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Content levels of various fruit metabolites in the 'Conference' pear response to branch bending

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Abstract

The response of 'Conference' pear subjected to branch bending in the content levels of various metabolites in its fruit was investigated. The fruits in commercial maturity were sampled in 2004 and 2005 from branches bent in the late summer of 2003 (the summer treatment), from branches bent in the late spring of 2004 (the spring treatment) and from control branches. The content levels of carbohydrates, organic acids and phenolic compounds were compared among treatments in two successive years. The fruit revealed various responses in content levels of metabolites. In the first year after bending, no significant differences were found in the content of each carbohydrate, but in 2005 by far the highest content level of glucose and fructose and the lowest content level of sucrose were found in fruits from the control. The control fruit showed significantly higher content levels of malic acid and lower content levels of some phenolics (chlorogenic and vanillic acid) in the first year after bending, but in the next year the opposite reaction occurred—the control fruit had the lowest content level of malic acid and the highest content level of epicatechin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside. The comparison of the two bending treatments alone in 2004 showed that the summer treatment often produced a slightly higher value of each phenolic in comparison to the spring treatment. However, in 2005 the significantly highest content of chlorogenic acid was in fruit from the spring treatment. Sorbitol, as well as citric acid, catechin and sinapic acid showed no clear tendency among treatments, neither in 2004 nor in 2005. It is suggested that these variations of 'Conference' fruit subjected to different bending treatments could not be the result of bending alone, but that they could be indirectly affected by other physiological responses of the fruit tree. However, it seemed that variations are affected by the time of bending and by the year-to-year, and such responses can be attributed to the 'Conference' genotype only.

Keywords: Pear; *Pyrus communis*; Branch bending; Fruits; Carbohydrates; Organic acids; Phenolic

1. Introduction

Pear (*Pyrus communis* L.) is a popular fruit in the temperate regions, and in addition to 'Williams', 'Conference' represents an important share of the cultivars grown in Slovenia (Hudina and Stampar, 2000). 'Conference' pear is a very fertile, high yielding cultivar that crops 80–90% on spurs from branches of 2- and 3-year of age as well as on older ones (Sansavini, 2002).

The necessity to regulate excessive vegetative (branch) growth and to increase flowering and fruiting becomes even more significant for economic reasons, i.e. cost reduction, since the ratio between production costs and market prices for fruit has increased in recent years. Among traditional methods of orchard management and cultural practices applied in an orchard to control growth and fruiting, branch bending has proved the most successful. Branch bending is a long established and widely used cultural practice in high-density orchards, and its concept has nowadays been integrated into the Solaxe training system (Costes et al., 2006). Lawes et al. (1997) report that bending resulted in higher floral precocity and in reduced shoot vigour of the 'Doyenne du Comice' pear. Apple and pear trees yielded more fruit and produced fruit earlier if regulated only by bending than those regulated by pruning alone (Goldschmidt-Reischel, 1997). However, Lauri and Lespinasse (2001) have shown that the tree's reaction to bending also varies with the genotype and the time of bending, as well as with the angle of bending, the duration of bending time, etc.

Fruit such as pears are an excellent source of carbohydrates for a diet. Despite the fact that pears contain a good quantity of

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sugars (on average 54% fructose, 18% sorbitol, and only 15% sucrose and 13% glucose) and dietary fiber (15–28 g/kg FW) (Blattný, 2003), they are a recommendable substitute for diabetics and the obese; moreover, dietary fiber together with phenolics helps to reduce the risk of developing cardiovascular disease (Gorinstein et al., 2002). In most pear genotypes, malic acid predominates among organic acids. However, Hudina and Stampar (2000) reported that 'Williams', 'Red Williams' and 'Rosired' pears contain a higher percentage of citric acid. Both acids mentioned are major contributors to the optimal degree of acidity, and their ratio (malic acid/citric acid ratio) correlates with the sensory evaluation of taste (Colaric et al., 2005).

Phenolic compounds have a great physiological role in fruit, as well in its resistance to mechanical and biological stress. Phenolic compounds in fruit are of great interest to the consumer, because they are an important factor in fruit quality: they contribute to their sensory qualities (aroma, astringency, bitterness and colour), some of them have pharmacological properties, too (anti-inflammatory, antitumor, antiallergic, etc.) (Macheix et al., 1990).

