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REVIEW

European pome fruit genetic resources evaluated for disease resistance

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Abstract Pome fruit genetic resources collections constitute a highly valuable resource not only for fruit breeding but also for direct use by nurseries, growers, and home gardeners. In order to use these resources efficiently and sustainably, reliable evaluation data on fruit and tree characteristics must be generated. Here we focus on pome fruit genetic resources evaluated phenotypically and genotypically for susceptibility to apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*), pear rust (*Gymnosporangium sabinae*) and storage diseases (e.g., *Penicillium expansum*). Examples are presented of several ongoing projects throughout Europe, with the aim to evaluate fruit genetic resources for disease susceptibility and potential use in breeding and for commercial use. The COST action 864 has fostered international cooperation in the evaluation of pome fruit genetic resources, and some of these evaluations therefore involve research groups from several of the participating countries.

Keywords Apple · Biodiversity · Disease resistance · Fire blight · Fruit genetic resources · Pear

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Introduction

Biodiversity has become an important global issue since the Convention on Biological Diversity (CBD) was adopted at the United Nations Conference on Environment and Development held in Rio de Janeiro in 1992 (http://www.biodiv.org). This convention has fostered international activities for the conservation and sustainable use of plant genetic resources for food and agriculture. Based on the CBD, a global plan of action for the conservation and sustainable use of plant genetic resources for food and agriculture was established in Leipzig in 1996 (http://www.fao.org). This global plan prompted the establishment of national action plans in several countries.

In many European fruit-growing areas, fungal diseases like scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and the bacterial disease fire blight (*Erwinia amylovora*) cause major problems in apple growing. In pear, scab (*Venturia pirina*) and fire blight are important diseases. In addition, pear rust (*Gymnosporangium sabinae*) is becoming increasingly important. Moreover, storage diseases and some other diseases and pests are also of relevance. Host resistance is a promising approach to achieve durable pome fruit production with low pesticide input and low risk of residues on the product.

In Europe, a rich heritage of pome fruit genetic resources is still present, either in traditional orchards or in genebank collections. These collections are, however, not much used for practical fruit-cultivation or for breeding compared to standard commercial cultivars. A thorough evaluation of disease resistance could lead to a wider use of the potentially very valuable fruit genetic resources. We will present an overview on recent activities in Europe, partly achieved under the umbrella of the COST 864 action "Pome Fruit Health" (2006–2011), and some results from specific trials aiming to evaluate the diversity of pome fruit genetic resources in Europe.

Evaluation of pome fruit genetic resources for disease resistance in Belgium, Germany, Sweden and Switzerland

In Belgium, a program on conservation, evaluation and promotion of the use of indigenous fruit tree genetic resources has been conducted since 1975 (Populer 1980). Until now, more than 1,600 accessions of apple and 1,200 of pear have been collected. Three quarters of these accessions are progressively submitted—since 1983, in unsprayed orchards—to a systematic evaluation and characterization process for disease resistance and other important agronomic features. The aim of this project is to (1) safeguard old cultivars (cvs) formerly bred or cultivated in Belgium as sources of diversity traits and as a cultural heritage (Populer et al. 1998), (2) organize a network of repository orchards for the unique and original material (Lateur 2003), (3) characterizing and evaluating the collected material for pest and disease resistance and agronomic traits (Lateur and Populer 1994, 1996), and (4) develop different approaches to identify the best performing genotypes either as cvs to be directly released to professional nurseries for amateur (Populer et al. 1998) or professional growers for dessert (Lateur et al. 2000) or processing purposes (Planchon and Lateur 1999), or as new parents to be used in breeding programs (Lateur et al. 1999, 2009).

In Germany, the conservation of fruit varieties reaches back to the early decades of the 20th century, and cvs of different fruit crop species are now preserved in public and private germplasm collections. The German Fruit Genebank was recently established as a decentralized network to coordinate the activities of the different germplasm collections (Flachowsky and Höfer 2010). The apple network was founded in 2009 and comprises 950 apple cvs held by six stakeholders. The main partner, the Institute for Breeding Research on Horticultural and Fruit Crops in Dresden-Pillnitz, maintains an apple genebank with 822 cvs, mostly old German cvs or cvs with a socio-cultural, local and historical relation to Germany. This genebank also contains 527 accessions of 26 primary *Malus* species and 20 hybrid species, the largest collection in Europe. Based on the "National Program for Genetic Resources of Agricultural and Horticultural Plants", the aim is to develop an effective conservation strategy of genetic resources for *Malus* in Germany, and to evaluate the fruit genetic resources in order to use these in fruit production, breeding and research. Current projects include comprehensive evaluation of resistance towards scab, mildew, and fire blight. In addition, characterization of fruit traits and the analysis of biochemical components as well as genetic characterization using SSR-markers are in progress (Höfer and Peil 2008).

In Sweden, the preservation of clonally propagated plants is presently managed by a governmentally appointed unit, the "National Program for Diversity of Cultivated Plants". In apple about 220 "mandate" cvs, defined as originating in Sweden or having a long history of being grown in the country, have been appointed, and in pear about 60. Material from most of these mandate cvs is now being conserved either in 11 clone archives, each with 10–50 mandate cvs, located at outdoor museums or other public places, and/or at Balsgård, now a part of the Swedish University of Agricultural Sciences. At Balsgård, many other apple and pear cvs are also being preserved, amounting to about 1,000 in total. Various pomological screenings relating to yield, fruit quality and disease resistance have been carried out in this collection since its establishment in the early 1950s (Nybom et al. 2008), together with DNA-based identification and diversity estimations (Garkava-Gustavsson et al. 2008, 2011).

