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# Tree vigor, yield, fruit quality, and antioxidant capacity of apple (*Malus* × *domestica* Borkh.) influenced by different fertilization regimes: preliminary results

**Tomo MILOŠEVIĆ[1,*], Nebojša MILOŠEVIĆ[2], Jelena MLADENOVIĆ[3]**

[1]Department of Fruit Growing and Viticulture, Faculty of Agronomy, University of Kragujevac, Čačak, Serbia
[2]Department of Pomology and Fruit Breeding, Fruit Research Institute Čačak, Čačak, Serbia
[3]Department of Chemistry and Chemical Engineering, Faculty of Agronomy, University of Kragujevac, Čačak, Serbia

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**Abstract:** Tree growth, productivity, fruit quality attributes, and antioxidant activity were tested in apple cultivar Idared under different fertilization treatments from the 22nd to the 23rd year after planting in a heavy acidic soil under western Serbian conditions. Treatments included the use of aged cattle manure, compound NPK (15:15:15) mineral fertilizer, a multinutrient fertilizer commercially named Italpolina (ITP) (NPK + organic carbon + humic acids), two types of N mineral fertilizers (calcium ammonium nitrate [CAN] and urea), and control (no fertilization). Results showed that Idared trees were more vigorous when fertilized with the two N fertilizers than when treated with another mineral, organic, or multinutrient fertilizer. Urea promoted the highest yield per tree and unit area, while yield efficiency was the highest in the control and those under treatment with urea, compound NPK, and manure. Fertilizer regimes had a significant effect on fruit weight and flesh firmness, but their effects on fruit size and dimension ratio were not significant. Compound NPK resulted in the greatest fruit weight, whereas firmness and soluble solids content were the highest in manure treatment. Titratable acidity (TA) was the highest in control trees and after CAN application, while urea induced the highest ripening index. Fertilizer treatments had no significant effect on the contents of invert sugars, sucrose, and total sugars (TS). The TS/TA ratio was highest under urea treatment and lowest in the control and under CAN application. ITP led to the highest values for total phenolics, total nonflavonoids, and total antioxidant capacity, while the highest total flavonoid content was obtained with compound NPK.

**Key words:** Apple fruit size, fertilization, phenolic compounds, productivity, sugars, vegetative growth

## 1. Introduction

Apples are the most important and most popular fruit crop in temperate climate zones, and their production is increasing. The apple is a convenient fruit available in retail throughout the world all year as a result of its high storability. Its beautiful appearance, crispy flesh, pleasant flavor, and sweetness attract consumers and fetch a high price (Asif Ali et al., 2004). Appearance, fruit size, uniformity, color, and freshness, as well as nonvisual attributes such as taste, aroma, flavor, firmness (texture), nutritional value, and health benefits, are components that determine the attractiveness of fruit to consumers (Nour et al., 2010). Firmness and aroma appear to be the most important factors for consumers. Some phytochemicals such as sugars, organic acids, and phenolic compounds contribute to the aroma of apples (Mikulič Petkovšek et al., 2009).

Intensive apple production is based on the use of a number of cultural techniques such as training, pruning, thinning, soil management, disease and pest management, weed control, irrigation, and fertilization (Milošević and Milošević, 2017). In the past few decades, intensive apple production worldwide has primarily focused on increasing productivity through intensification of the use of fertilizers and water, resulting in high production and environmental costs (Stefanelli et al., 2010). Therefore, fertilizers are an important tool for boosting apple yield and external and internal fruit quality attributes. However, excessive fertilization has been confirmed, especially in horticultural farming, with fertilizer cost accounting for almost 10% of variable costs (Huett and Dirou, 2000). In addition to the financial aspect, excessive fertilization has been associated with the contamination of soils and waters, as well as with increased pest and disease incidence (Marschner, 1995). Today the aim is to secure a profitable, internationally competitive food and farming sector that respects the environment and improves nutrition and public health (Traill et al., 2008). In sustainable agriculture, judicious use of fertilizers is an important issue.

* Correspondence: tomomilosevic@kg.ac.rs

A comparative fertilization trial was conducted in the Čačak region (western part of Serbia) to evaluate the impact of varying mineral, organomineral (multinutrient), and organic inputs on cultivar Idared budded on MM 106 rootstock and grown in a heavy acidic soil. The results place particular emphasis on tree growth habit/productivity and fruit quality attributes towards the obtainment of safe, healthy, nutritious fruits in economically justified, sustainable apple production.