In light of the positive findings for bending, the aim of this study is to determine the response of 'Conference' pear to branch bending with respect to the content of various metabolites in its fruit. The content levels of carbohydrates, organic acids and phenolic compounds, which determine the nutritional value of fruit, are compared according to treatments in two successive years. Apart from sensory evaluation and physical measurements of the fruit, the parameters analysed represent the main indicator of fruit quality.

2. Material and methods

2.1. Chemicals

Carbohydrates (sucrose, glucose, fructose and sorbitol), citric acid, shikimic acid, fumaric acid, (−)-epicatechin, vanillic acid, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside were obtained from Fluka Chemie GmbH (Buchs, Switzerland). The malic acid came from Merck KgaA (Darmstadt, Germany) and (+)-catechin from Carl Roth (Karlsruhe, Germany). Chlorogenic acid, sinapic acid, rutin (quercetin-3-rutinoside) and butylated hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was purchased from Riedel-de-Haën (Seelze, Germany) and acetonitrile from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Bidistilled water purified in a Milli-Q water purification system by Millipore (Bedford, MA, USA) was used. Methanol, acetonitrile and bidistilled water were of the HPLC grade. Sugar and organic acid standards were prepared in bidistilled water and phenolic standards in methanol.

2.2. Plant material

The study involved pear trees 'Conference'/Quince MA planted in 1987, trained to a spindle system and grown in the eastern part of Slovenia on sandy loam soil. Trees were spaced 3.8 m × 1.4 m, and the row orientation was south-north. The pear orchard was maintained according to standard commercial practice for integrated fruit production (orchard management and cultural practice, i.e. spraying, irrigation, etc.). Three different management treatments were applied to the trees, and each single treatment was repeated randomly on ten trees. Treatments were the following: (i) the summer treatment, where one 5-year-old branch per tree (on the tree's eastern side and of comparable properties) was bent to an angle of 120° from the vertical position in late summer 2003 (1st September 2003); (ii) the spring treatment, where one 5-year-old branch per tree was bent in late spring 2004 (15th May 2004); (iii) the control treatment, where labeled branches were not bent (but remained 45° from the vertical). Before bending, the bent branches were grown like the control branches—at an angle of 45° from the vertical position. All bent branches, as well control branches, were allowed to develop without pruning from 2003 to 2005. The undamaged pear fruit were harvested from those branches (three representative fruit from each branch, repeated on ten trees per treatment) at commercial maturity on 9th September 2004 and 1st September 2005. Immediately after harvest, the fruits were stored at −20 °C.

2.3. Extraction procedure

The samples were homogenized to a puree with the T25 basic Ultra Turrax homogeniser (IKA Labortechnik, Janke and Kunkel GmbH, Staufen, Germany). Then the puree was prepared separately for the carbohydrate and organic acid analyses, and for the phenolic compound analyses.

For sugars and organic acids extraction, bidistilled water up to a final volume of 50 ml was added to 10 g of puree and left for 45 min at room temperature, with occasional stirring. After extraction, the puree was centrifuged at 12,000 × *g* (Eppendorf Centrifuge 5810 R, Hamburg, Germany) for 7 min at 10 °C. The supernatant was then filtered through a 0.45 μm cellulose mixed esters filter (Macherey-Nagel, Düren, Germany) into a vial and used for the carbohydrate and organic acid analysis.

For phenolic analyses, 1 g of the homogenized sample was transferred to a test tube and extracted once with 10 ml of methanol for 45 min in an ultrasonic bath cooled with ice. The methanol contained 1% of the antioxidative agent BHT, which had no influence on the extraction process and the HPLC-analysis. The extracted sample was centrifuged 12,000 × *g* for 7 min at 10 °C. Then the supernatant was filtered through a 0.45 μm Chromafil (Macherey-Nagel) polyamide filter and used for the phenolic compound analysis.