In Switzerland, fruit genetic resources are conserved in decentralized collections mostly managed by NGO's but financially supported by the Federal Government. They are kept in collections of three levels. Neither accessions that are not yet characterized nor their identity assured are integrated in introduction orchards. The main conservation is assured by primary collections and duplication collections. The whole network is coordinated by the Fruit Coordinator of the Swiss Commission for the conservation of plant genetic resources. Following an inventory to detect the current Swiss fruit genetic resources diversity (Kellerhals and Egger 2004), a project for the characterization of fruit genetic resources preserved in the collections was initiated in order to allow for their use in fruit-growing and in breeding. Both the inventory and the characterization projects were run by the NGO Fructus (http://www.fructus.ch) in collaboration with other stakeholders. Further evaluation of selected accessions includes the testing of 600 apple accessions for scab and mildew susceptibility in the field, and the testing of about 200 different apple and pear accessions for fire blight susceptibility in a quarantine glasshouse.

Focus on fire blight

Fire blight is one of the most serious diseases in pome fruit cultivation, where it causes great yearly losses in North America and to an increasing extent also in Europe. Consequently, various aspects on fire blight research have been one of the main topics of the COST 864 action. Fire blight is also a threat to genetic resources collections (Peil et al. 2004). Among the *Malus* genetic resources, a broad range of resistance levels towards fire blight can be found (Aldwinckle et al. 1976; Szalatnay et al. 2010; Szobiczewski et al. 2011). Since fire blight first appeared in Switzerland in 1989, it has steadily spread from the original epicenter in the north-eastern cantons to the south-western regions (Duffy et al. 2005). In 2007, the worst outbreak so far took place, and several of the decentralized gene bank collections suffered from losses. Fire blight has not yet caused much damage in fruit orchards and gene banks in the countries of northern Europe. Small outbreaks are, however, noted in some years and are likely to increase both in size and frequency as the climate becomes warmer and moister.

Since quantification of fire blight damage in the field, or in greenhouses after artificial inoculation with bacterial suspensions of *Erwinia amylovora*, is very time-consuming and not always possible to perform, DNA markers have been developed and applied that help to discriminate between tolerant and susceptible cvs. Two markers, flanking a previously identified QTL (quantitative trait locus) for enhanced resistance to fire blight on linkage group 7 in apple (Calenge et al. 2005; Khan et al. 2006, 2007), have been screened on a total of 205 apple cvs in Sweden, many of which have an origin in the Nordic countries (Sehic et al. 2009; Nybom et al. (2011, this volume). This QTL can be tracked back down to “Cox’s Orange Pippin” (Khan et al. 2007). “Cox” descendants and other genotypes carrying the QTL are therefore more likely to show high tolerance compared to descendants not carrying the QTL. Both markers were present in 22% of the cvs, and both were lacking in 25% (Sehic et al. 2009; Nybom et al. 2011, this volume). However, 33% had only the marker AE10-375, while 20% had only GE-8019, suggesting that some cvs with both markers probably carry these on separate chromosomes and therefore may lack the QTL.

Materials and methods

Evaluation of pome fruit fire blight susceptibility in Switzerland and Germany

A screening of heritage cvs for relative fire blight tolerance was conducted in the quarantine glasshouse at the Research Station Agroscope Changins-Wädenswil (ACW) in Wädenswil, Switzerland. Scion material was grafted onto M9 rootstocks. In the spring, trees were planted in plastic deep-pots 60 from Stuewe and Sons (Corvallis, USA) with a depth of 35.5 cm and a diameter of 7 cm and then grown in the glasshouse for several weeks prior to inoculation. For each cultivar, 6–10 replicate trees were inoculated by puncturing the distal tip of 15–30 cm long shoots with a syringe containing an *E. amylovora* solution of 10^9 cfu/ml strain FAW610. Disease progress was evaluated weekly for 3 weeks by measuring the expansion of the necrotic lesion from the shoot tip in relation to the total shoot length. Artificial fire blight infections were performed in 2007, 2008, 2010, and 2011 with 40, 40, 80, and 20 different apple and pear accessions, respectively. Heritage cvs still grown for apple juice and cider production and commercial standard cvs were included.

In Germany, fire blight inoculations were performed in the greenhouse of the Institute for Resistance Research and Stress Tolerance Quedlinburg using a method modified to the assay of Kleinhempel et al. (1984). Currently, 82 cvs from the genebank of the Institute for Breeding Research on Horticultural and Fruit Crops in Dresden-Pillnitz have been tested. The inoculation was repeated in cvs that had a mean necrosis below 50% in the first experimental year. Five to twenty plants for each cultivar, grafted on M9, were inoculated by bisecting the tips of the two upper leaves of growing shoots (minimum length of 25 cm) with scissors dipped in a suspension of three highly virulent *E. amylovora* strains (10^9 cfu/ml). Length of necrosis in relation to total shoot length was measured after incubation for 4 weeks at 25–27°C (day), 20°C (night) and 85% air humidity in the greenhouse.

