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Influence of Nitrogen on the Contents of Carbohydrates and Organic Acids in Apples (*Malus domestica* Borkh.) cv. 'Golden Delicious'

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Summary

The aim of the experiment was to ascertain the dependence of the carbohydrates (sorbitol, sucrose, glucose and fructose) in fruits and leaves, organic acids in fruits, nitrogen content in leaves and leaf chlorophyll content according to the different rates of nitrogen supply. The analyses were conducted on 13-year-old cv. 'Golden Delicious' apple trees (*Malus domestica* Borkh.), which were either fertilized with 80 kg N ha^{-1} (N-80) or 200 kg N ha^{-1} (N-200) or not fertilized (N-0) as control. The leaf nitrogen content was significantly higher in the treatment N-200 than in the N-0 treatment. A seasonal decrease in leaf nitrogen content was noticed in all treatments. Leaf SPAD (indexed chlorophyll content) readings were increasing until the end of July and then stagnated. All over the season, the readings were significantly higher in treatment N-200 than in the N-0 treatment and from mid- June to end of July also higher than in the N-80 treatment. The nitrogen supply did not affect the content of single sugars nor did it affect total sugars in the leaves. The nitrogen fertilization had no effects the level of sorbitol in the fruits; increased N-supply resulted in lower contents of sucrose and fructose and higher contents of glucose in fruits. As for organic acids, the nitrogen supply caused higher concentrations of citric acid.

Key words. chlorophyll content – fructose – fruit – glucose – leaf – sorbitol – SPAD – sucrose

Introduction

Nitrogen (N) is a main nutrient which builds amino acids and is an important constituent of chlorophyll in plants. In the growing season, the plants' use of nitrogen depends on N availability in the soil, plant ability for N absorption and on the needs of growing and storage parts of the plant (Weinbaum et al. 2002). Higher nitrogen supply results in higher nitrogen contents in the plant. It is reflected in the greener ground colour and poorer red colour of the fruits but not in the soluble solids (Fallahi et al. 2001). Higher leaf and fruit nitrogen content results in reduced carbohydrates availability, delayed maturity and lower storage quality (Rease and Drake 1997). Dong et al. (2004) reported that higher N content affects the growth of leaves and shoots in the following growing season.

Up to 75 % of nitrogen in mesophyll cells can be located in the chloroplast (Peoples and Dalling 1988). Porro et al. (2001) reported that there is a significant link between the leaf N content and leaf chlorophyll. The chlorophyll content increased linearly with increasing leaf N content (Cheng 2003). In cv. 'Fuji', trees with less N had lower leaf photosynthesis than those with higher rates of N (Fallahi et al. 2001).

Nitrogen and carbon (C) metabolisms are interrelated. Carbon assimilation depends on the nitrogen metabolism to provide the photosynthetic machinery and N assimilation requires carbohydrate for the carbon skeleton and energy supply (Cheng and Fuchigami 2002). The authors showed that when tree N concentration increased, concentration of nonstructural carbohydrates decreased. Also Bi et al. (2004) found negative relationship between the total amino acids and the concentration of the total nonstructural carbohydrates. As much as 79 % C from nonstructural carbohydrates was found in free amino acids; the remaining (21 %) were used for respiration and other processes (Cheng et al. 2004).

Graham (2002) reported that many factors can affect carbohydrate production, storage, and loss in a tree. In cropping apple trees and modified biomass partitioning in the plants, fruits are strong sink of assimilates (Šturm and Štampar 1999; Veberic et al. 2002).

For apples, the sensory attributes of sweet and acid taste are important drivers of consumer preference (Harker et al. 2002). The sugar and acid content have a marked influence on the sensory quality of the fruit (Ackermann et al. 1992). The N supply affects the content of some sugars and amino acids, which have a certain influence on the taste and flavour of fruits. The highest correlations between sensory and objective measurements occurred for acid taste (Harker et al. 2002).

Some work has been done on nitrogen influence on carbohydrate content in vegetative parts of the plants, soluble solid in apple fruits, etc. In our experiment, we studied the influence of different N rations on sorbitol, glucose, sucrose and fructose in leaves and fruits, and on organic acids (malic, citric, fumaric and shikimic acid) in apple fruits during the growing season. During the experiment, we measured leaf N content, chlorophyll a and b contents and the SPAD (indexed chlorophyll content) values.

