC, Norelli JL, Aldwinckle HS (1999) Orchard performance of fire blight resistant Geneva apple rootstocks. *Acta Horticulturae* **489**, 287-291

Roemmelt S, Zimmermann N, Rademacher W, Treutter D (2003) Formation of novel flavonoids in apple (*Malus* x *domestica*) treated with the 2-oxoglutarate-dependent dioxygenase inhibitor prohexadione-Ca. *Phytochemistry* **64**, 709-716

Rushton PJ, Reinstädler A, Lipka V, Lippok B, Somssich IE (2002) Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signalling. *The Plant Cell* **14**, 749-762

Russo NL, Robinson T, Fazio G, Aldwinckle HS (2006) Rootstock blight in apple. *New York Fruit Quarterly* **14**, 9-12

Sanchez-Serrano JJ, Keil M, O'Connor A, Schell J, Willmitzer L (1987) Wound-induced expression of a potato proteinase inhibitor II gene in transgenic tobacco plants. *The EMBO Journal* **6**, 303-306

Sato Y, Kurihara A, Abe K, Ogata T, Kajiura I, Kotobuki K, Machida Y (1988) Studies on the mode of inheritance of self-compatibility in Japanese pear. *Journal of the Japanese Society of Horticultural Science* **5**, 76-77

Saygılı H, Türküsay H, Ünal A, Hepaksoy S, Can HZ (1999) Investigation on determining some pear varieties resistant to fire blight (*Erwinia amylovora* (Burr.) Winslow *et al*.). *Acta Horticulturae* **489**, 225-228

Schaart JG, Puite KJ, Kolova L, Pogrebnyak N (1995) Some methodological aspects of apple transformation by *Agrobacterium*. *Euphytica* **85**, 131-134

Schlangen K, Halbwirth H, Fischer T, Flachowsky H, Treutter D, Hanke V, Stich K (2007) Breeding for fire blight resistance in apple trees. *Journal of Biotechnology* **131**, 34-35

Schouten H, Krens FA, Jacobsen E (2006a) Do cisgenic plants warrant less stringent oversight? *Nature Biotechnology* **24**, 753

Schouten H, Krens FA, Jacobsen E (2006b) Cisgenic plants are similar to traditionally bred plants. *EMBO Reports* **7**, 750-753

Shrestha R, Koo JH, Park DH, Hwang I, Hur JH, Lim CK (2003) *Erwinia pyrifoliae*, a causal endemic pathogen of shoot blight of Asian pear tree in Korea. *Plant Pathology Journal* **19**, 294-300

Shulaev V, Korban SS, Sosinski B, Abbott AG, Aldwinckle HS, Folta KM, Iezzoni A, Main D, Arús P, Dandekar AM, Lewers K, Brown SK, Davis TM, Gardiner SE, Potter D, Veilleux RE (2008) Multiple models for Rosaceae genomics. *Plant Physiology* **147**, 985-1003

Shwartz H, Shtienberg D, Vintal H, Kritzman G (2003) The interacting effects of temperature, duration of wetness and inoculum size on the infection of pear blossoms by *Erwinia amylovora*, the causal agent of fire blight. *Phytoparasitica* **31**, 174-187

Silfverberg-Dilworth E, Matasci CL, van de Weg WE, van Kaauwen MPW, Walser M, Kodde LP, Soglio V, Gianfranceschi L, Durel C-E, Costa F, Yamamoto T, Koller B, Gessler C, Patocchi A (2006) Microsatellite markers spanning the apple (Malus x domestica Borkh.) genome. *Tree Genetics and Genomes* **2**, 202-224

Sobiczewski P, Żurawicz E, Bereczyński, Lewandowski M (2006) Fire blight susceptibility of new apple cultivars and clones from Poland. *Acta Horticulturae* **704**, 551-555

Spinelli F, Ciampolini F, Cresti M, Geider K, Costa G (2005a) Influence of stigmatic morphology on flower colonization by *Erwinia amylovora* and *Pantoea agglomerans*. *European Journal of Plant Pathology* **113**, 395-405

Spinelli F, Speakman JB, Rademacher W, Halbwirth H, Stich K, Costa G (2005b) Luteoforol, a flavan 4-ol, is induced in pome fruits by prohexadione-calcium and shows phytoalexin-like properties against *Erwinia amylovora* and other plant pathogens. *European Journal of Plant Pathology* **112**, 133-142

Spotts RA, Mielke EA (1999) Resistance of pear cultivars in Oregon to natural fire blight infection. *Fruit Varieties Journal* **53**, 110-115