Analysis of molecular markers related to enhanced fire blight tolerance in Switzerland and Sweden

The apple accessions tested for fire blight at Wädenswil since 2008 were analyzed for the presence or absence of two molecular markers flanking a fire blight resistance QTL which was described by Calenge et al. (2005) and Khan et al. (2007). The two SCAR markers AE 10-375 and GE-8019 were used to identify accessions that carry both of these markers and conclusively also the FBF7 fire blight resistance QTL as described by Baumgartner et al. (2010). Similar analyses have been performed at Balsgård in Sweden. Due to the Swedish plant quarantine regulations, it has not been possible to verify the marker studies by inoculation tests with *E. amylovora* in Sweden. A joint study was therefore undertaken in 2008 together with the Research Station Agroscope Changins-Wädenswil, Switzerland (Sehic et al. 2009). Budsticks of apple cultivars with and without the two DNA markers flanking the FBF7

QTL were collected at Balsgård, Sweden, and sent to Switzerland for bench-grafting on M9 rootstocks. Terminal shoots of the resulting plants, belonging to 17 different cultivars, were inoculated with a highly virulent strain of *E. amylovora* using scissors. Observation and measurements of the progress and severity of fire blight symptoms were performed 10 and 20 days after inoculation. The susceptibility of genotypes was evaluated as percent length of the necrotic regions in the inoculated shoot. Another joint study was undertaken between Sweden and the Institute of Horticulture in Skierniewice, Poland, during 2009–2010 (Nybom et al. 2011, this volume).

Evaluation of scab and powdery mildew susceptibility in Switzerland and Germany

An experimental orchard comprising 600 apple accessions on M9 rootstock was planted in the spring of 2008 at Horgen near Wädenswil, Switzerland**.** Planting distance was 3.5 m × 0.7 m. Each accession was represented by two randomly replicated trees. As controls and to ensure uniform spreading of the disease, a total of 36 trees of “Golden Delicious” and “Gravensteiner” were regularly distributed across the plot. This plot was not sprayed with fungicides. Scoring was performed each year in June according to a scale adapted from Lateur and Blazek (2004) (Table 1).

At the Institute for Breeding Research on Horticultural and Fruit Crops, Dresden-Pillnitz, scab and mildew assessments were conducted twice on leaves during the vegetation period (June and August) and once on the fruit (scab only) in September–October. A quantitative nine-step scoring scale was used for leaves: 1 (no scab and mildew visible) and 9 (majority of leaves with very heavy infection). Fruit scab was scored from 1 to 9, with 1 (no scab at all) and 9 (totally covered and crumbled). Altogether 778 cultivars were scored in the years 1997, 1999 and 2006; in these years no fungicides were applied.

Field evaluation of pear rust susceptibility in Belgium

In Belgium, a systematic evaluation process of pear rust susceptibility on leaves started in 2004 in the evaluation orchards using a 1–9 incidence assessment scale as described above for scab (Lateur and Blazek 2004). The results presented here originate from an unsprayed evaluation orchard planted in 1983 with 246 old Belgian pear accessions grafted on quince A with “Beurré Hardy” used as interstock, and with a planting distance of 2 m × 4.5 m. The pear rust disease evaluations were performed during the late summer time period.

Evaluation of storage diseases in apple in Sweden

Infections by the fungi *Pezicula malicorticis* and *Pezicula alba* (bull’s eye rot, synonyms: *Neofabraea malicorticis* and *Neofabraea alba*), *Penicillium expansum* (blue mould), and *Colletotrichum gloeosporioides* (bitter rot) belong to the main causes of decay during apple storage (Tahir 2006). The problem is especially serious in countries like Sweden where post-harvest dipping or fumigation is entirely prohibited. Problems with storage diseases are large also in other countries where apples are grown organically. In a recent Ph D thesis from Balsgård about apple production methods, organically produced “Aroma” fruits suffered a 20-fold increase in bull’s-eye rot compared to fruit grown according to the IP (integrated production) concept (Jönsson et al. 2010). A set of inoculation tests was performed at Balsgård, SLU in the fall of 2010 (Ahmadi-Afzadi et al. manuscript in preparation). A total of 94 apple cultivars were inoculated with *P. expansum* and *Colletotrichum gloeosporioides*. For each fungus, a spore suspension was injected into the fruit at two opposite points. Length and width of the resulting damage was measured after cold storage, and—in the case of *Colletotrichum*—a few days of subsequent storage at ambient temperature.

Results

Phenotypic glasshouse fire blight tests in Switzerland and Germany

The results of fire blight testing of different apple cvs in the glasshouse at ACW revealed a wide range of tolerance and susceptibility among the apple and pear accessions tested (pear data not presented). Figure 1 shows the results for 18 different apple accessions tested in 2011 with cvs “Enterprise” as resistant and “Gala” as susceptible control. The apple variety “Alant” was highly resistant in our trials.

“Alant” and other tolerant cvs such as “Danziger Kantapfel” have already been used in crosses to develop new apple cvs with high fruit quality and increased fire blight tolerance. Further tests are being performed to confirm these results and they will contribute to find outstanding accessions in respect to fire blight tolerance among pip fruit genetic resources.