Materials and Methods

The measurements were carried out from May to September 2002 on 13-years-old 'Golden Delicious' apple trees grafted on M9 rootstock, grown in an experimental orchard in central Slovenia. In the middle of April, the N content in soil was defined by N-min method. No difference was shown between trees included in experiment. At the end of April, we added different rates of nitrogen:

N-0: 0 kg N ha^{-1} (control),
N-80:80 kg N ha^{-1},
N-200: 200 kg N ha^{-1}.

Each treatment encompassed four trees. On each of the trees all measurements and samplings were done.

Crop load of individual trees was similar and did not significantly differ between treatments.

Nitrogen content in leaves

The leaf samples were taken every two weeks from May 14 to September 11. For the N analysis, 10 leaves per tree were taken and were quickly brought into the laboratory. The leaves were washed with distilled water to remove the remaining pesticides, which could contain N. The foliar samples were dried (at 40 °C) to constant weight, grounded to powder and analysed for N concentration using modified Kjeldahl method.

The SPAD (indexed chlorophyll content) readings were taken with a chlorophyll meter SPAD-502 (Minolta Corporation, Japan) on three random chosen leaves of each tree without tearing the leaves off. The SPAD values were compared with spectrophotometrically determined chlorophyll in the leaves.

Spectrophotometric determination of the chlorophyll in the leaves

The part of the leaf (4 mm in diameter) where SPAD reading was measured was taken. The leaf discs were placed individually into the paper bags, labelled and kept on ice in a cooler, in which they were brought into the laboratory, and stored at −20 °C until the analyses for chlorophyll and carotenoids content were carried out. For the extraction of chlorophyll and carotenoids from fresh foliage dimethyl sulfoxide (DMSO) was used. In microcentrifuge tubes 0.5 ml DMSO was poured and one leaf disc added together with the crystals of magnesium hydroxide carbonate to assure the sufficient concentration of Mg^{2+} ions in the solution. The disc was mashed for better extraction and another 0.5 ml DMSO was added. The samples were left in the water bath (65 °C) in the dark for two hours. After the extraction, the samples were cooled down to room temperature and decanted into vessels. Immediately afterwards, the absorptions were measured on spectrophotometer (Lambda Bio 20, Perkin Elmer) at the wavelength of 480 nm (carotenoids), 649 nm (chlorophyll b) and 665 nm (chlorophyll a). Concentrations of single photosynthetic pigments (chlorophyll a and b, and carotenoids) in extracts were determined by using Wellburn (1994) equations.

Content of carbohydrates and organic acids in leaves and fruits

The fruit samples were taken in the period from June 19 to September 11. To perform sugar (sorbitol, sucrose, fructose and glucose) analyses in the leaves, ten leaves without petioles were taken from each tree at the same sampling dates as for the N analysis. They were lyophilised, ground to powder, and 1.0 g of each sample was poured with 80 ml bi-distilled water for the analyses.

The apple fruits were sampled from mid-June to the harvest time in September and analysed for carbohydrates (sorbitol, sucrose, glucose and fructose) and organic acids (malic, citric, fumaric and shikimic acids) contents. Every two weeks, three fruits were taken from each tree. In the laboratory, stalk, sepal and core were removed from the fruit and 10 g of the fresh mass poured over with 80 ml bi-distilled water and homogenized with Ultra-Turrax T-25 (Ika-Labortechnik).

The leaf and fruit samples were left for extraction for 30 min at room temperature, meanwhile frequently mixed. Afterwards the samples were centrifuged at 4200 rpm for 7 min. The supernatants were filtered through 0.45 μm filter (Macherey-Nagel), poured into vials and analysed according to method described by Dolenc and Štampar (1997) using HPLC (High Performance Liquid Chromatography). For each analysis of sugars and organic acids 20 μl of sample were used. Sugars were analysed in the column Aminex − HPX 87C with flow of 0.6 ml min^{-1} and at 85 °C. For the mobile phase bi-distilled water was used and a RI detector for identification. Organic acids were analysed in the column Aminex − HPX 87H with flow of 0.6 ml min^{-1} and at 65 °C. For the mobile phase 4 mM sulphuric acid (H_2SO_4) was used together with UV detector with wavelength at 210 nm for identification. The concentrations of carbohydrates and amino acids were calculated with the help of a corresponding external standard.

