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Connection between the disease resistance of sour cherry genotypes and the carbohydrate content of the leaf and phloem tissues

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Abstract

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The objective of the present study was to establish a possible connection between disease resistance and the carbohydrate content of plant tissues by examining sour cherry genotypes with different tolerance levels in homeostasis. Research on the sour cherry – *Monilinia laxa* interaction involved the comparison of two Hungarian cultivars ('Érdi bőtermő' and 'Csengődi') and their offsprings (8) by measuring the quantity of homeostatic carbohydrate fractions in their leaves and phloem tissues. The results demonstrated that the glucose quantity and the ratio of glucose and fructose to sucrose were correlated with the disease resistance of sour cherry cultivars and their hybrids. The glucose content was higher in susceptible genotypes and lower in tolerant genotypes. The hexose:sucrose ratios of susceptible genotypes were significantly higher than those of tolerant genotypes.

Keywords: *Monilinia laxa resistance*; OPLC, homeostasis; sour cherry breeding; carbohydrate

Sour cherry (*Prunus cerasus* L.) is one of the most important fruit crops cultivated in Hungary. A significant aim of breeding is to increase disease resistance, a factor that determines the success of cultivation. Blossom blight [*Monilinia laxa* (Aderh. & Ruhland) Honey] greatly reduces the productivity of infected trees (Holb et al. 2008). 'Érdi bőtermő', the most important Hungarian cultivar, is extremely susceptible to the *M. laxa*, which causes twig dieback (Rozsnyay, Sződi 2009). The cultivar 'Csengődi', on the other hand, has good tolerance to *M. laxa* and has therefore been used as a resistance donor in the Hungarian breeding programme (Apostol, Véghelyi 1993).

Many endogenous chemical compounds or groups of compounds, including carbohydrates, play a role in the tolerance and self-protection of plants against various biotic and abiotic factors. Plants have the potential to recognise and respond to micro-organisms. The process of recognition leads to significant reprogramming in plant cells to activate and deploy defence responses to halt pathogen growth. The launching of defence reactions is a material- and energy-intensive process (Heil, Bostock 2002; Swarbrick et al. 2006), resulting in the depletion of the carbohydrates provided by primary metabolic pathways (Bolton 2009). Pathogenic infections also cause changes in the secondary metabolism by activating self-protection processes, and modify the primary metabolism, affecting the growth and development of the plant. Pathogen attacks thus cause yield losses even if the infection does not cause disease

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symptoms or the death of the plant (Berger et al. 2007). In addition, the high-molecular-weight polysaccharides making up the cell wall form a network consisting of ionic and covalent bonds, which is able to physically prevent pathogen intrusion (Vowerk et al. 2004). Thus, sugars not only function as substrates for growth but also activate sugar-sensing systems that initiate changes in gene expression in plants. Both the abundance and depletion of carbohydrates may enhance or repress the expression of genes affecting the sugar metabolism (Koch 1996). A low carbohydrate content provides unfavourable conditions for the growth of bacteria (Milčevičová et al. 2010). Similar observations were made by Sárdi et al. (1999) in snap bean (*Phaseolus vulgaris* L.) – *Pseudomonas* interactions. The diverse stress reactions of different beans genotypes to *Pseudomonas* inoculation can be associated with differences in the sucrose:glucose ratio of the leaf tissues. Among other things *Rhizoctonia solani* inoculation in potato influences the quantity of carbohydrates (Aliferis, Jabaji 2012). Hevesi et al. (2004) studied the utilization of carbohydrate resources by the bacterium *Erwinia amylovora* in pome fruits. The optimal concentrations of glucose, fructose and sucrose and the consumption and degradation time of the different carbohydrates utilized by *Erwinia amylovora* were determined in artificial nectar. Beniken et al. (2011) compared citrus rootstocks exposed to water deficit and to inoculation by *Citrus tristeza virus* and *Phytophtora* spp. The combined effect of the virus, bacterium and water deficit increased the quantity of soluble carbohydrates and amino acids in the leaves of rootstocks. Goncalves et al. (2005) studied the leaf symptoms caused by *Sugarcane yellow leaf virus* (ScYLV) in sugarcane (*Saccharum* spp.) hybrids. The carbohydrate contents in the leaves were increased by ScYLV inoculation. Relative to the leaves of healthy plants, sucrose was the sugar that accumulated most in the leaves of infected plants, followed by total soluble sugars and reducing sugars.