The results of fire blight testing in Germany also revealed a wide range of tolerance and susceptibility among the 80 tested apple accessions tested (Table 2), with only 15 accessions showing less than 50% necrosis. The scab resistant varieties “Remo” and “Rewena” were highly resistant to fire blight in our trial followed by six old German cvs: “Altländer Pfannkuchenapfel”, “Edelborsdorfer”, “Altmärker Goldrenette”, “Roter Altländer Pfannkuchenapfel”,

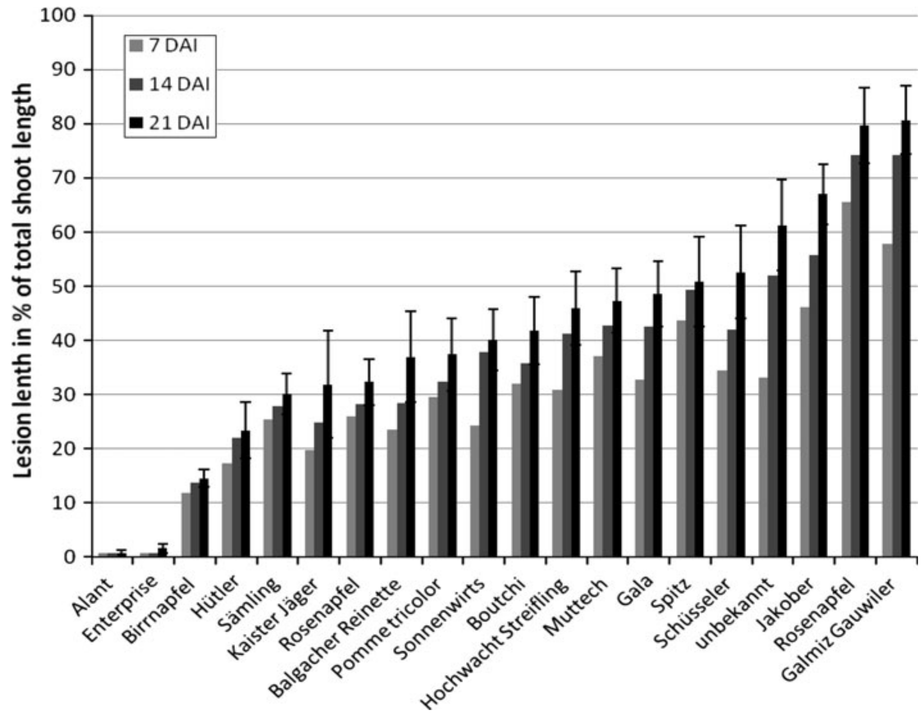

Fig. 1 Fire blight glasshouse screening of 18 traditional apple cultivars with "Gala" and "Enterprise" as standards, year 2011, Wädenswil, Switzerland (scoring 7, 14 and 21 days after infection (DAI)). *Bars* represent standard deviation

"Altländer Rosenapfel", and "Jacob Fischer". These had an average necrosis length below 35% of total shoot length. The high tolerance of "Schneiderapfel" confirmed the results obtained at ACW for the same cultivar.

Marker analysis for the fire blight resistance QTL FBF7 in Switzerland and Sweden

In Switzerland, both SCAR markers AE and GE, and therefore the FBF7 resistance QTL, were present in four out of 39 tested cvs in 2008, namely "Dettighofer", "Bernecker Wildling", "Schweizer Orangenapfel", and "Sternapi". These cvs displayed an average necrosis length of 39.8% of the total shoot length compared to 44.5% for cvs carrying none ore only one of the flanking SCAR markers.

In the Swiss inoculations of Swedish-grown apple material, cvs carrying the two DNA markers were significantly less damaged than cvs lacking the markers, $p < 0.001$. The least damaged cvs had both markers and belonged to the "Cox" family. In 2009 and 2010, terminal shoots of greenhouse-grown grafted trees of 23 apple cvs (only 22 in 2010), including the very susceptible "Idared" and the highly tolerant "Enterprise", were inoculated with *E. amylovora* in another co-operative project involving Balsgård, SLU, this time together with the Institute of Horticulture at Skierniewice, Poland (Nybom et al. 2011, this volume). Amount of damage caused by the inoculations were correlated when data from the 2 years were compared. A significant relationship between the presence of the two DNA markers and the level of fire blight tolerance was found both in 2009 ($p < 0.01$) and 2010 ($p < 0.05$).

Field evaluation of scab and mildew susceptibility in Switzerland and Germany

The distribution of disease scores for apple cvs evaluated for scab and mildew susceptibility in their third leaf Switzerland is presented in Figs. 2, 3 (Table 1). A majority (536, 89%) were scored in scab susceptibility classes 1–3, corresponding to a maximum scab incidence of 5%. Among these, 196 cvs did not show any scab symptoms at all. On the other hand, 18 cvs showed symptoms similar to, or even higher than those in "Golden Delicious".

The corresponding results for susceptibility to powdery mildew (*P. leucotricha*) are displayed in Fig. 4. A total of 380 accessions (65%) showed no symptoms. Again relatively few accessions displayed high susceptibility.