Statistic evaluation

The results were statistically analysed with the Statgraphics plus 4.0 program using the analysis of variance (ANOVA). Differences between the treatments were estimated with a multiple range test using least significant difference (LSD) or Duncan test at $\alpha<0.05$. Simple linear regression was used to determinate the relationship between SPAD readings and the chlorophyll content.

Results and Discussion

Nitrogen content in the leaves

At each sampling, the nitrogen content was significantly lower in the N-0 treatment compared to the N-200 treatment, and until the end of June also lower than in the N-80 treatment (Table 1). Also Klein (2002) in cv. 'Golden Delicious' and 'Granny Smith', Fallahi et al. (2001) in 'Fuji', and Neilsen et al. (1995) in 'Fuji', 'Spartan', 'Fiesta' and 'Gala' reported that higher N supply results in higher N content in the leaves.

In all treatments, the nitrogen concentration in the leaves was lower at harvest than at the first sampling. Aichner and Stimpfl (2002) established that the nitrogen content in the leaves slightly decreased from the second week after full bloom until harvest. Also Neilsen et al. (1995) noticed out in four different apple cultivars that the nitrogen content in the leaves decreased during the growing season.

Table 1. Nitrogen content and concentrations of total sugars (average ± standard error) in leaves of apple 'Golden Delicious', fertilized with different rations of nitrogen. N-0 = non-fertilized trees, N-80 = fertilized with 80 kg N ha^{-1}; N-200 = fertilized with 200 kg N ha^{-1}. Values in a horizontal row followed by a different letter are significantly different at $\alpha<0.05$ by LSD multiple range test.

Date	Nitrogen fertilization N-0	N-80	N-200
	Nitrogen content (% DW)		
14.05.02	2.16 ± 0.05 a	2.19 ± 0.05 a	2.71 ± 0.14 b
21.05.02	2.27 ± 0.02 a	2.36 ± 0.10 a	2.68 ± 0.12 b
29.05.02	2.15 ± 0.07 a	2.19 ± 0.09 a	2.55 ± 0.09 b
17.06.02	2.12 ± 0.08 a	2.32 ± 0.09 a	2.68 ± 0.11 b
01.07.02	2.20 ± 0.05 a	2.35 ± 0.07 ab	2.56 ± 0.07 b
15.07.02	2.28 ± 0.04 a	2.44 ± 0.13 ab	2.71 ± 0.09 b
29.07.02	2.18 ± 0.05	2.45 ± 0.19	2.47 ± 0.07
13.08.02	2.16 ± 0.03 a	2.30 ± 0.06 ab	2.45 ± 0.08 b
29.08.02	2.05 ± 0.10 a	2.29 ± 0.07 ab	2.40 ± 0.06 b
11.09.02	2.02 ± 0.05 a	2.15 ± 0.07 ab	2.35 ± 0.13 b
	Concentrations of total sugars (g kg^{-1} DW)		
14.05.02	193.0 ± 7.3	178.6 ± 7.8	181.1 ± 10.8
21.05.02	173.9 ± 2.8	169.3 ± 13.0	171.4 ± 6.5
29.05.02	176.0 ± 5.3	181.5 ± 3.5	179.2 ± 3.3
17.06.02	159.8 ± 15.4	194.7 ± 10.7	193.7 ± 11.5
01.07.02	166.5 ± 2.8	169.0 ± 3.7	169.2 ± 3.1
15.07.02	158.6 ± 2.4	149.8 ± 4.2	155.5 ± 5.7
29.07.02	131.9 ± 2.2	134.0 ± 7.1	138.3 ± 6.5
13.08.02	129.8 ± 5.4	138.6 ± 4.0	133.6 ± 2.6
29.08.02	132.8 ± 7.0 ab	157.3 ± 5.5 b	120.9 ± 11.7 a
11.09.02	126.5 ± 5.2	138.2 ± 3.4	126.1 ± 14.2