The publications cited in this paper use different approaches, but most of them confirm that carbohydrates play a role in defense responses. Against this background, and on the basis of results obtained after many years of research into host-pathogen interactions, it can be hypothesised that, in the case of sour cherry, the measurable quantity of certain carbohydrates in stress-free plants may be associated with the disease resistance of cultivars with different resistance levels (Sárdi et al. 1996; 1999; Szarka et al. 2002; Kovács-Nagy et al. 2008; Németh et al. 2009). The present study was undertaken in order to characterize sour cherry cultivars and hybrids representing different levels of *M. laxa* resistance in homeostasis, based on the carbohydrate concentration in the leaves and phloem tissue.

MATERIAL AND METHODS

Testing the resistance level using artificial *in vivo* inoculation. The *in vivo* inoculations were based on the method elaborated by Rozsnyay (1977) for *in vitro* inoculation of apricot with cytospora canker (*Cytospora cincta* Sacc.), which is also appropriate for inoculation by *M. laxa* (Crossa-Raynaud 1969; Sződi et al. 2008). The method involves determining the phloem necrosis of infected twigs. The *in vivo* artificial inoculations were performed in the field immediately after blooming on 10-15 cm one-year-old twigs, 20 per cultivar, using 14-day-old *M. laxa* culture. Round wounds, each 6 mm in diameter, were made on each twig and agar plugs inoculated with the pathogen were inserted into them. The round wounds and agar plugs were made by hole-puncher. The infected wounds were covered with wet cotton wool and fixed with aluminium foil to avoid drying. The evaluations were done 20 days after inoculation. During evaluation the bark was removed and the phloem necrosis was measured in mm. The level of resistance was tested in three consecutive years (2012–2014). The OPLC (Overpressured Layer Chromatographic separation) technique and densitometry evaluation were used for the quantitative and qualitative determination of the carbohydrates associated with resistance. The cultivars 'Csengődi' and 'Érdi bőtermő' and hybrids found to have the greatest resistance and susceptibility were compared on the basis of quantifiable carbohydrates in the leaf and phloem tissue. The comparative examinations were performed in three replications in 2015 and 2016.

Determination of carbohydrate content. The comparative examination was carried out in spring and winter (in homeostasis, free of extreme environmental conditions). Leaf and phloem tissues were collected in spring and phloem tissues in winter from one-year-old non-infected twigs in 2015 and 2016. The samples were frozen in liquid nitrogen, powdered and extracted with methanol

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(300 mg plant powder/800 ml of methanol : H_2O, 80 : 20, v/v). This suspension was centrifuged at 1,000 g for 10 min at room temperature. The clear supernatants were used for overpressured layer chromatographic separations (OPLC chromatograph developed by OPLC-NIT Co., Ltd., Budapest, Hungary). The OPLC separations were carried out on TLC and HPTLC silica gel 60 F254 (Merck Co.) precoated chromatoplates using acetonitrile:H_2O (85:15, v/v). Staining was performed with aniline-diphenyl amine-phosphoric acid reagent. A Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), λ = 540 nm, was used for densitometric determination (Sárdi et al. 1996, 1999).

Statistical analysis. The data obtained after artificial *in vivo* inoculation were analysed using the Kruskal-Wallis model, and means comparison and significant differences were calculated using Dunn's non-parametric comparison (P = 0.05). The carbohydrate data were analysed with the ANOVA model, and means comparison and significant differences were calculated using Duncan's test (P = 0.05). Statistical analysis was carried out using the 22.0 software (SPSS Inc., Chicago USA).

RESULTS AND DISCUSSION

Artificial *in vivo* inoculations

The largest twig dieback was measured on infected twigs of the hybrid 9/79-80, where, on average, more than 50 mm phloem necrosis was detected, while in the case of 'Érdi bőtermő' phloem necrosis averaged 44 mm. The extent of phloem necrosis was 30.9 mm for hybrid 9/24, an average of 20 mm for hybrids 9/5-6 and 9/91 and still more than 10 mm for hybrid 9/21, but in hybrids 7/47, 7/141 and 7/6-68 and the cultivar 'Csengődi' phloem necrosis measured less than 10 mm. The tolerance of hybrids 7/67-68 and 7/141 was similar to that of 'Csengődi', with a level of inoculation of around 1.5 mm. Hybrids with a level of inoculation of over 40% compared to 'Érdi bőtermő' were regarded as susceptible (9/5-6, 9/91, 9/24, 9/79-80), while those with infection of under 40% were regarded as tolerant (7/67-68, 7/141, 7/47, 9/21, 'Csengődi') (Fig. 1). In all the figures, the cultivars and their selected hybrids are listed in ascending order of disease resistance.