Similar observations have been carried out over a longer time period in the apple genebank at Dresden-Pillnitz, Germany. Among the 778 cvs evaluated, only 25 remained below a score of 3 (1–5% of organs, directly apparent) for mildew and scab both on leaf and fruit in the 3-year trial with no application of fungicides (Table 3). This group includes new scab resistant cvs such as "Remo", "Ariwa" or "Hana" but also old German, traditional cvs like "Roter Münsterländer", "Bittenfelder Sämling", "Riesenboiken",

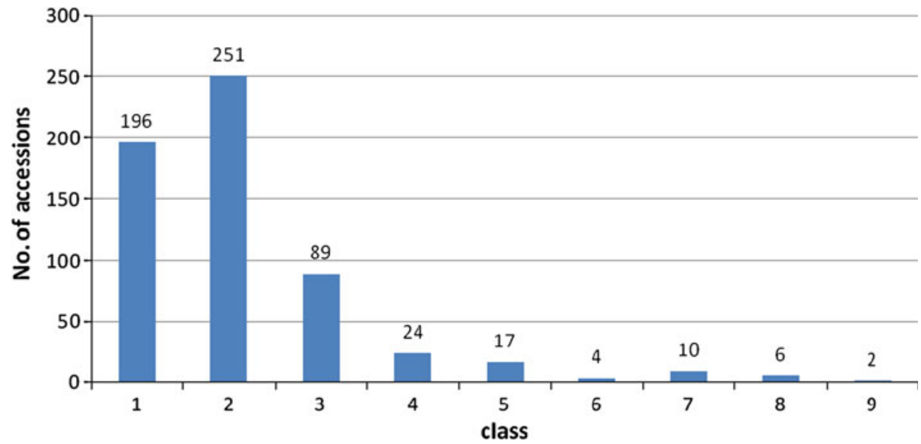

Fig. 2 Distribution of 599 apple cvs in different susceptibility classes for apple scab (*V. inaequalis)* in 2010 (3rd leaf) with "Gravensteiner" (class 2) and "Golden Delicious" (class 7) as reference cultivars

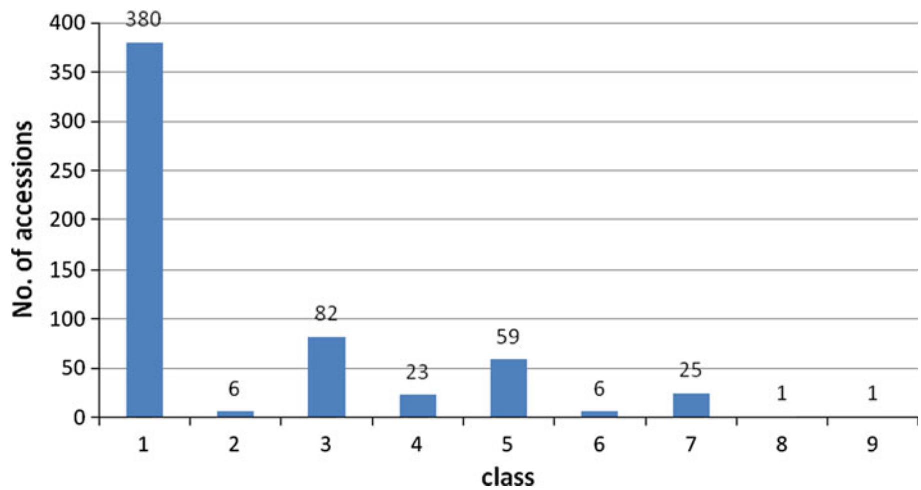

Fig. 3 Distribution of 583 apple cvs in different susceptibility classes for powdery mildew (*P. leucotricha*) in 2010 (3rd leaf) with "Gravensteiner" (class 5) as reference variety

Table 1 Field evaluation scale for scab and mildew susceptibility in Switzerland derived from Lateur and Blazek (2004)

1	No visible symptoms
2	1% of organs on close scrutinity
3	1–5% of organs, directly apparent
4	Intermediate
5	About 25% of organs infected
6	Intermediate
7	Heavy infection of 50% of organs
8	Intermediate
9	Very heavy infection, more than 90% of organs infected

and "Rote Sternrenette". All cultivars have not yet been included in the fire blight tests, but "Remo" has received excellent results in all parts of the resistance evaluation. "Engelhofer", "Gewürzluiken", and "Ariwa" have already been used in crosses to develop new apple cultivars with high fruit quality and multiple resistances.

Storage disease analyses in Sweden

Large differences in the size of disease symptoms resulting from inoculations were noted among the investigated apple cvs. A strong association was found between harvesting time and amount of blue mould damage; the later-ripening cvs showed significantly higher tolerance. Significant correlations were found also between the amount of blue mould damage and initial firmness as well as softening; especially the late-ripening (from mid-September) cvs with high levels of firmness and little softening were less affected by blue mould (Fig. 4). Similar tendencies but less pronounced were found also for bitter rot.