DW: dry weight

According to the literature, there are different optimal concentrations of nitrogen in the leaves. Fallahi et al. (2001) recommended 2.3 % of dry weight (DW) as optimal concentration of N in leaves of 'Fuji' apple, Klein (2002) in 'Golden Delicious' and 'Granny Smith' 2.4 % of DW, and Faust (1989) in apple tree 2.0 % of DW. The results of our research were compared with the results by Aichner (2002), where optimal concentrations in the period 20 days after full bloom in 'Golden Delicious' apple were 2.55–3.00 %, and in July and August 2.30–2.60 % of DW. In our experiment, the optimal values on the first sampling date were only achieved in the N-200 treatment (Table 1), while in July and August the optimal values were achieved in both N-80 and N-200 treatments and only once (July 15) the values were higher in the N-200 treatment. Due to the fact that the optimal nitrogen concentrations in the leaves in July and August were achieved in the N-80 treatment, we concluded that 80 kg nitrogen per ha was sufficiently effective. The N concentrations at the control trees were slightly deficient. Moreover, Neilsen et al. (1995) did not find big differences in nitrogen content in the leaves of trees, which were low or high N supplied. This means that the leaf nutrient content does not depend only on the nitrogen supply, but also on the environmental factors.

Chlorophyll content in the leaves

The SPAD values are correlated with the nitrogen supply (Porro et al. 2001). In all sampling periods, the values were significantly higher in the N-200 treatments than in the control treatments, and from Mid-June to the end of July the values were also higher than in the N-80 treatment (Table 2). The SPAD values in all treatments were increasing until the end of July, and stagnating afterwards. Neilsen et al. (1995) got similar results in the experiment on four different apple cultivars. The authors reported that the increasing SPAD values do not depend only on nitrogen fertilization but also on leaf thickness, which increases during the growing season; the SPAD values and chlorophyll content in the leaves were higher when thickness of leaves increased.

The content of photosynthetic pigments in the leaves was significantly higher in the N-200 treatment than in other treatments in the most of sampling periods (Table 2). The concentrations were also higher in the N-80 treatment than at the control trees, but the differences were not significant. We can conclude that higher N supply probably results in a higher content of photosynthetic pigments in the leaves. Cheng (2003) established that higher nitrogen supply resulted in higher chlorophyll content in leaves.

Table 2. SPAD values and concentrations of chlorophyll a and b (average ± standard error) in leaves of apple 'Golden Delicious', fertilized with different rations of nitrogen. N-0 = non-fertilized trees, N-80 = fertilized with 80 kg N ha^{-1}; N-200 = fertilized with 200 kg N ha^{-1}. Values in a horizontal row followed by a different letter are significantly different at $\alpha<0.05$ by LSD multiple range test.

Date	Nitrogen fertilization N-0	N-80	N-200
	SPAD values		
14.05.02	29.6 ± 1.1	31.0 ± 1.3	33.0 ± 1.4
21.05.02	32.7 ± 1.4 a	34.6 ± 0.8 a	39.1 ± 0.9 b
29.05.02	37.8 ± 0.8 a	40.1 ± 1.1 ab	42.7 ± 1.1 b
17.06.02	36.4 ± 1.1 a	40.5 ± 0.8 b	43.0 ± 0.7 b
01.07.02	40.5 ± 0.8 a	43.6 ± 0.9 b	47.3 ± 0.8 c
15.07.02	42.6 ± 0.7 a	46.4 ± 0.7 b	50.4 ± 1.0 c
29.07.02	44.4 ± 1.0 a	47.3 ± 1.1 b	50.5 ± 0.7 c
13.08.02	43.1 ± 0.6 a	48.2 ± 1.0 b	52.4 ± 1.4 c
29.08.02	44.7 ± 1.5 a	48.5 ± 1.2 ab	51.8 ± 1.4 b
11.09.02	45.5 ± 0.9 a	48.3 ± 1.4 a	53.0 ± 1.1 b
	Concentrations of chlorophyll a (μg mm^{-2})		
14.05.02	0.32 ± 0.01	0.36 ± 0.03	0.36 ± 0.02
21.05.02	0.38 ± 0.03 a	0.37 ± 0.02 a	0.48 ± 0.02 b
29.05.02	0.44 ± 0.02 a	0.47 ± 0.02 ab	0.53 ± 0.02 b
17.06.02	0.50 ± 0.02 a	0.53 ± 0.02 a	0.58 ± 0.03 b
01.07.02	0.55 ± 0.03 a	0.53 ± 0.04 a	0.72 ± 0.04 b
15.07.02	0.53 ± 0.03 a	0.58 ± 0.04 a	0.68 ± 0.04 b
29.07.02	0.58 ± 0.03 a	0.67 ± 0.03 ab	0.77 ± 0.03 b
13.08.02	0.50 ± 0.02 a	0.58 ± 0.04 a	0.71 ± 0.03 b
29.08.02	0.54 ± 0.03 a	0.64 ± 0.03 ab	0.69 ± 0.04 b
11.09.02	0.56 ± 0.04 a	0.65 ± 0.03 ab	0.68 ± 0.03 b
	Concentrations of chlorophyll b (μg mm^{-2})		
14.05.02	0.117 ± 0.005 a	0.149 ± 0.013 ab	0.137 ± 0.006 b
21.05.02	0.131 ± 0.008 a	0.130 ± 0.007 a	0.168 ± 0.006 b
29.05.02	0.150 ± 0.006 a	0.163 ± 0.009 a	0.186 ± 0.007 b
17.06.02	0.208 ± 0.012 a	0.204 ± 0.007 a	0.238 ± 0.018 b
01.07.02	0.203 ± 0.013 a	0.222 ± 0.015 ab	0.251 ± 0.012 b
15.07.02	0.164 ± 0.008 a	0.209 ± 0.015 b	0.235 ± 0.014 b
29.07.02	0.189 ± 0.014 a	0.213 ± 0.021 ab	0.244 ± 0.012 b
13.08.02	0.189 ± 0.008 a	0.203 ± 0.014 a	0.244 ± 0.014 b
29.08.02	0.192 ± 0.008 a	0.230 ± 0.013 b	0.242 ± 0.016 b
11.09.02	0.187 ± 0.011 a	0.230 ± 0.011 b	0.236 ± 0.013 b

