Ideotype of apples with resistance to storage diseases

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ABSTRACT: Upon the completion of a three-year evaluation of natural occurrence of storage diseases within a range of cultivars or advanced selections and a simultaneous assessment of their other characteristics, an apple ideotype has been proposed that could be used in breeding of new cultivars resistant to these diseases. The ideotype combines the potential of apples for long-term storage with higher skin thickness and toughness, a lower production of ethylene, a higher natural content of calcium, a higher content of total phenolic compounds and antioxidant capacity, a higher flesh firmness, and a higher acidity of juice expressed in pH values. For some of these characteristics, threshold values have been suggested in the paper. The genotypes preselected according to these criteria should be chosen on the basis of the final screening with *Pezicula alba* inoculum test. The result of the inoculation test should be equal to or better than the standard cultivar Gala. Future research is required to determine the feasibility of the ideotype in routine breeding programs.

Keywords: apples; storage diseases; ideotype; cultivars; advanced selections; bitter rot; grey mould; blue mould; resistance

A majority of apple cultivars presently grown in the Czech Republic is susceptible to storage diseases. Bitter rot caused by *Pezicula alba* Guthrie [*Gloeosporium album* (Osterw)], [*Pezicula malicorticis* (H.S. Jackson)], [*Gloeosporium perennans* (Zeller et Childs)]; grey mould caused by *Botryotinia fuckeliana* (de Bary ex de Bary), [*Botrytis cinerea* (Pers: Fr)]; and blue mould caused by *Penicillium expansum* (Link. Thom) are the most deleterious when apples are stored in cold air storages (Blažek et al. 1999; Kůdela, Kocourek 2002; Blažek 2004; Kloutvorová, Kupková 2007).

Besides normal protection against fungal diseases in integrated apple growing systems in the Czech Republic, two special fungicide treatments are recommended for susceptible cultivars – one at the beginning of September and the second 10 days before harvest (Lánský, Kneifl 2000; Lánský at al. 2003). These treatments are not allowed if apples are grown organically; other ways of protection against storage diseases applicable within the systems are technically demanding and finally much more expensive (DeEll, Prange 1993; Belz, Ruess 2001; Darnhofer, Wurm 2005). Chemical protection against storage diseases is hardly practicable in orchards of amateur growers, especially if cultivars with different times of ripening are planted close to one another at one place.

For the reasons mentioned above, breeding apples with resistance to storage diseases seems to be increasingly important for the future. An evaluation of the current knowledge of resistance within cultivars however indicates a wide range of factors influencing it. Among the active agents playing an important role in the resistance, the phenolic compounds and relevant enzymatic systems are often mentioned (Lattanzio et al. 2001; Torres et al. 2002; Valentines et al. 2005). Skin thickness and its resistance to breakage is considered an important factor as well (Spotts et al. 1999).

It is obvious that the heritability of the characteristic is rather complicated. With the aim to improve breeding procedures focused on the resistance to storage diseases, we have tried to define an ideotype for apples possessing higher levels of these characteristics. For its construction, we used the results

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of our previous work, in which apple cultivars with different levels of resistance to storage diseases were chosen (Blažek et al. 2006).

MATERIAL AND METHODS

Apples of selected cultivars and advanced selections grown on M 9 rootstock with different levels of resistance to storage diseases (Blažek et al. 2003) were collected between 2004–2006 in Holovousy experimental orchards. The location is climatically characterised by average yearly temperature of 8.1°C, average rainfall about 650 mm, and altitude about 300 m above sea level. Randomised samples of fruits picked at the stage of their optimum harvest ripeness were placed in a chamber of air storage; together with many samples of other cultivars and selections they were kept at the temperature of 1 or 2°C and RH > 95%.

Shortly after harvest the following characteristics were assessed among the evaluated samples: production of ethylene, fruit weight, skin colour, skin blush, skin waxiness, skin russeting, flesh firmness, ascorbic acid content, total phenolic compounds, antioxidant capacity, total acid content, pH value, total sugars, dry matter and calcium content.