Field evaluation of pear rust susceptibility in Belgium

In 2001, the first real epidemic of pear rust (*G. sabinae*) was observed on pear leaves in Belgian collections. Scoring results differed considerably between years (Fig. 5); the years 2004 and 2009 were characterized by a medium to high level of pear rust symptoms (average of 4), whereas the years

Table 2 Fire blight glasshouse screening of 80 apple cultivars with "Idared" as standard (in bold), between years 2006 and 2010, Germany (scoring 4 weeks after inoculation)

Accession (variety)	Lesion length (cm)	Standard deviation
Remo	13.25	7.85
Rewena	16.51	15.77
Altländer Pfannkuchenapfel	22.65	17.96
Edelborsdorfer	26.88	22.32
Altmärker Goldrenette	29.93	13.81
Ilga	30.69	26.46
Altländer Pfannkuchenapfel, Roter	32.05	28.86
Altländer Rosenapfel	33.22	18.38
Jacob Fischer	33.92	24.73
Schneiderapfel	37.17	19.77
Prinzenapfel	39.92	30.84
Akane	42.96	17.85
Golden Resistent	43.83	30.08
Ahra	47.97	19.30
Regunde	49.58	34.35
Erwin Baur Roba	52.33	
Rebella	53.21	
Bittenfelder Sämling	53.76	
Adersleber Kalvill	54.29	
Hibernal	55.09	
Aneta	55.50	
Gewürzluiken	56.94	
Dalinred	57.17	
Riesenboiken	59.65	
Börtlinger Weinapfel	59.92	
Blenheim	60.06	
Aiwaniya	60.37	
Discovery	60.82	
Peasgoods Goldrenette	61.54	
Welschisner	62.02	
Dayton	62.13	
Fürst Blücher	62.20	
Engelshofer	62.37	
Rote Alkmene	62.63	
Hagloe Crab	63.17	
Roter Münsterländer	63.71	
Erbachhofer Mostapfel	64.30	
Alkmene	66.20	
Finkenwerder Herbstprinz	67.19	
Ahrista	67.23	
Kardinal Bea	68.53	
Engelsberger Weinapfel	70.36	
Reglindis	70.53	
President Decour	70.92	
Bellefleur Krasniy	71.04	
Deutscher Goldpepping	71.12	

Table 2 continued

Accession (variety)	Lesion length (cm)	Standard deviation
Allington Pepping	73.57	
Mauks Sämling	74.05	
Idared	**74.26**	
Amerikanischer Gestreifter Süßapfel	75.82	
Früher Victoria	76.28	
Altenstädter Roter	77.81	
Ahrina	78.26	
Rote Sternrenette	78.72	
Juliane	78.79	
Kirschweinling	79.02	
Großer Brünnerling	79.44	
Court Pendu Plat	81.64	
Adams Parmäne	83.90	
Antonovka	84.81	
Antonvka Kamienna	85.66	
Altländer Jakopsapfel	86.28	
Elise Rathke	86.55	
Cellini	87.38	
Anacuta	87.62	
Königinapfel	88.38	
Ashmaeds Kernel	89.04	
Gloria Mundi	91.93	
Antonovka polutorafuntovaja	92.10	
Ananasrenette	92.94	
Margol	94.13	
Alantapfel	94.34	
President Roulin	94.90	
Demokrat	95.30	
Roter Ausbacher	95.52	
Mauks Hybride	96.30	
Externtaler	96.77	
Grahams Jubiläumsapfel	98.33	
Altenburger Bergapfel	100.00	
Pommerscher Krummstiel	100.00	

Standard deviation indicated with cultivars tested a second year after primary results gave less than 50% necrosis

2007 and 2006 showed even higher (average of 4.5) and lower (average of 2.7) disease symptoms, respectively. No cvs were observed to be totally resistant or immune against pear rust. In contrast, some cvs were so susceptible that the leaf symptoms reduced photosynthesis while symptoms on fruits and twigs caused necroses and abscissions. These cultivars suffered severely when planted in unsprayed standard orchards. A data compilation, presenting pear rust susceptibility in a set of old pear cultivars, is currently being prepared within the BIODIMESTICA trans-border INTERREG project.

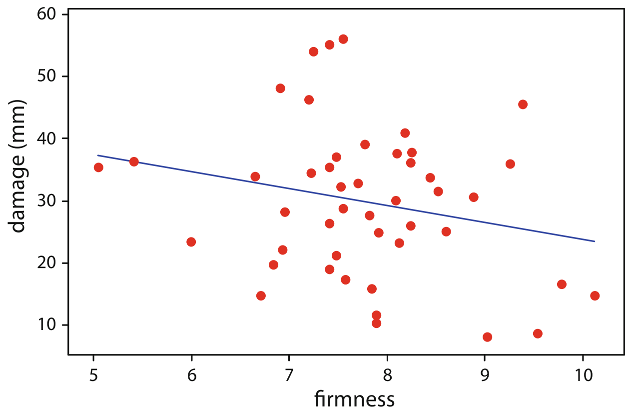

Fig. 4 Negative correlation ($r = -0.31$, $v = 43$, $p < 0.05$) between fruit firmness (kg/cm^2) at harvest, and fruit damage after inoculation with *Penicillium expansum* in late maturing apple cvs

Discussion

For sustainable exploitation of plant genetic resources in fruit cultivation and breeding, phenotypic and genotypic characterization is necessary. This characterization should be based on well-established guidelines for description, and augmented by additional tests related to disease resistance and fruit quality as well as modern molecular tools. As illustrated by the evaluation of pear rust (Fig. 5), a field evaluation process usually requires several repeated observations in order to produce representative data. Indeed, it often takes several years before the quantitative and qualitative components of disease inoculum become fully established in unsprayed orchards. Therefore, evaluations conducted immediately after a long period with standard fungicide application, are seldom representative of a normal epidemic. In addition, year-to-year variation in disease pressure in often high, depending on climatic conditions, inoculum pressures and growing conditions. Similarly, the spatial distribution of disease pressure can vary widely within the same orchard. As discussed by Lateur and Populer (1996), field evaluation data should be collected during at least five seasons before being used for objective conclusions.