Carbohydrate content

In the spring plant samples, glucose, fructose, sucrose and xylose were detected reproducibly. The quantity of both monosaccharides was higher in the leaves than in the twigs for all the cultivars and hybrids. In the leaves the glucose concentration showed a clear correlation with disease resistance, as it was significantly higher (averaging 4.73 mg/g) in susceptible hybrids than in tolerant hybrids (3.02 mg/g on average). The glucose concentration in phloem tissue was negatively correlated with disease resistance, but significant differences and correlation could only be measured for fructose concentration, the quantity of fructose was almost twice in susceptible cultivars and hybrids (1.97 mg/g on average) than in tolerant ones (1.02 mg/g on average). The sucrose content in the leaves was higher (averaging, 6.2 mg/g) than in phloem tissues (averaging, 3.72 mg/g) in all the hybrids and cultivars. In the leaves of susceptible genotypes, the sucrose content was lower (averaging, 5.37 mg/g) than in the leaves of tolerant ones (7.03 mg/g on average), while in the twigs no cor-

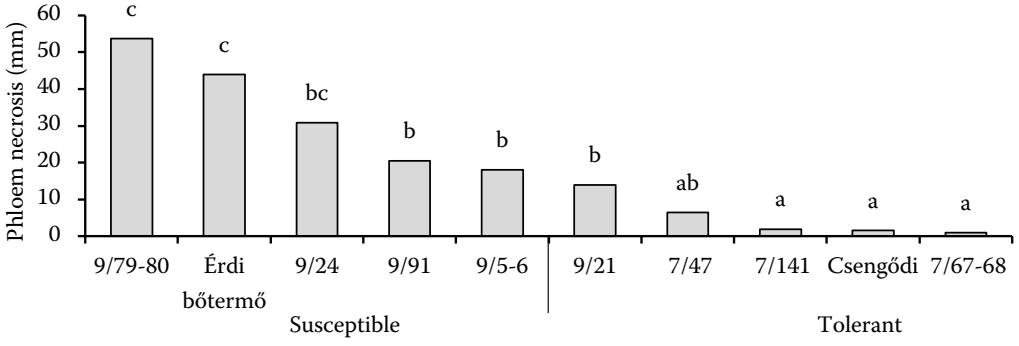

Fig. 1. Artificial *M. laxa in vivo* inoculations of sour cherry hybrids in the field
different small letters represent significant differences at P = 0.05

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Table 1. The most abundant carbohydrates in the leaves and twigs (phloem) in homeostasis

Carbohydrates	Cultivars and hybrids									
	Susceptible					Tolerant				
	9/79-80	Érdibőtermő	9/24	9/91	9/5-6	9/21	7/47	7/141	Csengődi	7/67-68
	Leaf (mg/g)									
Fructose	2.45^{b}	2.67^{b}	3.54^{c}	2.84^{bc}	3.46^{c}	1.08^{a}	1.23^{a}	2.18^{b}	2.19^{b}	2.65^{b}
Glucose	5.06^{c}	4.59^{c}	4.69^{c}	5.06^{c}	4.25^{bc}	1.95^{a}	3.25^{b}	3.45^{b}	3.15^{b}	3.32^{b}
Sucrose	6.48$^{b–e}$	5.65$^{a–d}$	4.71^{ab}	4.24^{c}	5.76$^{a–d}$	5.28^{abc}	7.02^{cde}	7.17^{cde}	8.29^{e}	7.38^{de}
Xylose	0.51^{bc}	0.45^{b}	0.69^{d}	0.79^{d}	0.76^{d}	0.15^{a}	0.62^{cd}	0.64^{b}	0.75^{d}	0.44^{bcd}
	Phloem (mg/g)									
Fructose	1.86^{bc}	1.83^{bc}	2.2^{c}	1.9^{bc}	2.04^{bc}	1.7^{b}	0.97^{a}	0.85^{a}	0.78^{a}	0.77^{a}
Glucose	4.44^{e}	3.75^{de}	2.99^{cd}	3.25^{cd}	2.98^{cd}	2.77^{bc}	2.04^{ab}	2.54^{abc}	2.53^{abc}	1.79^{a}
Sucrose	4.73^{d}	4.15^{cd}	2.71^{a}	2.61^{a}	3.53^{abc}	2.98^{ab}	3.65$^{a–d}$	4.18^{cd}	4.71^{d}	3.99^{bcd}
Xylose	0.75$^{a–d}$	0.6^{ab}	0.69^{abc}	0.85^{cd}	0.93^{d}	0.55^{a}	0.67^{abc}	0.82^{bcd}	0.57^{a}	0.63^{abc}

mean values followed by different letters within a column are significantly different by Duncan's multiple range test at $P = 0.05$

relation was observed. Xylose was not correlated with disease resistance (Table 1).