2.4. HPLC analyses

Chromatographic separation of the carbohydrates and organic acids was performed using a Thermo Separation Products HPLC system (Riviera Beach, FL, USA). The HPLC-analyses of carbohydrates were made using a Rezex RCM-monosaccharide column (300 mm × 7.8 mm) operated at 65 °C from Phenomenex (Torrance, CA, USA). The mobile phase was bidistilled water and the flow rate was 0.6 ml/min. The duration of the analysis was 60 min, and a refractive index (RI) detector (Shodex RI-71, Showa Denko K.K., Kawasaki, Japan) was used for monitoring eluted carbohydrates. The

HPLC-analyses of organic acids were carried out using an Aminex HPX-87H column (300 mm × 7.8 mm) (Bio-Rad, Hercules, CA, USA) operated at 65 °C and associated with a Knauer K-2500 UV–vis detector (Knauer, Berlin, Germany) set at 210 nm. The mobile phase was 4 mM sulphuric acid in bidistilled water at a flow rate of 0.6 ml/min, and the total run time was 30 min (Colaric et al., 2006).

Separation of phenolic compounds was carried out using the Surveyor HPLC system (Thermo Finnigan, San Jose, CA, USA) with a photo diode array (PDA) detector, and it was controlled by the ChromQuest 4.0 Chromatography workstation software system. A Chromsep HPLC column SS (250 mm × 4.6 mm, Hypersil 5 ODS) coupled with a Chromsep guard column SS (10 mm × 3 mm) from Chrompack (Middelburg, the Netherlands) operated at 25 °C, was used. The chromatographic conditions were identical to those recommended by Schieber et al. (2001). The mobile phase consisted of solvent A: 2% acetic acid in bidistilled water (v/v), and of solvent B: 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1, v/v), with a flow rate of 1 ml/min. The elution gradient was as follows: 0–50 min, 10–50% B; 50–60 min, 55–100% B; 60–65 min, 100–10% B. The total run time was 65 min, and between each analysis 15 min of equilibration treatment (10% B) was performed. Analysed compounds were identified and quantified in a manner similar in that previously described by Colaric et al. (2006). Identification of phenolics was done at 280 nm, except for chlorogenic and sinapic acid, which were identified at 320 nm.

2.5. Data analyses

All data were tested by one-way analysis of variance (ANOVA; general linear model) using the Statgraphics Plus 4.0 program (Manugistics, Inc., Rockville, MD, USA). Means among treatments were separated by the least significance difference (LSD) test ($P < 0.05$). Data in Tables 1–6 are presented as means with standard errors (S.E.) and LSD values. Contents of carbohydrates, as well as malic and citric acid in ‘Conference’ fruit are given in g/kg of fresh fruit weight (FW), while the contents of shikimic and fumaric acid and phenolics are given in mg/kg FW.

3. Results and discussion

3.1. The content of carbohydrates

Among carbohydrates, sucrose, glucose, fructose and sorbitol were determined in ‘Conference’ fruit, and their ratio was in accordance with Blattný (2003), who reported the average ratio in pears to be 15% sucrose, 13% glucose, 54%

Table 1
Average content of carbohydrates in ‘Conference’ fruit (the mean ± S.E., g/kg FW) harvested in 2004 regarding branch bending

Sugar	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Sucrose	15.20 ± 1.60a	17.03 ± 2.48a	18.53 ± 1.73a	6.22
Glucose	10.03 ± 1.67a	8.35 ± 1.88a	7.63 ± 1.20a	5.07
Fructose	51.38 ± 1.98a	50.24 ± 1.49a	48.47 ± 1.45a	5.03
Sorbitol	24.11 ± 1.62a	24.38 ± 2.19a	23.85 ± 2.30a	6.10

No values are significantly different (LSD test, $P < 0.05$).

Table 2
Average content of carbohydrates in ‘Conference’ fruit (the mean ± S.E., g/kg FW) harvested in 2005 regarding branch bending

Sugar	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Sucrose	25.62 ± 2.45b	21.78 ± 1.26b	6.60 ± 0.61a	6.53
Glucose	10.43 ± 0.35a	11.40 ± 0.24b	14.75 ± 0.13c	0.96
Fructose	73.65 ± 1.39ab	68.88 ± 1.46a	76.23 ± 4.69b	7.00
Sorbitol	34.90 ± 3.02a	37.04 ± 1.33a	33.58 ± 1.48a	7.33

Different letters within each row: significantly different (LSD test, $P < 0.05$).