The chlorophyll a content in the leaves was increasing until mid-July and then slightly decreasing until harvest in all treatments (Table 2). From the second sampling period on, the concentrations were significantly higher in the N-200 treatment than in the control samples and sometimes also higher than in the N-80 treatment. The chlorophyll b content was increasing until mid-June in all treatments and stayed relatively unchanged until the last sampling (Table 2). In the N-200 treatment, the concentrations of chlorophyll b were significantly higher than in the control samples.

The interrelation between total concentration of photosynthetic pigments in the leaves and SPAD values was significant. The strongest correlation (R^2 = 86.66 %) was found between the SPAD values and chlorophyll b, a little weaker correlation (R^2 = 80.47 %) was measured between the SPAD and chlorophyll a, and the weakest correlation (R^2 = 77.85 %) was between the SPAD values and carotenoids (data not shown). Some other authors also described positive correlation between the chlorophyll content and SPAD values (Van den Berg et al. 2004).

Carbohydrate content in the leaves

Sugar alcohol sorbitol, which is present in larger amount in the leaves (Berüter 1985), is the primary photosyn-

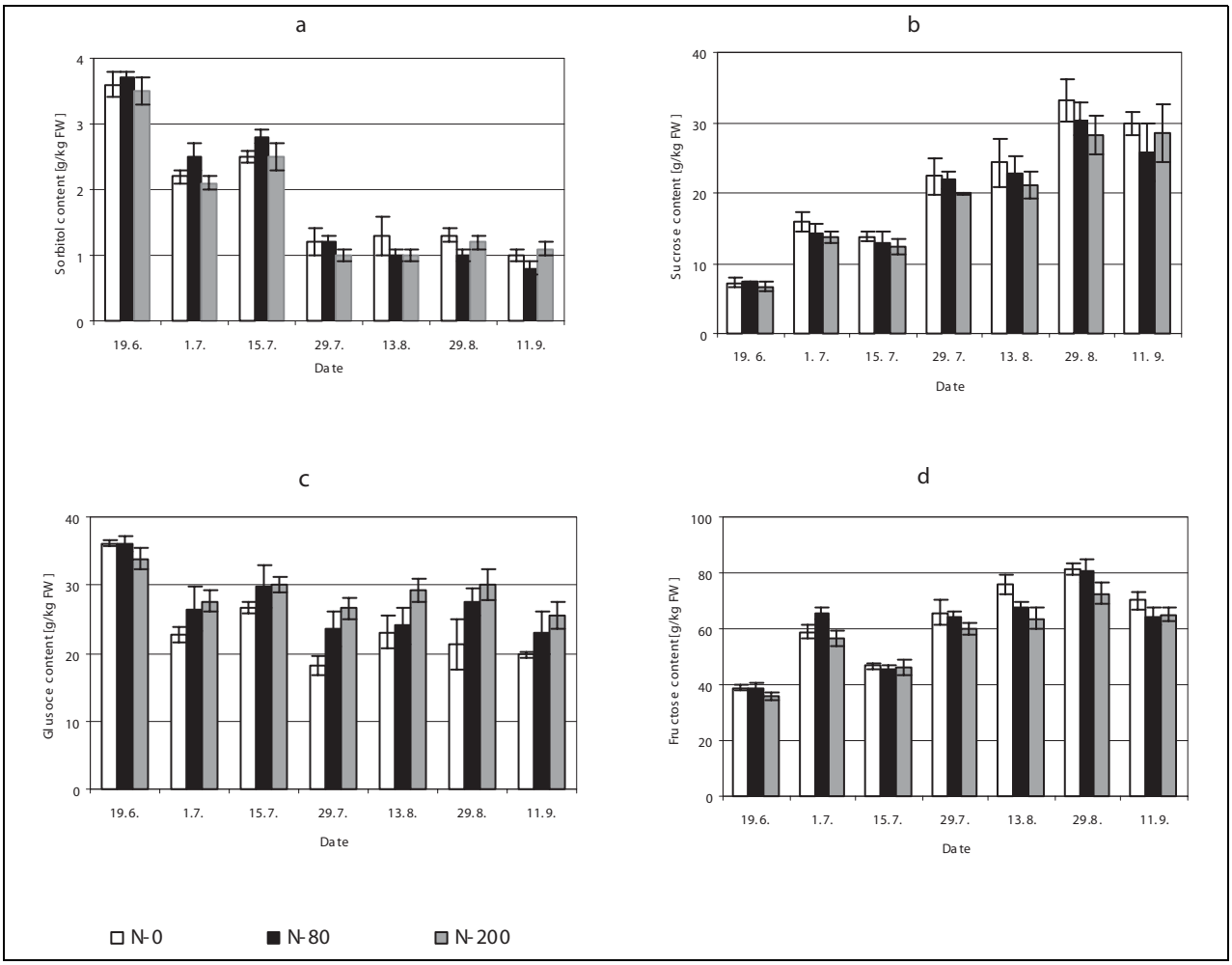


Fig. 1. Content of sorbitol (a), sucrose (b), glucose (c) and fructose (d) in fruits of different rations N fertilized apple trees. N-0 = non-fertilized trees, N-80 = fertilized with 80 kg N ha^{-1}; N-200 = fertilized with 200 kg N ha^{-1}. The vertical bars indicate standard errors.