Ethylene concentration was measured by the gas chromatograph (Agilent 4890 D, with FID, column 1.2 m length, 3 mm I.D. filled by Porapak Q). Three or four fruits were closed in a 4 l glass and had a 2-hour exposure at room temperature. Measured values were adjusted to 1 kg of fruit and 1-hour exposure.

Skin colour was rated using a 1–9 rating scale (1 completely green and 9 deep yellow). Skin blush was estimated as a percentage of the total skin area. Skin waxiness was rated using a 1–9 rating scale (9 – no waxiness). Flesh firmness was measured with the penetrometer (Model FT 327; R. Byrce, Alfosine, Italy) using the 11 mm tip supplied with the pressure tester and penetrated to the depth of 7.9 mm as marked on the plunger. Values were assigned by an *N*-test on each apple (both on the blush side and the non-blush side) and then both readings were averaged. The content of ascorbic acid sugars and acids were rated by titration (utilising standard curves). The content of total soluble solids was determined on the basis of weighing before and after drying. The content of calcium was determined by the flame atomic spectroscopy. The amount of total phenolic compounds was determined separately in the fruit skin and flesh by the spectrophotometric method, using Folin-Ciocalteu's reagent. The DPPH (diphenylpicryl-hydrazyl) method based on radical scavenging was used for the evaluation of antioxidant activity.

The length of storage life of each cultivar was estimated by sensory tasting, which was repeated several times during the storage period of the evaluated samples. The end of the storage life was based on a distinct decline of flesh taste, its firmness and several other fruit characteristics. During these sensory sessions, skin thickness of each cultivar was scored using a 1–9 rating scale; it was further measured directly on slices (adjusted as microscopic permanent preparations) using a 100× enlargement with a Nikon microscope attached to a digital camera DS – 5 M – L1. Similarly, the exocarp was measured as composed of the cuticle, one layer of epidermis and two or three layers of adjacent cells of hypodermis (Homutová, Blažek 2006).

Inoculation tests for screening the susceptibility of apples to *Pezicula alba* were done according to the procedures described by Kloutvorová and Kupková (2007). In the method, mycelium of the fungus was transferred under the fruit cuticle. The fungal isolate used in the study was selected and maintained on potato dextrose agar in Petri dishes. Inoculated fruits after a 2-month incubation period in a cold storage were rated according to the diameter of the lesions.

These results were tested by analysis of variance. Means were separated by Tukey's "least significance difference" test at $P < 0.05$ (LSD). The differences between cultivars were tested according to the occurrence of diseases on fruits during three years as replications.

Table 1. Impact of the length of storage life on resistance of apple cultivars and advanced selections to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Mean length of storage life (days)
Very susceptible	139.7
Susceptible	191.4
Moderately resistant	227.2
Resistant	252.0
LSD at $P < 0.05$	24.1

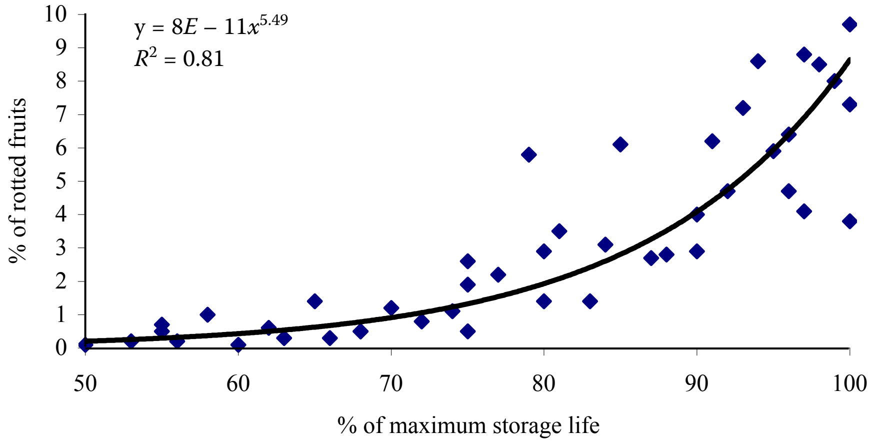

Fig. 1. Relationship between percentage of rotted fruits and the stage of storage life in several cultivars

