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Influence of Frost Damage on the Sugars and Organic Acids Contents in Apple and Pear Flowers

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Summary

Spring frost often reduces the yield because of damaged flowers or flower parts. The contents of primary metabolites in both the flowers (whole flower) that were damaged by frost and the undamaged flowers (control) were investigated in the apple cultivar 'Idared' and the pear cultivar 'Passe crassane'. The contents of carbohydrates (glucose, fructose, sucrose and sorbitol) and organic acids (malic, citric, shikimic and fumaric) in flowers were determined by High Performance Liquid Chromatography. The results showed higher contents of glucose, fructose, sucrose, sorbitol and total sugars in the flowers, which were damaged by frost (completely or the vital part of the flower – ovary). This was the case in apple flowers as well as in pear flowers. The glucose/fructose ratio remained unchanged whether or not the flowers were damaged by frost. The content of individual and total organic acids in pear flowers was not influenced by flower frost.

Key words. apple – pear – flower – frost damage – HPLC – organic acids – sugars

Introduction

Irregularity of yield is one of the main problems in fruit production. Year-to-year variations in crop production have been traditionally related to weather conditions in spring (Rodrigo and Herrero 2002). Frost is a dangerous climatic hazard that can be responsible for yield losses and serious injuries to orchard trees (Zinoni et al. 2000; Rossi et al. 2002). The resistance to frost damage is associated with the nutritional status of plant and the carbohydrates content in the bark (Veberič et al. 2002, 2003). In temperate climates, important damages on deciduous fruit trees are caused in buds, flowers and developing fruits after dormancy, and the losses due to frosts during the blooming are usually more important than those due to low winter temperatures (Rodrigo 2000). The organs of the flower, more sensitive to low temperature stress, are the ovule, pistil, and ovary; stamens are more resistant (Sekozawa et al. 2003).

The ability to tolerate cold climate is under genetic control (Li et al. 2004). Cold-responsive genes likely to be involved in the development of cold tolerance include those encoding enzymes responsible for the synthesis of sugars or sugars derivates (Taji et al. 2002; Wisniewski et al. 2003). Although a causal relationship has not been established, the carbohydrate content has also been associated with plant hardiness. Carbohydrates have been frequently related to cold hardiness and seem to have several functions including cryoprotection of cellular constituents and the ability to retard the development of ice crystals. Indeed, the accumulation of water-soluble carbohydrates reduces the freezing point of the cell sap and thus the freezing temperature (Sakai and Larcher 1987). In woody species, carbohydrate levels have been correlated with flower bud hardiness (Flinn and Ashworth 1995). It is suggested that sucrose determinations may act as a reliable indicator of the extent of hidden frost damage (Rutherford and Whittle 1981). The increase in the total sugar content during cold hardening may not to be of primary importance as the ratio between particular sugars might prove to be crucial for cryoprotection (Liu et al. 1998; Svobodova et al. 2000).

The aim of our research was to examine how frost changes the content of carbohydrates and organic acids in damaged apple and pear flowers and what occurs with primary metabolites in undamaged flowers.

Material and Methods

Plant material

In this study we investigated the apple cultivar 'Idared' and pear cultivar 'Passe crassane'. The sugar (glucose, fructose, sucrose and sorbitol) and organic acid (citric, malic, shikimic and fumaric) contents of apple and pear flowers (whole flower with peduncle) were studied in the same commercial orchard on 4th April 2002 – 24 hours after spring frost (−6 °C) during full bloom [all flowers were open – F2 stage according to Fleckinger (Winter 2002)]. In the experiment we included flowers with frost damage (damaged flowers) and flowers without frost damage (undamaged flowers). We cut the flower vertically. If the ovary was green, such a flower was examined in the treatment of flowers without frost damage. If the ovary was brown or black, the flower belonged to the treatment of flowers with frost damage, because the first symptom observed after thawing in apple flowers at full