thetic product in many species in the *Rosaceae*, including genera *Malus* (apples), and accounts for 60 to 90 % of the carbon exported from the leaf (Loescher 1987). In the research we performed, sorbitol was also present in the highest concentrations. The glucose and sucrose contents were lower, and fructose was present in the lowest concentrations. In our study, the contents of sorbitol, sucrose, glucose, fructose (data not shown) and total sugars (Table 1) in leaves were not statistically different between treatments. Cheng et al. (2004) found similar results in the case of sorbitol content, but not in the contents of other sugars in the leaves. Total sugar content in the leaves was decreasing during the growing season and slightly increased again in September in all treatments. Veberic et al. (2003) noted similar results in their study.

Carbohydrate content in the apple fruits

Sorbitol is the main translocate entering the apple fruit (Berüter and Studer Feusi 1997) and is the major substrate for glucose accumulation during the sugar storage phase (Berüter 1985). The sorbitol content was the lowest in the N-200 treatment until mid-August, and at later samplings higher only when compared to the N-80 treatment. The differences between the treatments were not significant. The concentration of sorbitol in fruits decreased from mid-June to the end of July (Fig. 1a). Similar sharp decline of fruit sorbitol in the same period was observed by Veberic et al. (2003) in cv. 'Golden Delicious' and by Šturm and Štampar (1999) in cv. 'Idared'. The concentration was then increasing again until September, what was observed also by Berüter (1985). The author describes it as a consequence of enzyme sorbitol dehydrogenase activity, which transforms sorbitol to fructose.