Steinberger EM, Beer SV (1988) Creation and complementation of pathogenicity mutants of *Erwinia amylovora*. *Molecular Plant-Microbe Interactions* **1**, 135-144

Strittmatter G, Gheysen G, Gianinazzi-Pearson V, Hahn K, Niebel A, Rohde W, Tacke E (1996) Infections with various types of organisms stimulate transcription from a short promoter fragment of the potato *gst1* gene. *Molecular Plant-Microbe Interactions* **9**, 68-73

Suleman P, Steiner PW (1994) Relationship between sorbitol and solute potential in apple shoots relative to fire blight symptom development after infection by *Erwinia amylovora*. *Phytopathology* **84**, 1244-1250

Szalatnay D, Eder-Bauermeister, Duffy B, Kellerhals M (2009) Characterization of fruit genetic resources in Switzerland. *Acta Horticulturae* **814**, 143-148

Szankowski I, Briviba K, Fleschhut J, Schönherr J, Jacobsen HJ, Kiesecker H (2003) Transformation of apple (*Malus domestica* Borkh.) with the stilbene synthase gene from grapevine (*Vitis vinifera* L.) and a PGIP gene from kiwi (*Actinidia deliciosa*). *Plant Cell Reports* **22**, 141-149

Taylor RK, Ranatunga C, Bus V, Alspach PA (2002) Host-pathogen interactions of *Erwinia amylovora* on apple and pear in New Zealand. *Acta Horticulturae* **590**, 363-367

Thibault B, Belouin A, Lecomte P (1989) Sensibilité variétale du poirier au feu bactérien. *L'Arboriculture Fruitière* **421**, 29-34

Thibault B, Lecomte P, Hermann L, Belouin A (1987a) Comparison between two methods of selection for resistance to *Erwinia amylovora* in young seedlings of pear. *Acta Horticulturae* **217**, 265-272

Thibault B, Lecomte P, Hermann L, Belouin A (1987b) Assessment of the susceptibility to *Erwinia amylovora* of 90 varieties or selections of pear. *Acta Horticulturae* **217**, 305-307

Thomas TM, Jones AL (1992) Severity of fire blight on apple cultivars and strains in Michigan. *Plant Disease* **76**, 1049-1052

Thompson JM, Zimmerman RH, van der Zwet T (1975) Inheritance of fire blight resistance in *Pyrus*. I. A dominant gene, *Se*, causing sensitivity. *Journal of Heredity* **66**, 259-264

Thompson SS, Janick J, Williams EB (1962) Evaluation of resistance to fire blight of pear. *Journal of the American Society of Horticultural Science* **80**, 105-113

Thomson SV, Gouk SC (2003) Influence and age of apple flowers on growth of *Erwinia amylovora* and biological control agents. *Plant Disease* **87**, 502-509

Thornburg RT, An G, Cleveland TE, Johnson R, Ryan CA (1987) Wound-inducible expression of a potato inhibitor II-chloramphenicol acetyltransferase gene fusion in transgenic tobacco plants. *Proceedings of the National Academy of Sciences USA* **84**, 744-748

Tóth M, Kása K, Göndör M, Honty K, Hevesi M (2006) First results of fire blight resistance screening in a Hungarian apple breeding programme. *Acta Horticulturae* **704**, 545-547

Tsiantos J, Psallidas P (2004) Fire blight resistance in various loquat, apple and pear cultivars and selections in Greece. *Journal of Plant Pathology* **86**, 227-232

Uyeda E (1903) On the causal bacteria of the fire blight of apple. *Dai-Nippon Nokai-ho* **260**, 1-3 (In Japanese)

van der Scheer HATh (1984) The experimental garden for research on fire blight at Ouwerkerk, The Netherlands. *Acta Horticulturae* **151**, 291-292

van der Zwet (1970) New outbreaks and current distribution of fire blight of pear and apple in Northern Europe. *FAO Plant Protection Bulletin* **18**, 83-88

van der Zwet T, Beer SV (1991) Fire blight - its nature, prevention, and control: a practical guide to integrated disease management. *Agriculture Information Bulletin* **631**, Washington DC, 83 pp

van der Zwet T, Bell RL (1990) Fire blight susceptibility in *Pyrus* germplasm from Eastern Europe. *HortScience* **25**, 566-568

van der Zwet T, Bell RL (1995) Response of Central European *Pyrus* germplasm to natural fire blight infection and artificial inoculation. *HortScience* **30**, 1287-1291

van der Zwet T, Bell RL, Blake RC (1981) Some factors affecting selection for fire blight resistance in pear. *Acta Horticulturae* **117**, 55-61

van der Zwet T, Keil HL (1979) Fire blight, a bacterial disease of rosaceous plants. *Agriculture Handbook* **510**, USDA, Washington DC, USA, 200 pp

van der Zwet T, Oitto WA, Brooks HJ (1970) Scoring system for rating the severity of fire blight in pear. *Plant Disease Reporter* **54**, 835-839

van der Zwet T, Oitto WA, Westwood MN (1974) Variability in degree of fire blight resistance within and between *Pyrus* species, interspecific hybrids, and seedling progeny. *Euphytica* **23**, 295-304