RESULTS AND DISCUSSION

Among the evaluated characteristics, the following were more or less related to resistance to storage diseases: length of storage life, skin thickness or toughness, ethylene production, content of calcium in fruits, content of phenolic substances, antioxidant capacity, flesh firmness, pH values and inoculation tests.

Length of storage life

The impact of storage life length on resistance of apple cultivars and advanced selections to storage diseases is given in Table 1. From these figures, it is obvious that cultivars that endured in a good state in atmospheric storage till the end of April or even longer were less susceptible to storage diseases than cultivars with a limited storage potential. Similarly, according to hitherto results in Holovousy, shares of rotted apples increase very rapidly towards the end of the normal storage life of any particular cultivar (Fig. 1). As far as this characteristic is concerned in relation to the ideotype of a cultivar with improved resistance to storage diseases, at least 7-month natural storage potential in cold air storage should be required. The impact of the storage potential on apple storage rot incidence was formerly observed by Alston (1988).

Skin thickness or toughness

Despite certain variation in these characteristics, cultivars with thicker skin and greater skin toughness are generally more resistant to apple storage diseases and vice versa (Table 2). It is evident that sufficiently thick and tough fruit skin is a significant factor of fruit protection against infection by storage diseases (Spott et al. 1999; Homutová, Blažek 2006). Concerning the ideotype, skin thickness should

Table 2. Influence of skin thickness and skin toughness on resistance of apple cultivars and advanced selections to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Skin thickness (μm)	Sensory assessment of skin toughness (1–9)
Susceptible	50.2	4.49
Moderately resistant	57.4	4.95
Resistant	62.3	6.35
LSD at $P < 0.05$	3.8	0.53

Table 3. Production of ethylene shortly after harvest in fruits of apple cultivars and advanced selections with different resistance to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Production rate of ethylene (μl/kg/h)	
	span	average
Susceptible	0.55–12.70	4.37
Moderately resistant	0.60–6.86	2.06
Resistant	0.29–1.08	0.63
LSD at $P < 0.05$		1.09

be greater than 60 μm and its toughness should be above grade 6 of the 1–9 rating scale. Thickness of the skin should be uniform and without any breaks in the calyx area or around the stem.

Ethylene production

Cultivars that are resistant to storage diseases were distinguished by a lower production of ethylene on average (Table 3). However, the production of ethylene has probably only an indirect effect on the incidence of storage diseases; in higher concentrations, it accelerates fruit ripening and shortens the duration of the storage period. The production of ethylene in fruits of the ideotype at the optimum picking time should not be higher than 1 μl/kg/h. Below standard values of gas production in fruits should also be recorded during the whole storage period.

Content of calcium in fruits

Calcium content in fruits varied greatly according to years and sites (different soil conditions), and even within the same cultivar. Despite the larger variability, apples of cultivars classified as resistant to storage diseases had on average a higher content of calcium than the susceptible ones (Table 4). Desirable genotypes should have calcium content in fruits higher than 350 mg per kg of dry matter without previous foliar application of the element. The effect of higher calcium content on storage diseases is probably also indirect. A tendency was found that apples with higher calcium content were firmer during storage and produced less ethylene (Tomala, Soska 2004). The relation between higher content of calcium in fruits within seedling families and bitter pit was also studied previously (Volz et al. 2006).