The total quantity of monosaccharides (glucose and fructose) in the leaves and twigs was found to be significantly correlated with disease resistance, since these carbohydrates were found in greater quantities in the susceptible cultivar and hybrids than in tolerant ones (Fig. 2).

The ratio of total monosaccharides (glucose and fructose) to sucrose was significantly higher in the leaves of the susceptible cultivar and hybrids than in tolerant ones. This correlation was also found when examining phloem tissues (Fig. 3).

In winter, when twig examinations were performed in the dormancy period, which is free of extreme stress effects, differences could again be shown between the genotypes. The total glucose and fructose content was significantly higher in susceptible genotypes. Genotypes in different resistance categories could be distinguished on the basis of the ratio of monosaccharides (glucose and fructose) to sucrose in the twigs (Fig. 4).

In spring, the quantity of glucose and the hexose (glucose and fructose) to sucrose ratio were mainly associated with *M. laxa* resistance. In the susceptible cultivar, the glucose concentration in the leaves was significantly higher than in the tolerant cultivar, and similar results were found for the hybrids. These results confirm the observations of Sárdi et al. (1996, 1999) who compared bean cultivars, susceptible and resistant to *Pseudomonas* and those obtained in studies on the pepper-*Xanthomonas* (Szarka et al. 2002) and grape-*Botrytis* (Kovács-Nagy et al. 2008; Németh et al. 2009) host-pathogen interactions.

The comparison was repeated in winter, during the dormancy period, on two cultivars ('Érdi

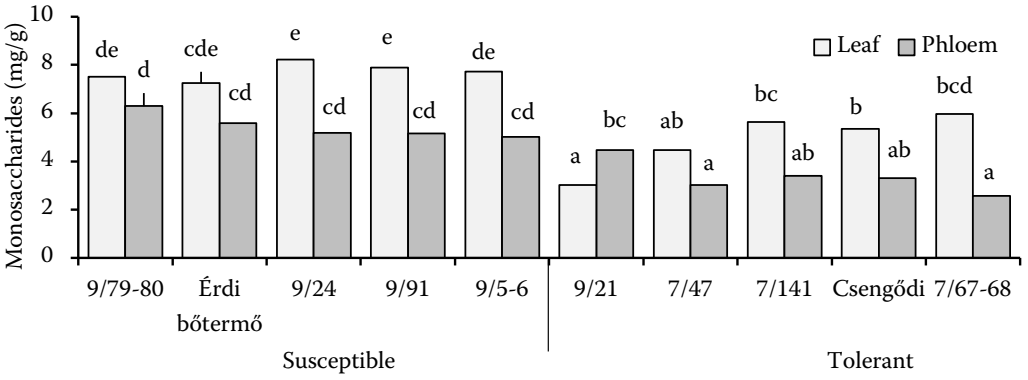

Fig. 2. Sum of the most abundant monosaccharides (glucose and fructose) in leaves and twigs in homeostasis different small letters represent significant differences at $P = 0.05$

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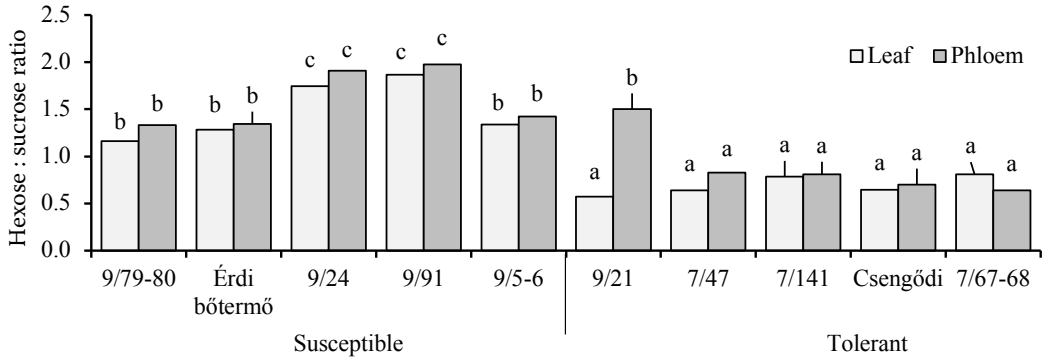