Field evaluation is especially difficult for fire blight, sometimes also impossible due to phytosanitary restrictions. Evaluation in the glasshouse with a shoot inoculation test is a reasonable alternative. However, it is recommended to extend glasshouse shoot testing also to glasshouse flower

Table 3 Evaluation of scab and mildew in the apple genebank at Dresden-Pillnitz in 1997, 1999 and 2006, showing a selection of cvs with a maximum score of 3 on the nine-step scoring scale during all three years

Cultivar	1997			1999			2006		
	Mildew	Scab leaf	Scab fruit	Mildew	Scab leaf	Scab fruit	Mildew	Scab leaf	Scab fruit
Engelshofer	2	1	1	2	1	1	1	1	1
Erbachhofer Mostapfel	2	1	1	2	1	1	1	1	1
Präsident Decour	2	1	1	1	1	ND	1	1	2
Roter Münsterländer	2	1	ND	2	1	ND	2	1	1
Bittenfelder Sämling	2	1	1	1	1	1	2	1	2
Kirschenweinling	2	1	1	3	1	1	2	1	2
Hana	3	1	1	2	1	1	2	1	1
Rotfrüchtiger Suislepper	3	1	1	2	1	ND	2	1	1
Roter Ausbacher	3	1	ND	2	1	1	2	1	1
Riesenboiken	2	1	1	3	2	1	2	2	ND
Externtaler	1	1	ND	3	3	1	3	1	1
Gewürzluikenapfel	2	1	1	2	1	1	3	1	1
Discovery	2	1	2	3	1	1	3	1	1
Plovdiv 1713	3	1	1	3	1	1	3	1	1
Genitor 82	3	1	ND	2	1	ND	3	1	1
Nela	3	1	ND	2	1	1	3	1	1
Ariwa	ND	ND	ND	3	1	1	3	1	1
Antonovka Eineinhalb Pf.	3	1	1	2	1	1	3	1	2
Rote Sternrenette	3	1	1	2	2	2	3	1	2
Realka	3	1	1	2	1	1	3	1	2
Golden Resistent	2	1	2	3	1	2	3	2	3
Remo	3	1	1	3	1	1	3	2	3

ND not determined

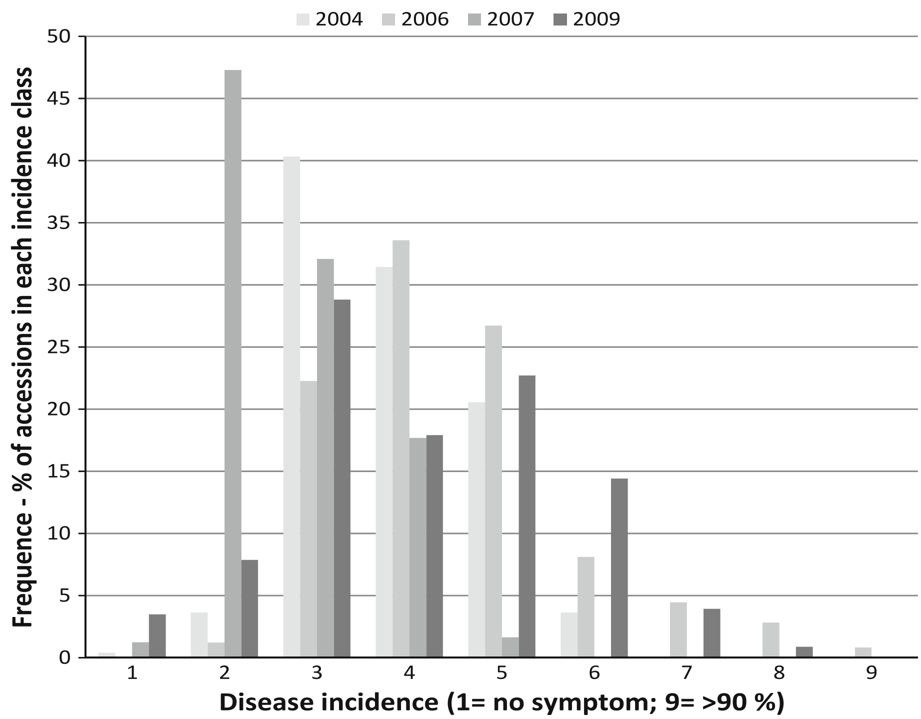

Fig. 5 Field evaluations of pear rust symptoms in 246 pear cvs in Belgium 2004–2009

testing and, if possible, to include field evaluation similar to the field evaluation of new biological control agents. Szobiczewski et al. (2011) reported that phenotypic evaluation of fire blight shoot susceptibility of several common genotypes conducted within the frame of the COST 864 action in Poland and Germany, in general showed that highly resistant and highly susceptible genotypes react in the same manner independently of place and inoculation procedure. Observed inconsistency problems could be related to the testing procedure, i.e., stage of shoot development, the number of individuals per genotype, the method of inoculation, the type of inoculum and the influence of environment.