Table 3
Average content of organic acids in ‘Conference’ fruit (the mean ± S.E., g/kg FW; except for shikimic and fumaric acids; their values are in mg/kg FW) harvested in 2004 regarding branch bending

Organic acid	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Citric acid	0.23 ± 0.01a	0.25 ± 0.01a	0.22 ± 0.02a	0.04
Malic acid	3.78 ± 0.08a	3.77 ± 0.09a	4.25 ± 0.18b	0.42
Shikimic acid	73.13 ± 7.75a	92.57 ± 8.11a	85.22 ± 5.58a	22.29
Fumaric acid	2.75 ± 0.13ab	2.59 ± 0.13a	3.09 ± 0.23b	0.50

Different letters within each row: significantly different (LSD test, $P < 0.05$).

Table 4
Average content of organic acids in ‘Conference’ fruit (the mean ± S.E., g/kg FW; except for shikimic and fumaric acids; their values are in mg/kg FW) harvested in 2005 regarding branch bending

Organic acid	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Citric acid	0.21 ± 0.08a	0.17 ± 0.01a	0.21 ± 0.02a	0.13
Malic acid	2.76 ± 0.22b	2.70 ± 0.07b	1.97 ± 0.01a	0.41
Shikimic acid	9.56 ± 3.17a	10.71 ± 0.49a	47.09 ± 6.15b	6.32
Fumaric acid	2.83 ± 0.42a	2.54 ± 0.21a	2.42 ± 0.23a	1.06

Different letters within each row: significantly different (LSD test, $P < 0.05$).

Table 5
Average content of phenolic compounds in ‘Conference’ fruit (mean ± S.E., mg/kg FW) harvested in 2004 regarding branch bending

Phenolic compound	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Chlorogenic acid	240.32 ± 8.20b	212.58 ± 4.66ab	196.66 ± 21.86a	42.36
Catechin	1.75 ± 0.10a	1.31 ± 0.29a	1.68 ± 0.20a	0.62
Epicatechin	38.13 ± 4.43a	37.75 ± 5.57a	30.13 ± 3.35a	13.59
Sinapic acid	0.13 ± 0.02a	0.16 ± 0.03a	0.11 ± 0.02a	0.07
Vanillic acid	3.84 ± 0.41b	2.78 ± 0.18a	2.52 ± 0.35a	1.02

Different letters within each row: significantly different (LSD test, $P < 0.05$).

fructose, 18% sorbitol and the sum of those sugars about 124–158 g/kg fruit FW.

In both successive years, fructose predominated among carbohydrates, as well as among all analysed compounds; it was followed by sorbitol with about one half the fructose content (Tables 1 and 2). In the first year after bending, no significant differences were found in carbohydrate content among treatments (Table 1). However, the glucose and fructose contents were a little higher in fruit harvested from branches bent in the late summer of 2003 (the summer treatment), whereas the lowest sucrose content was noticed. Some lower values of other carbohydrates were found in the control fruit.

In the following year, the carbohydrate content – sucrose, glucose and fructose – significantly differed among treatments (Table 2). Sucrose content was lowest in fruit from the control treatment, and it differed significantly from both bending treatments. Just the opposite, the highest content levels of glucose and fructose were attained in fruit from the control.

Sanyal and Bangerth (1998) claimed that branch bending imposes mechanically induced stress. Moreover, Hudina and Stampar (1999) reported that sorbitol content increased in pear fruit from trees that were subjected to stress conditions. Nevertheless, on the basis of these statements and our results showing the opposite, it is suggested that no stress occurred in our study, since no significant differences between bending treatments and the control were observed for sorbitol in both years.