Fig. 3. Ratio of monosaccharides (glucose and fructose) to sucrose in leaves and twigs in spring
different small letters represent significant differences at $P = 0.05$

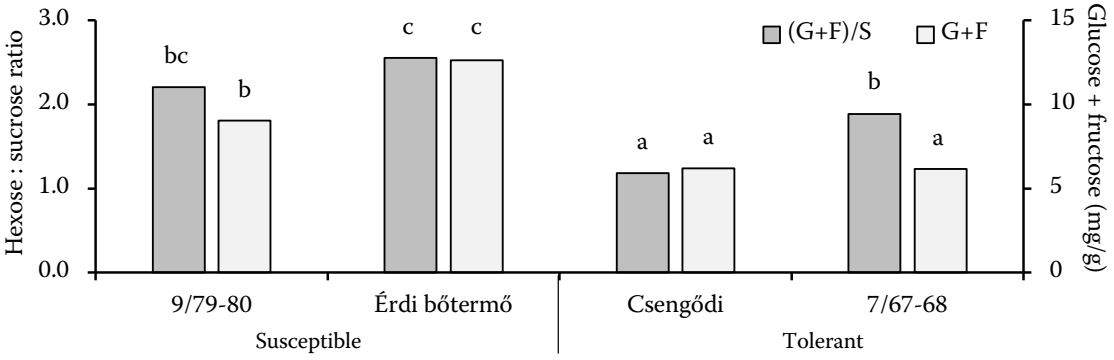

Fig. 4. Total glucose (G) and fructose (F) content and the ratio of monosaccharides (G +F) to sucrose (S) in leaves and twigs in winter
different small letters represent significant differences at $P = 0.05$

bőtermő' – susceptible, 'Csengődi' – resitant) and two hybrids (9/79-80 – susceptible, 7/67-68 – tolerant) and, the glucose and sucrose concentrations of the of phloem tissue were found to exceed those of the spring phloem samples. Among the carbohydrates measurable in the deep dormancy period, significant differences were mainly detected in the total glucose and fructose quantity, which was higher in susceptible genotypes than in tolerant genotypes. The hexose to sucrose ratio was also correlated with disease resistance. These results were in agreement with the results of Bolouri-Moghaddam et al. (2010) and Xiang et al. (2011), who reported that the cellular sucrose:hexose ratio is an important parameter determining cellular responses. The present results demonstrated that genotypes with different levels of disease resistance could be clearly distinguished based on the monosaccharide (glucose + fructose) to sucrose ratio. The sucrose quantity had a significant effect on the level of resistance. Sucrose is the main product of photosynthesis and plays an important role as a transport carbohydrate in plants (Koch 2004). It influences regulatory mechanisms, including growth and development, differential gene expression and stress-related responses (Wind et al. 2010).

The results agree well with previous observations on herbaceous plants, suggesting that the analysis of individual carbohydrates could be suitable for characterising the disease resistance of genotypes and for pinpointing significantly different defence levels.

It should be emphasised, however, that the basic criterion for effective comparisons is the strict observance of sampling rules, especially in the field. The long-term goal is to develop a methodology suitable for routine use in sour cherry breeding.

References

Aliferis K.A., Jabaji S. (2012): Deciphering plant pathogen interactions applying metabolomics: principles and applications**.** Canadian Journal of Plant Pathology, 34: 29–33.

Apostol J., Véghelyi K. (1993): Results of testcrossing in order to get disease resistant sour cherry varieties. In: Schmidt

https://doi: 10.17221/26/2017-HORTSCI

H., Kellerhals M. (ed.): Progress in temperate fruit breeding. Developments in Plant Breeding. Dordrecht, Kluwer Academic Publishers: 53–56.

Berger S., Sinha A.K., Roitsch T. (2007): Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. Journal of Experimental Botany, 58: 4019–4026.

Beniken L., Beqqali M., Dahan R., Benkirane R., Omari F.E., Benazouz A., Benyahia H., Gaboun F. (2011): Évaluation de la résistance de dix porte-greffes d'agrumes résistants à la tristeza vis-à-vis du déficit hydrique. Fruits, 66: 373–384.

Bolton M.D. (2009): Primary metabolism and plant defense – fuel for the fire. Molecular Plant-Microbe Interactions Journal, 22: 487–497.