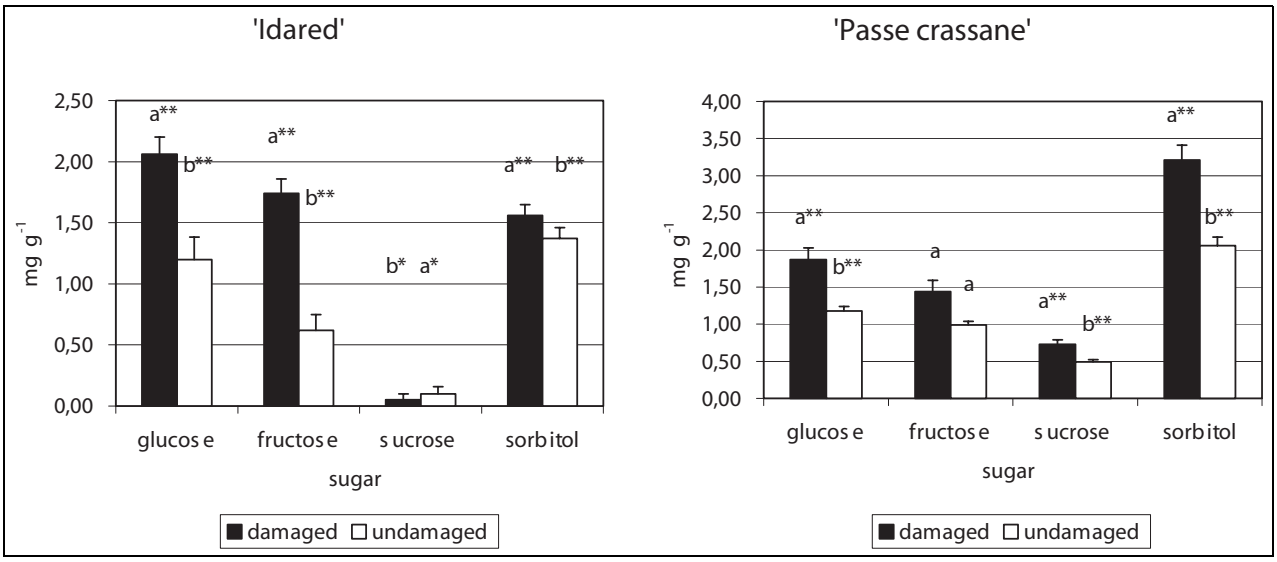


Fig. 1. The content of glucose, fructose, sucrose and sorbitol in fresh flowers of the apple cultivar 'Idared' and the pear cultivar 'Passe crassane' with SE (standard error). Bars within the same sugar labelled with different letters were significantly different at p<0.05 (marked with one asterisk) and at p<0.01 (marked with two asterisks).

bloom is a brown discoloration at the base of the style and the damage may extend both to the style and to the ovary, resulting in death of the placenta, abortion of ovules or formation of large breaks in the cortical tissue. In pear, the presence of symptoms of damage may be observed in the ovary before they are observed in the style (RODRIGO 2000). Flowers were collected randomly from 30 trees for each cultivar. For each cultivar we prepared 10 samples. For each sample we weighted 10 g of flowers (1 g of fresh flowers approximately consisted of 6 to 7 flowers).

Chemical analysis

The samples of sugars (glucose, fructose, sucrose and sorbitol) and organic acids (malic, citric, fumaric and shikimic) for the HPLC analysis were prepared out of 10 g of fresh apple and pear flowers. 10 g flowers were homogenized with 30 ml bi-destilled water, with an Ultra-Turrax T-25 (Ika-Labortechnik) and clarified by centrifugation at 6000 rotation min^{-1} for 15 min. Before injection into the column, the samples were filtered through a 0.45 µm Minisart filter (RC-25, Sartorious). The samples of 20 µl were used for the current HPLC analysis of sugars and organic acids (HUDINA and ŠTAMPAR 2000).

The HPLC (High Performance Liquid Chromatography) system consisted of Thermo Separation Products (TSP) equipment with a model P1000 pump, autosampler model AS1000, column heater and OS/2 Warp IBM Operation system (1994) work station. Solute elution had been monitored by using a differential refractive index RI (model Shodex-71RI).