Sucrose is the second carbohydrate translocated from the apple leaf into fruit (Veberic et al. 2003). The concentrations of sucrose were the highest in the control samples and the lowest in the N-200 treatment during the growing season, but differences were not significant. The sucrose content was increasing until the end of August, and then stagnating (Fig. 1b). Sucrose accumulation in the apple fruits has been described by other authors as well (Šturm and Štampar 1999; Veberic et al. 2003). Berüter (1985) explained the accumulation of sucrose towards the end of the growing season with decreasing activity of the enzyme acid invertase.

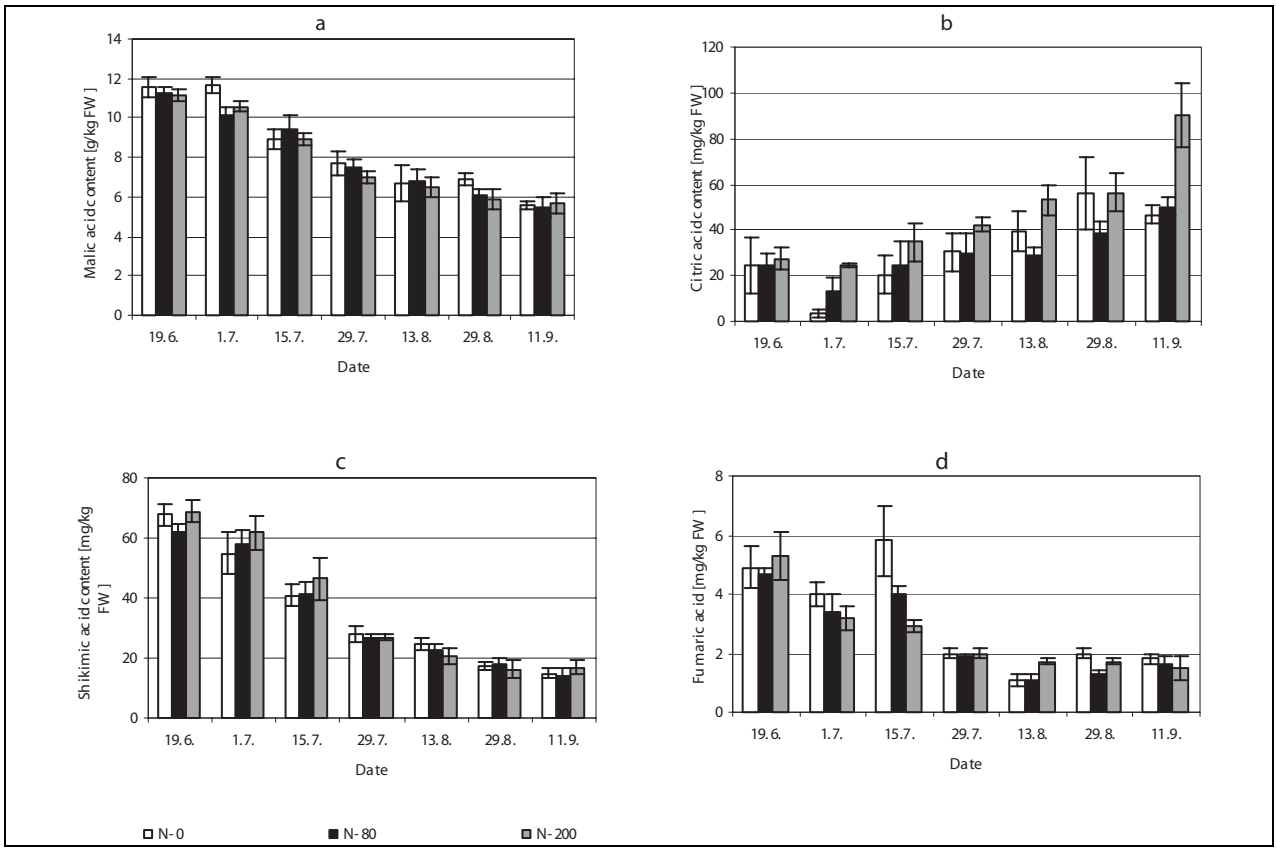


Fig. 2. Content of malic (a), citric (b), shikimic (c) and fumaric acid (d) in fruits of different rations N fertilized apple trees. N-0 = non-fertilized trees, N-80 = fertilized with 80 kg N ha^{-1}; N-200 = fertilized with 200 kg N ha^{-1}. The vertical bars indicate standard errors.