Bolouri - Moghaddam M.R., Le Roy K., Xiang L., Rolland F., Van den Ende W. (2010): Sugar signalling and antioxidant network connections in plant cells. The FEBS Journal, 277: 2022–2037.

Crossa-Raynaud P.H. (1969): Evaluating resistance to *Monilinia laxa* [(Aderh. & Ruhl.) Honey] of varieties and hybrids of apricots and almonds using mean growth rate of cankers on young branches as a criterion of susceptibility. Journal of the American Society for Horticultural Science, 94: 282–284.

Gonçalves M.C., Vega J., Oliveira J.G., Gomes M.M.A. (2005): Sugarcane yellow leaf virus infection leads to alterations in photosynthetic efficiency and carbohydrate accumulation in sugarcane leaves. Fitopatologia Brasileira, 30: 10–16.

Heil M., Bostock R.M. (2002): Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Annals of Botany, 89: 503–512.

Hevesi M., Farkas Á., Kása K., Orosz-Kovács Z. (2004): Carbohydrate utilization of Erwinia amylovora in vitro. International Journal of Horticultural Science 10: 31–34.

Holb I.J., Szabó T., Thurzó S., Nyéki J., Drén G., Racskó J., Szabó Z., Soltész M., Veres Z. (2008): Incidence of brown rot blossom blight (*Monilinia laxa* [Aderhold & Ruhland]) and fruit rot in organic sour cherry production in Hungary. Acta Horticulturae (ISHS), 795: 913–918.

Koch K.E. (1996): Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 47: 509–540.

Koch K.E. (2004): Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology, 7: 235–246.

Kovács-Nagy E., Bilek A., Lacz E., Bodor P., Sárdi É. (2008): Grape variety comparison of different stress tolerance based on the quantitative measurement of carbohydrates. International Journal of Horticultural Science, 14: 7–10.

Milcevicova R., Gosch C., Halbwirth H., Stich K., Hanke M.V., Peil A., Flachowsky H., Rozhon W., Jonak C., Oufir M., Hausman J.F., Matusikova I., Fluch S., Wilhelm E. (2010): *Erwinia amylovora*-induced defense mechanisms of two apple species that differ in susceptibility to fire blight. Plant Science, 179: 60–67.

Németh Zs.I., Sárdi É., Stefanovits-Bányai É. (2009): State dependent correlations of biochemical variables in plants. Journal of Chemometrics, 23: 197–210.

Rozsnyay Z.D. (1977): Cytospora cancer and dieback of apricots. EPO Bulletin 7: 69–88.

Rozsnyay Zs., Sződi Sz. (2009): Meggyfajták monília-fogékonysága. [Susceptibility of sour cherry trees to monilia]. Kertészet és Szőlészet, 58: 12–13. Sárdi É., Velich I., Hevesi M., Klement Z. (1996): The role of endogenous carbohidrates in the *Phaseolus-Pseudomonas* host-plant age interaction. 1. Bean ontogenesis and endogenous carbohydrate components. Horticultural Science 28: 65–69.

Sárdi É., Velich I., Hevesi M., Klement Z. (1999): Ontogenesis- and biotic stress-dependent variability of carbohydrate content in snap bean (*Phaseolus vulgaris* L.). Zeitschrift für Naturforschung, 54: 782–787.

Swarbrick P.J., Schulze-Lefert P., Scholes J.D. (2006): Metabolic consequences of susceptibility and resistance in barley leaves challenged with powdery mildew. Plant Cell & Environment, 29: 1061–1076.

Szarka J., Sárdi É., Szarka E., Csilléry G. (2002): General defense system in the plant kingdom III. International Journal of Horticultural Science, 8: 45–54.

Sződi S., Rozsnyay Z., Rózsa E., Turóczi G. (2008): Susceptibility of sour cherry cultivars to isolates of *Monilia laxa* (Ehrenbergh) Saccardo et Voglino. International Journal of Horticultural Science, 14: 83–87.

Vowerk S., Somerville S., Somerville C. (2004): The role of plant cell wall polysaccharide composition in disease resistance. Trends in Plant Sciences, 9: 204–209.

Xiang L., Le Roy K., Bolouri-Moghaddam M.R., Vanhaecke M., Lammens W., Rolland F., Van den Ende W. (2011): Exploring the neutral invertase-oxidase stress defense connection in *Arabidopsis thaliana*. Journal of Experimental Botany, 62: 3849–3862.

Wind J., Smeekens S., Hanson J. (2010): Sucrose: metabolit and signalling molecule. Phytochemistry, 71: 1610–1614.

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