Sugars analyses were performed isocratically on Aminex-HPX 87C cartridge at a flow rate 0.6 ml min^{-1} at temperature 85 °C with bi-destilled and on-line degassed water used as eluent. Attenuation of the refractive index detector was set at 16 x. Organic acids were analysed in the column Aminex-HPX 87H with flow of 0.6 ml min^{-1} and at 65 °C. For mobile phase 4 mM sulphuric acid (H_2SO_4) was used and UV detector with wavelength at 210 nm for identification.

Authentic samples of glucose, fructose, sucrose, sorbitol, and citric, fumaric and shikimic acid were from Fluka Chemical (New York, USA), and malic acid was from Merck Chemicals (Darmstadt, Germany).

Statistical analysis

The one-way analysis of variance (ANOVA) and Student–Newman-Kreuls test at p=0.05 and p=0.01 were used for the statistical analysis of the sugars and organic acids between the two treatments. A statistical software package Statgraphics 4.0 was used to perform the analysis.

Results and Discussion

Apple

In the presenting work we analysed the content of primary metabolites (sugars and organic acids) in flowers after frost. It is known that soluble solids and sorbitol increase rapidly in all parts of the flowers from bud burst until full bloom (VEMMOS 1995), and flower and early fruitlet abscission are not caused by carbohydrate shortage (Ruiz el al. 2001) if there is no frost damage.

The contents of individual sugars in apple flowers in the cultivar 'Idared' with and without frost damage are presented in Fig. 1. The content of glucose was almost two-fold higher in flowers with frost damage (2.06 mg g^{-1} of fresh flowers) than in the undamaged flowers (1.20 mg g^{-1} of fresh flowers). The difference between damaged and undamaged flowers was significant also at p=0.01. The content of fructose and sorbitol was significantly higher in the flowers with frost damage than at the control. Sucrose contents differed significantly between damaged and undamaged flowers and the content was

Table 1. The content of malic, citric, fumaric and shikimic acid in fresh flowers of the apple cultivar 'Idared' and the pear cultivar 'Passe crassane'.

Cultivar	Flowers	Malic acid		Citric acid		Fumaric acid		Shikimic acid	
		mg g^{-1}	SE	mg g^{-1}	SE	mg g^{-1}	SE	mg g^{-1}	SE
'Idared'	damaged	0.05a	0.01	1.13b**	0.28	0.0005a	0.0001	0.0024a	0.0007
	undamaged	0.04a	0.01	4.00a**	0.65	0.0008a	0.0001	0.0022a	0.0004
'Passe crassane'	damaged	0.06a	0.01	0.05a	0.006	0.0016a	0.0003	0.176a	0.014
	undamaged	0.06a	0.01	0.04a	0.004	0.0009a	0.0002	0.174a	0.013

Significant differences are marked with different letters at p<0.05 (marked with one asterisk) and at p<0.01 (marked with two asterisks).
SE: standard error

Table 2. Contents of total sugar, glucose/fructose ratio, and total acids inf fresh flowers of the apple cultivar 'Idared' and the pear cultivar 'Passe crassane'.

Cultivar	Flowers	Total sugar		Glucose/fructose ratio		Total acids	
		mg g^{-1}	SE		SE	mg g^{-1}	SE
'Idared'	damaged	5.41 a**	0.3	1.18 a	0.03	1.18 b**	0.27
	undamaged	3.29 b**	0.4	1.94 a	0.07	4.05 a**	0.66
'Passe crassane'	damaged	7.12 a**	0.5	2.09 a	0.67	0.28 a	0.02
	undamaged	4.73 b**	0.2	1.19 a	0.03	0.27 a	0.01

Significant differences are marked with different letters at p<0.05 (marked with one asterisk) and at p<0.01 (marked with two asterisks).

doubled in the undamaged flowers (0.10 mg g^{-1} of fresh flowers).