Content of phenolic substances

The content of total phenolic compounds significantly differed in apple skin and flesh of all the studied cultivars; their concentration in skin was substantially higher (Table 5). With increased susceptibility of fruits to storage diseases, the quantity of phenolics decreased both in skin and in flesh. In a detailed study of phenolic composition, the more resistant cultivars showed higher content of epicatechin, catechin and chlorogenic acid in the skin.

Table 4. Content of calcium (mg/kg d.m.) in fruits of apple cultivars and advanced selections with different resistance to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Span	Average
Susceptible	203–492	284.4
Moderately resistant	240–491	342.5
Resistant	227–522	359.3
LSD at $P < 0.05$		50.8

Table 5. Content of phenolics (mg/kg f.m.) in the skin and flesh of fruits of apple cultivars and advanced selections with different resistance to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Skin		Flesh	
	span	average	span	average
Susceptible	5,244–2,048	3,122.6	1,331–250	752.5
Moderately resistant	4,645–2,065	3,455.5	1,320–217	887.8
Resistant	5,937–2,595	3,991.2	2,913–541	1,280.7
LSD at $P < 0.05$		414.4		313.6

Table 6. Antioxidant capacity of apple cultivars and advanced selections with different resistance to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Chlorogenic acid equivalent (g/kg f.m.)	
	span	average
Susceptible	6.3–0.6	2.2
Moderately resistant	8.5–0.4	3.1
Resistant	12.8–0.3	4.2
LSD at $P < 0.05$		1.1

Table 7. Flesh firmness (N) of apple cultivars and advanced selections with different resistance to storage diseases

Groups of cultivars or advanced selections according to their response to storage diseases	Span	Average
Susceptible	45–81	59.3
Moderately resistant	56–105	75.5
Resistant	53–102	77.1
LSD at $P < 0.05$		8.5
Juice acidity (pH) of apple cultivars and advanced selections with different resistance to storage diseases		
Susceptible	4.51–3.60	3.93
Moderately resistant	4.38–3.43	3.81
Resistant	4.22–3.43	3.74
LSD at $P < 0.05$		0.16

Antioxidant capacity

The antioxidant activity and capacity of radical scavenging varied greatly with the year and within the cultivar. Nevertheless, slightly higher antioxidant activity was determined in the cultivars with higher resistance to storage diseases (Table 6).

Flesh firmness

On average cultivars resistant to storage diseases were characterised by significantly firmer flesh than cultivars classified as susceptible (Table 7). This relationship, however, seems not to be very relevant as several cultivars with resistance to storage diseases had rather soft flesh, whereas some susceptible cultivars had flesh that was firm enough.

Acidity of apple juice (pH)

The actual acidity measured in pH values varied greatly within all groups of apples with different resistance to storage diseases, but the cultivars with higher resistance were generally more acid (Table 7). A similar relationship might be indicated according to the content of titrable acids in flesh.

Inoculation tests

Artificial screenings of chosen cultivars and advanced selections using inoculation of fruits by isolates of fungi that generate apple rots during storage were applied in this study with various efficiency and reproducibility of the results. Moderate reproducibility of the laboratory tests was reported before (Biggs, Miller 2005). Among these tests, inoculations of apples by *Pezicula alba* fungus were the most encouraging; with respect to the storage disease susceptibility or resistance based on 3-year storage tests, the results complied approximately up to 50% with the final classification of cultivars (Fig. 2). An apple collection in the National Fruit Trials in England was artificially screened for this fungus 40 years ago (Alston 1967). Therefore, an inoculation test should be used for accomplishing the most suitable ideotype; in this test, a desirable genotype should have equal or better performance than the standard cultivar Gala.