Vanneste JL (Ed) (2000) *Fire Blight, the Disease and its Causative Agent, Erwinia amylovora*, CABI Publishing, New York, 370 pp

Vavilov NI (1951) The origin, variation, immunity and breeding of cultivated plants; selected writings. *Chronica Botanica* **13**, 1-366

Venisse J-S, Gullner G, Brisset M-N (2001) Evidence for the involvement of an oxidative stress in the initiation of infection of pear by *Erwinia amylovora*. *Plant Physiology* **125**, 2164-2172

Venisse JS, Malnoy M, Faize M, Paulin J-P, Brisset MN (2002) Modulation of defense responses of Malus spp. during compatible and incompatible interactions with *Erwinia amylovora*. *Molecular Plant-Microbe Interactions* **15**, 1204-1212

Venter M (2007) Synthetic promoters: genetic control through *cis* engineering. *Trends in Plant Science* **12**, 118-1124

Viseur J (1990) Evaluation of fire blight resistance of somaclonal variants obtained from the pear cultivar 'Durondeau'. *Acta Horticulturae* **273**, 275-284

Viseur J, Tapia y Figueroa M (1987) *In vitro* co-culture as a tool for the evaluation of fire blight resistance in pears and apples. *Acta Horticulturae* **217**, 273-282

Volz RK, Rikkerink E, Austin P, Lawrence T, Bus VGM (2009) "Fast-

breeding" in apple: a strategy to accelerate introgression of new traits into elite germplasm. *Acta Horticulturae* **814**, 163-168

Waleron M, Waleron K, Geider K, Lojkowska E (2008) Application of RFLP analysis of *recA*, *gyrA* and *rpoS* gene fragments for rapid differentiation of *Erwinia amylovora* from *Erwinia* strains isolated in Korea and Japan. *European Journal of Plant Pathology* **121**, 161-172

Webster AD (2003) Breeding and selection of apple and pear rootstocks. *Acta Horticulturae* **662**, 499-512

Welander M, Maheswaran G (1992) Shoot regeneration from leaf explants of dwarfing apple rootstocks. *Journal of Plant Physiology* **140**, 223-228

Wilson J (1907) Investigations by the pathologists. *USDA Yearbook* 1906. 1907, 41 pp

Wilson M, Epton HAS, Sigee DC (1990) *Erwinia amylovora* infection of hawthorn blossom. *Acta Horticulturae* **273**, 207-210

Woods AF (1909) The wastes of the farm: pome fruits. In: *United States Department of Agriculture Yearbook (1908)*, Washington DC, pp 209

Yamamoto T, Terakami S, Kimura T, Sawamura Y, Takada N, Hirabayashi T, Imai T, Nishitani C (2009) Reference genetic linkage maps of European and Japanese pears. *Acta Horticulturae* **814**, 599-602

Yao JL, Cohen D, Atkinson R, Richardson K, Morris B (1995) Regeneration of transgenic plants from the commercial apple cultivar Royal Gala. *Plant Cell Reports* **14**, 407-412

Yoder KS, Miller SS, Byers RE (1999) Suppression of fire blight in apple shoots by prohexadione-calcium following experimental and natural inoculation. *HortScience* **34**, 1202-1204

Zeller W (1974) Der Feuerbrand des Kernobstes. *Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft* **158**, 121 pp

Zeller W, Zeller V (1998) Resistance of perry pears in Germany against fire blight (*Erwinia amylovora*). *Beiträge zur Züchtungsforschung* **5**, 35-36

Zhang Y, Geider K (1997) Differentiation of *Erwinia amylovora* by pulsed-field gel electrophoresis. *Applied and Environmental Microbiology* **63**, 4421-4426

Zhu B, Chen THH, Li PH (1995) Activation of two osmotin-like genes by abiotic stimuli and fungal pathogen in transgenic potato plants. *Plant Physiology* **108**, 929-937

Zhu LH, Li XY, Ahlman A, Xue ZT, Welander M (2004) The use of mannose as a selection agent in transformation of the apple rootstock M26 via *Agrobacterium tumefaciens*. *Acta Horticulturae* **663**, 503-506