The contents of malic, shikimic and fumaric acids did not significantly differ between the damaged and undamaged flowers (Table 1). Only the citric acid content in undamaged flowers (4.00 mg g^{-1} of fresh flowers) was almost four-fold higher than in the damaged flowers (1.13 mg g^{-1} of fresh flowers).

Table 2 displays that there were no significant differences in glucose/fructose ratio, while the content of total sugars was significantly higher in the frost-damaged flowers. The content of total acids due to a higher content of citric acid was significantly higher in the undamaged flowers.

Pear

Like in the apple frost-damaged flowers in the cultivar 'Idared' we also found significantly higher contents of glucose, sucrose and sorbitol (Fig. 1) in the pear flowers in the cultivar 'Passe crassane'. Only the fructose content did not significantly differ between damaged and undamaged flowers in spite of a higher fructose content in damaged flowers.

The content of individual organic acids in frost damaged and undamaged flowers did not significantly differ between them (Table 1).

Also in the pear flowers, which were damaged by frost, the content of total sugars was higher, as in apple flowers (Table 2). The glucose/fructose ratio was higher in the flowers with frost damage than at the control, but the differences between damaged and undamaged flowers were not significant. Neither were the differences in the total acids contents.

Frost damage is mainly caused by ice formation rather than by low temperatures per se. The fate of the cell depends on the place, where the formation of ice crystals occurs: extra- and intra-cellular formation. During spring frosts, the cellular damage is usually produced by extra-cellular freezing. The anatomical effects caused by spring frosts in reproductive organs usually result in internal and external morphological abnormalities that affect the normal development of the fruit or even cause abscission (Rodrigo 2000). Extra-cellular ice is formed in freezing-intolerant as well as freezing-tolerant species and causes cellular dehydration. The most important cause of freezing-damage is when this dehydration exceeds what the cell can tolerate (Pearce 2001).

The content of individual sugars and also the total sugars in apple and pear flowers with frost damage was higher than in flowers without frost damage and could be the consequence of dehydration of cell. While ice is crystallizing, it has the same effect as dry air because the vapour pressure above ice is lower than that above a super-cooled solution. In the cold, less metabolic energy is available, the uptake of water and nutrients is restricted, less biosynthesis takes place, assimilation is reduced and growth stops (Larcher 2003).

The citric acid content in undamaged apple flowers was almost four-fold higher than in damaged flowers. The reason could be that during cold stress and dehydration of the protoplast, organic acids and salt ions in the

remaining unfrozen solutions reached abnormally high concentrations (LARCHER 2003). At pear cultivar 'Passe crassane' we did not observe significant differences in the content of citric acid in damaged and undamaged flowers.

LIU et al. (1998) and SVOBODOVA et al. (2000) state that in flowers damaged by low temperatures the ratio between individual sugars may be more important than the content of total sugars. We did not find differences in glucose/fructose ratio between damaged and undamaged flowers but damaged and undamaged flowers differed significantly in the content of total sugars in apple and pear flowers.

Conclusion

Spring frost hardiness is a complex phenomenon depending on a number of factors that range from inherited genetic characteristics and the stage of development to impact of environmental conditions and cultural practices on the individual genotype. Fruit trees with higher content of carbohydrates reserves in bark as well as in other parts of the tree may be more tolerant to different forms of frost damages. Therefore a very good supply with nutrients as well as other cultural practices during previous season could be very important for praxis. After frost damage of a whole flower or only the part of a flower – ovary, the contents of glucose, fructose, sucrose, sorbitol and total sugars were significantly higher in apple and also in pear flowers compared to the undamaged flowers, what is probable the consequence of cell dehydration in the frost damaged flowers. The glucose/fructose ratio did not differ between the damaged and undamaged flowers. The content of individual organic and total acids in pear flowers was not influenced by whether or not the flowers were damaged by frost. For future research it would be interesting to attend the sugars and organic acids content before and during the on set of low temperatures. Also the loss of water in flowers after frost damage should be investigated.

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