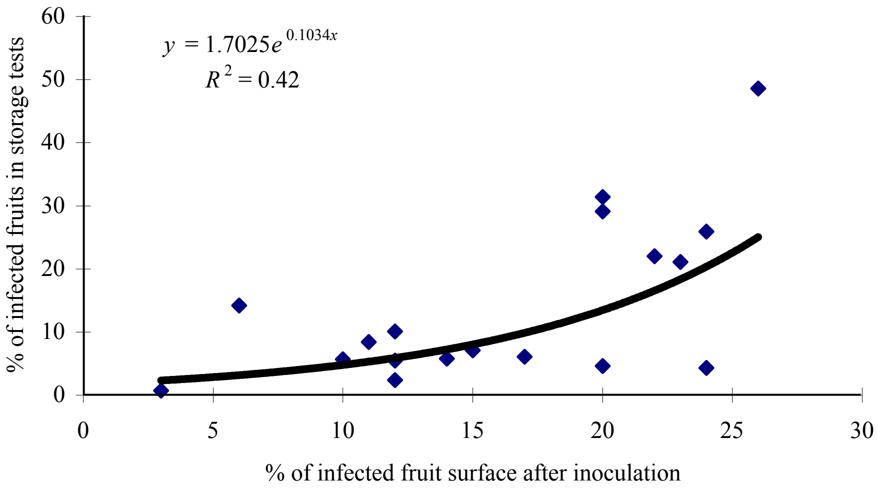

Fig. 2. Relationship between the mean percentage of infected fruits in storage tests with selected cultivars and the percentage of infected fruit surface in *Pezicula alba* inoculation tests (Holovousy 2006)

CONCLUSIONS

In terms of the hitherto results, an apple ideotype with resistance to storage diseases has been defined as fulfilling the below-mentioned criteria:

1. Suitability for long-term storage. Minimal storing potential in the case of ambient atmospheric cold storage is keeping fruits in marketing quality for 7 months.
2. Sufficiently thick and firm skin. The skin should be thicker than 60 µm and/or its toughness should exceed grade 6 on the 1–9 rating scale.
3. Generally lower level of ethylene production in fruits. At the time of harvest maturity the production of ethylene in fruits should not be higher than 1 µl/kg/h. Below standard values of gas production in fruits should be recorded during the whole storage period.
4. Higher natural content of calcium in fruits. These fruits should contain more than 350 mg of calcium per kg of dry matter without previous foliar application of the element.
5. Higher content of total phenolic compounds both in skin and in flesh.
6. Higher antioxidant capacity of fruits.
7. Higher flesh firmness. After fruit picking the flesh firmness should be higher than 75 N.
8. Higher acidity of juice indicated by the pH value lower than 3.8.
9. Results of inoculation tests by *Pezicula alba* should be equal to or better than standard cultivar Gala.

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Ideotyp jabloně s odolností proti skládkovým chorobám

ABSTRAKT: Na základě tříletého hodnocení přirozeného výskytu skládkových chorob u dlouhodobě skladovaných plodů širšího souboru odrůd nebo selektovaných novošlechtění a současného hodnocení jejich dalších charakteristik je navržen ideotyp jabloně, který by se mohl uplatnit ve šlechtění odrůd odolných proti těmto chorobám. Genotypy jabloní s tímto šlechtitelským cílem by se měly vyznačovat delší přirozenou skladovatelností plodů, jejich větší tloušťkou a pevností slupky, nižší tvorbou etylenu, přirozeným vyšším obsahem vápníku, vyšším obsahem fenolických látek, větší antioxidační kapacitou, větší pevností dužniny a nižšími hodnotami pH šťávy v plodech. U některých těchto charakteristik jsou navrženy prahové hodnoty. Genotypy předběžně vybrané podle těchto kritérií by měly být posléze selektovány na základě inokulačních testů houbou *Pezicula alba*. Napadení plodů v těchto testech by nemělo být větší než u standardní odrůdy Gala. Dalším výzkumem by měla být prověřena použitelnost navrženého ideotypu v praktickém šlechtění.

Klíčová slova: jablka; skládkové choroby; ideotyp; odrůdy; perspektivní hybridy; kruhová hořká hniloba; šedá hniloba; měkká hniloba; odolnost

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