To achieve durable disease resistance in fruit breeding, it is advisable not to rely exclusively on single resistance genes. The *Vf* scab resistance gene in apple has been widely used in breeding but has now been overcome by new races of *V. inaequalis* (Parisi et al. 1993; Roberts and Crute 1994). Several apple breeders are therefore trying to pyramid different resistance genes against the same pathogen in one genotype (Kellerhals et al. 2011). An alternative approach is to incorporate partial resistances present in the germplasm (Lateur et al. 2009). Laurens et al. (2004) have screened local European cultivars as sources for durable scab resistance in apple. Glasshouse tests were carried out at different sites with a set of 36 varieties and subjected to inoculation with local inocula or with monoconidial strains. Some accessions displayed consistently low susceptibility. Information on the differential resistance and susceptibility of apple and pear genetic resources towards diseases is important for breeding and for replanting traditional varieties.

Molecular markers

Molecular marker-based information on the genetic diversity of fruit genetic resources is presently being developed around the world. Molecular markers are also being used to fingerprint fruit germplasm for identification. DNA-based fingerprinting has thus been carried out for all the Swedish mandate cultivars of apple and pear (Garkava-Gustavsson et al. 2008, unpublished results). Unfortunately, up to 30% of this material is, in all likelihood, mislabeled according to the molecular data. Similarly high levels of identification problems have been reported from other gene banks with clonally propagated crops (reviews in Nybom and Weising 2010; van Treuren et al. 2010), clearly demonstrating the need for proper molecular marker-based identification as a useful approach for all phenotyping projects. Similar work is performed at ACW in Switzerland.

Using standard sets of markers for fingerprinting, reliable comparisons can be made between laboratories and datasets, and collections can be screened cost-effectively. Evans et al. (2009) appointed a set of 17 microsatellite loci (SSR) and eight reference genotypes to allow comparisons between *Pyrus* germplasm collections within ECPGR. At a collaborative workshop, the ECPGR *Malus*/*Pyrus* Working Group recently also agreed on 12 SSR markers for use in apple, and eight reference genotypes (Fernandez-Fernandez 2010 personal communication). Harmonized allele labeling systems and standardized record sheets for the databases were also proposed so that microsatellite fingerprints can be used effectively to characterize accessions in collections, and to facilitate comparison of datasets between laboratories to detect duplicates and synonyms.

The application of molecular markers to evaluate fruit germplasm is still hampered by the fact that we are mainly dealing with quantitative characters and rarely single genes. The example of the application of the FBF7 QTL, conferring enhanced resistance to fire blight, shows that it is still not possible to rely solely on molecular selection while dealing with quantitatively inherited characters. A significant relationship between presence of the two FBF7 QTL and the level of fire blight tolerance was found in the present studies in some years and it was also highlighted by Baumgartner et al. (2010) for progeny plants as well. However, this QTL confers only partial resistance.

Germplasm collections in Europe and overseas may contain a high level of genetic diversity which will be further exploited with advanced genomic tools in the near future. The low level of linkage disequilibrium (LD) in germplasm collections allows for association studies which should lead to the detection of genomic regions affecting traits of interest such as disease resistances (Laurens et al. Fruitbreedomics, this volume).

Implications for pome fruit breeding programs

Genetic diversity provides the raw material for breeding and plant improvement. It allows breeders to react to new arising requirements of the consumers and markets as well as to climate change. Traditionally, evaluation of the genetic resources and subsequent crossing products in fruit breeding programs, were based exclusively on phenotypic characterization. However, at present a combined analysis and application of both phenotyping and genotyping is common practice (Kellerhals et al. 2009).

At ACW, genetic resources have been integrated in the apple breeding program for many years (Table 4). "Sternapi" is supposed to be a very old cultivar dating back to the time of the Romans. It is characterized by a relatively small fruit with five pronounced ribs giving it the shape of a star. This feature might be interesting also for a new, distinguishable commercial cultivar. Other interesting cvs include "Dülmener Rosenapfel" which has proved to be resistant to all scab inocula used in the trials of Laurens et al. (2004), and "Krimskoe", and "Korastojnka" which originate from Ukraine and Bulgaria, respectively. These were introduced for their high level of polygenic scab resistance. Progeny plants have been selected for scab resistance, mildew resistance and other phenotypic features. Selected genotypes have been grafted on M27 rootstock and recently planted in a trial for evaluation of fruit and tree characters.

Table 4 Examples of the incorporation of traditional cvs (in bold) in the apple breeding program at ACW (crosses conducted in 2006 and 2007)

Mother	Father	Seeds	Nb in step 1
ACW 12556	**Sternapi**	468	10
Dülmener Rosenapfel	ACW 12309	141	21
ACW 11309	**Roter Herbstcalville**	96	–
Rucliva	**Gelber Bellefleur**	644	370
Milwa	**Krimskoe**	1167	50
Milwa	**Korastojnka**	979	40

In Belgium, a breeding program was initiated in 1988 focused specifically on polygenic disease resistance, fruit quality, and low input cultivation with emphasis on organic farming (Lateur et al. 2009). Screening methods adapted for partial disease resistance mechanisms, low input cultivation and easy tree training have been developed and used in the genetic resource collections (Lateur et al. 2000), and the best performing cvs are now being used in the breeding program together with a selection of commercial cvs.

Old and disease-tolerant cvs are also being used in the breeding programs in Germany and Sweden. Due to the modern molecular techniques, it has also become easier to identify and subsequently introduce interesting genes from wild species into the breeding.

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