The glucose content in growing fruits was the highest at the first sampling date and decreased a bit during the summer. Similar results were described by ŠTURM and ŠTAMPAR (1999). The concentrations from the second sampling period on were the highest in the N-200 treatment and the lowest in the control trees samples. The differences were not significant, except for the lowest values at the end of July (Fig. 1c). BERÜTER (1985) explains a decrease in glucose in July with the accumulation of starch, which is the most intensive at that time.

The most prominent sugar in the apple fruits is fructose (ACKERMANN et al. 1992). The concentrations of fructose were increasing in the growing fruits until the beginning of July and were significantly higher in the N-80 treatment than in the lowest concentrations in the N-200 treatment (Fig. 1d). The fructose content slightly decreased during the following weeks, and then increased again until the end of August. At mid-August, the concentrations were significantly lower in the N-200 treatment than in the N-0 treatment. Before harvest, the fructose content in the apple fruits decreased a bit (Fig. 1d), what was noted by ACKERMANN et al. (1992) as well. Lower fructose content was found when sorbitol content was higher and vice versa. Similar results were measured in some other studies as well (VEBERIC et al. 2003; ŠTURM and ŠTAMPAR 1999). During the growth of apple fruit, steady accumulation of fructose occurs and is closely connected with activity of enzyme sorbitol dehydrogenase (BERÜTER 1985).

Organic acids in the fruits

The content of organic acids in the apple fruits depends on the stage of fruit maturity, plant genotype, tree growing conditions, climatic factors and growing technologies (ŠTURM and ŠTAMPAR 1999; ŠTURM et al. 2003).

We ascertained the content of malic, citric, fumaric and shikimic acid in the apple fruit. Malic acid is the principal metabolic substrate together with the sugars (ACKERMANN et al. 1992). Malic acid is present in the apple fruits in higher concentrations than other organic acids and decreases during the fruit growth and ripening. In our study, the concentrations decreased from 11 g kg^{-1} FW (fresh weight) to about 4 g kg^{-1} FW at harvest time (Fig. 2a). An influence of different nitrogen amounts was not detected in our study, except in the second sampling period when the concentrations were significantly higher in the samples of the control trees than in the N-200 treatment.

In the apple fruits, the content of the citric acid was much lower compared to the malic acid. In the first sampling period, the concentrations were about 24 mg kg^{-1} FW (Fig. 2b). The concentrations were decreasing until the beginning of July and then increasing until harvest. ŠTURM and ŠTAMPAR (1999) reported that the dynamics of citric acid is similar to dynamics of malic acid; this was not confirmed in our study. The concentrations were higher in the N-200 treatment; they were significant at the beginning of July, mid-August and mid-September.

Shikimic and fumaric acids were found in lower quantities in apple fruits. The contents of both acids decreased during the growing season (Fig. 2c and 2d), what is also reported by ŠTURM and ŠTAMPAR (1999). In general, nitrogen influence on shikimic acid in apple fruit was not seen in our study, meanwhile influence on fumaric acid was found at samplings in mid-July and in the second half of August.

The concentration of titratable acid was sometimes the highest in the fruits from low nitrogen plots and decomposition of the acids seemed to progress slower in low nitrogen than in high nitrogen fruits (LINK 1980). In our experiment, the nitrogen influence on malic acid, which is the main constituent of titratable acid in apple fruit, was not indicated. The influence of nitrogen was the most perceivable at citric acid, but it is present in apple fruits in very low quantities. Thus, the effect of nitrogen on total organic acids was not proved.

Conclusions

The higher nitrogen supply resulted in higher concentrations of leaf N content, in higher SPAD values and in higher concentrations of photosynthetic pigments (chlorophyll a and b). The correlations between the SPAD values and chlorophyll a and between the SPAD values and chlorophyll b were determined. Total sugars in leaves were not dependent on different nitrogen supplies. In fruits, higher nitrogen supply resulted in lower sucrose content until the end of August, in lower fructose content in all sampling periods, and in higher glucose content from the beginning of July to harvest. Also concentrations of citric acid were higher when nitrogen supply was increasing. Whereas, the sorbitol contents did not depend on nitrogen supply, nor did the contents of malic and shikimic acid. Fertilization with 80 kg N ha^{-1}, which is used in integrated production, compared to control did not result in lower contents of sugars in fruits, meanwhile 200 kg N ha^{-1} generally resulted in decreasing concentrations (except glucose), which could result in lower fruit quality.

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