To date, Colaric et al. (2006) have researched the influence of branch bending on ‘Williams’ fruit at the chemical level, and they obtained different responses from those for ‘Conference’ fruit in the first year, although the study lasted for 1 year only. ‘Williams’ fruit picked from branches bent in the previous summer (2003) showed the lowest content levels of each carbohydrate, and the highest ones were found in the fruit from branches bent in the current spring (2004), except for glucose. Probably, branches bent in the spring had more photosynthates that were exported to the fruit. Ito et al. (2004) studied

Table 6
Average content of phenolic compounds in ‘Conference’ fruit (mean ± S.E., mg/kg FW) harvested in 2005 regarding branch bending

Phenolic compound	Bending treatment			LSD value
	Summer 2003	Spring 2004	Control	
Chlorogenic acid	64.45 ± 10.61a	174.14 ± 22.61b	116.51 ± 15.09ab	106.7
Catechin	10.41 ± 2.76a	8.57 ± 0.97a	9.69 ± 1.74a	5.73
Epicatechin	5.96 ± 0.84a	7.03 ± 0.81a	17.58 ± 3.63b	5.47
Sinapic acid	0.93 ± 0.12a	0.88 ± 0.08a	1.18 ± 0.11a	0.38
Vanillic acid	1.09 ± 0.39a	3.33 ± 0.48a	2.55 ± 1.01a	2.46
Rutin	3.61 ± 0.95a	2.65 ± 0.21a	3.68 ± 0.11a	1.52
Q-3-D-galactoside	0.92 ± 0.53a	0.94 ± 0.29a	4.51 ± 0.35b	1.43
Q-3-β-D-glucoside	2.73 ± 0.90a	2.88 ± 0.53a	6.09 ± 0.34b	2.62

Different letters within each row: significantly different (LSD test, $P < 0.05$).

carbohydrate metabolism in the lateral buds and in the shoot internodes of Japanese pear. They observed that bending influenced higher contents of sorbitol and sucrose in the central internode of the bent branch in comparison to that of the control, i.e. the vertical branch. Sorbitol and sucrose are the main translocating carbohydrates found in rosaceous fruit trees, the group to which pear belongs.

3.2. The content of organic acids

‘Conference’ pear is classified into the group of pear genotypes where, among organic acids, malic acid usually predominates in the fruit (Hudina and Stampar, 2000), and this was also confirmed in our study. The content of malic acid in the fruit from the control was significantly higher than in the fruit from both bending treatments in 2004, where similar content levels were found (Table 3). In the following year, however, significantly lower values of malic acid were measured in the control compared to those from the bending treatments (Table 4). In both years, a similar trend was noticed: the highest malic acid content corresponded with the highest sucrose content and with the lowest glucose content and vice versa.

Fumaric and shikimic acids were present in very small amounts. In 2004, the highest values of fumaric acid were found in fruit from the control, whereas citric and shikimic acids showed no significant differences in content levels among treatments. In the following year, the highest values of shikimic acid were found in the control, whereas some lower values of fumaric acid were found. Citric acid showed no clear tendency among treatments, neither in 2004 nor in 2005.

However, ‘Williams’ pears contained more citric than malic acid (Hudina and Stampar, 2000). Moreover, the content levels of all organic acids except citric (its content level was the lowest in the same treatment) proved the highest in the ‘Williams’ fruit from the branches bent in summer, whereas the same fruit were the poorest in carbohydrates (Colaric et al., 2006), as mentioned before. Both major acids (malic and citric) are the most important contributors to the optimal degree of acidity, and it was found that their ratio correlated with sensory evaluation of taste (Colaric et al., 2005).

3.3. The content of selected secondary compounds

Three phenolic acids (chlorogenic, sinapic and vanillic acids) and two flavonoids from the flavan-3-ols subclass (catechin and epicatechin) were detected in the first year after bending; moreover, three additional flavonoids from the flavonol glycosides subclass (rutin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside) were detected in the next year. The separation of phenolics (chromatographic conditions, mobile phases) was done according to Schieber et al. (2001), who determined rather similar content levels of flavonol glycosides in pear fruit. However, chlorogenic acid was the major phenolic in the ‘Conference’ fruit, and its content level is more in accordance with Macheix et al. (1990), who reported values from 10 to 516 mg/kg FW.

Generally, in the first year, some lower values of each determined phenolic compound, except for catechin, were observed in fruit from the control (Table 5). Content levels of chlorogenic acid and vanillic acid in fruit from the control differed significantly from the fruit involved in the summer treatment. The comparison of both bending treatments resulted in an observation that the summer treatment often showed a slightly higher value of each phenolic in comparison to the spring treatment. In the second year, epicatechin, sinapic acid and flavonol glycosides appeared in higher levels in fruit from the control, and among them epicatechin, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside differed significantly from both bending treatments (Table 6). Andreotti et al. (2006) reported that the accumulation of flavan-3-ols and flavonol glycosides is affected by wounding. However, the content levels of chlorogenic and vanillic acids in 2005 were the highest (significant for the chlorogenic acid) in the fruit from branches bent in the late spring of 2004 and lowest in the fruit from branches bent in the late summer of 2003, but the content levels of sinapic acid were the opposite.

It is supposed that some lower values of phenolics in fruit from the control (in 2004) could be connected to some lower values of carbohydrates (except for sucrose) in the same treatment, despite the fact that phenolics are mostly produced from carbohydrates (Macheix et al., 1990). The key enzyme that links primary and secondary metabolism is phenylalanine ammonialyase (PAL). It was reported that PAL activity reaches its maximum in very young fruit, and that corresponds to an increase in the accumulation of phenolic compounds; therefore, higher content levels of phenolics appear in young fruit. However, the phenolic metabolism is also greatly dependent on many external (light, temperature, stress) and internal (hormones, nutrients) factors (Macheix et al., 1990). Macheix et al. (1990) reported that fruit phenolics were also increased by lighting, which raises primary production (carbohydrates), which then supports higher PAL activity, and thus causes an increase in the accumulation of phenolic compounds, especially flavonoids.

Treutter (2001) stated that stress situations induce synthesis of phenolic compounds, and, according to Sanyal and Bangerth (1998), mild stress is imposed by branch bending. Considering only the phenolics content levels in ‘Conference’ fruit from the bending treatments (in 2004), we can confirm the statement of Sanyal and Bangerth (1998). Nevertheless, one can judge that branch bending was not functioning as a type of stress factor, since the content levels of most phenolics in the ‘Williams’ fruit involved in the summer treatment were among the lowest and in the fruit from the spring treatment among the highest (Colaric et al., 2006). What is more, ‘Williams’ leaves that responded to branch bending had almost the lowest phenolic content levels in the summer treatment (Colaric et al., 2007).

It seems that the response of fruit to bending varies most with genotype (since ‘Williams’ and ‘Conference’ pears responded differently), as well as with the time of bending, by the year-to-year variations, etc. Moreover, we should not forget that the variations observed in that study could also be a consequence of the physiological responses of the fruit tree and the fruit. Lauri and Lespinasse (2001) showed that the response in growth and fruiting of apples subjected to shoot bending also varied with genotype and time of bending. Furthermore, Sansavini (2002) reported that ‘Conference’ differed from ‘Williams’ in growth and fruiting habits. ‘Conference’ has the advantage that it is regarded as regular bearing cultivar, which bears 80–90% on spurs (model 3 of five fruiting models), mostly from branches of 2–3 years in age, but it never stops cropping on the spurs of the oldest branches, while ‘Williams’ crops less than 50% on the spur buds.

The decision about the timing of branch bending still remains open for ‘Conference’ pears, which showed an unclear tendency between both bending treatments, although some differences were found among the fruit involved in bending and those in the control treatments (Tables 1–6). But for the ‘Williams’ pears it can be inferred that branch bending in spring is more recommended (Colaric et al., 2006). However, it seems from the above results that branch bending had a positive effect on the level of phenolics in ‘Conference’ fruit in 2004.

4. Conclusion

The focus of the research was the effect of branch angle change on ‘Conference’ fruit in two successive years at the chemical level. The fruit revealed various responses in carbohydrates, organic acids and phenolics content levels. Nevertheless, it was shown that fruit from the control reached significantly higher content levels of malic acid and lower content levels of certain phenolics in the first year after bending, but in the next year the exactly opposite reaction occurred. Sorbitol, as well as citric acid, catechin and sinapic acid showed no clear tendency among treatments, neither in 2004 nor in 2005. It can be inferred that those variations of ‘Conference’ fruit among bending treatments could not be the result of bending alone, but that they could be indirectly affected by other physiological responses of the fruit tree. However, it seems that variations are affected by bending time and by the year-to-year variations, and such responses can be attributed to the ‘Conference’ genotype only.

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