## 2. Materials and methods

### 2.1. Plant material and orchard management

The field experiment was carried out in a privately owned apple orchard located in the village of Prislonica (43°53′N, 20°21′E; 340 m a.s.l.) near the town of Čačak (western Serbia) (Figures 1A and 1B). Apple cultivar Idared grafted onto MM 106 rootstock was used. The orchard was established in the spring of 1994. Planting distance was 3.0 m × 1.6 m or 2083 trees $ha^{-1}$. Trees were trained to a vertical axis system. Tree vigor was controlled by summer pruning. Standard cultural practices were used (hand thinning, soil management, pest and disease management, weed control) without irrigation due to the unavailability of a water supply source. Orchard floor management involved grass alleyways and 1-m-wide herbicide strips in the tree rows. Glyphosate as a total systemic herbicide was used for weed control.

The experimental procedure included soil fertilization with urea containing 46% N total ($N_{TOT}$) (0.015 kg $m^{-2}$), CAN with 27% $N_{TOT}$ (0.03 kg $m^{-2}$), compound NPK as a 15:15:15 (0.05 kg $m^{-2}$) mineral fertilizer, ITP multinutrient fertilizer (0.04 kg $m^{-2}$), and aged cattle manure consisting of 0.5% $N_{TOT}$, 0.3% Al-$P_2O_5$, 0.6% Al-$K_2O$, and 25% organic matter on a dry weight basis (5 kg $m^{-2}$) (Milošević et al., 2013; Milošević and Milošević, 2017). The control treatment received no fertilization. ITP is a granular pH-neutral multinutrient fertilizer comprising NPK + organic carbon + humic acids and containing 12% $N_{TOT}$, 2% N-$NH_4$, 7% N-$NH_2$, 3% organic N, 5% available Al-$P_2O_5$, 15% available Al-$K_2O$, 21% organic carbon, and humic acid of high biological value. The high levels of active and humidified ingredients provided a short-term improvement in the microbiological, physical, and chemical properties of the soil and increased its buffering capacity. The organic matter prevented N, P, K, and microelements from being washed out of the soil (manufacturer's instructions).

Aged cattle manure, compound NPK, and ITP were added to the soil in late autumn in both 2015 and 2016, whereas CAN and urea were applied in early spring, i.e. before the start of the growing seasons in 2016 and 2017. Fertilizer treatments were conducted in a randomized complete block design with 6 trees per cultivar-fertilizer combination in 4 replicates (n = 24). A total of 144 trees were included in the trial alongside the untreated control. Investigations were carried out in 2016 and 2017.

### 2.2. Soil characteristics and weather conditions

Soil chemical analyses were conducted prior to the experiment, i.e. in November 2015. The soil had a sandy-clay texture and a low pH in nKCl (4.92); it contained 1.91% organic matter, 0.17% $N_{TOT}$, 5.43 mg 100 $g^{-1}$ available Al-$P_2O_5$, 23.96 mg 100 $g^{-1}$ available Al–$K_2O$, and 0% $CaCO_3$. Results revealed that the soil had an unfavorable pH value, a good supply of organic matter and available Al-$P_2O_5$ and Al-$K_2O$, and moderate levels of $N_{TOT}$. No chlorosis was observed, as lime was not found in the soil.

Weather data during the experimental period were consistent with the long-term averages and were characterized by the mean annual temperature of 11.3 °C and total annual rainfall of 690.2 mm. The air temperature


**Figure 1.** The experimental site at Prislonica, Čačak (Serbia), before flowering (A) and at full flowering (B).

during the growing season was 17.0 °C on average. The absence of rainfall in July and the first half of August in 2017 had a potentially adverse effect on the traits evaluated.

### 2.3. Measurements

During the research period, tree growth, yield attributes, and physical and chemical fruit properties were monitored.

#### 2.3.1. Tree growth and productivity

For tree growth, trunk circumference at 20 cm above ground level was measured at the end of the growing season using a Starrett 727 digital caliper gauge (Athol, USA) and converted into trunk cross-sectional area (TCSA, $cm^2$). The final TCSA obtained in 2017 was analyzed. An ACS electronic scale (Zhejiang, China) was used to measure yield per tree in each year (kg). Yield per unit area (t $ha^{-1}$) was calculated. Yield efficiency (YE) was calculated as the ratio of average yield per tree to final TCSA (kg $cm^{-2}$).

#### 2.3.2. Physical properties of the fruit

Measurements of the physical properties of fruits were made immediately after harvest. Samples of 25 fruits per fertilizer treatment in 4 replicates (n = 100) were hand-harvested at random for the experiment. The iodine test and the Streif index (Streif, 1983) were used to estimate an optimum harvest time for the apple cultivar. Fruit weight (g) was measured with a MAULsteel 5000 G digital balance (Jakob Maul GmbH, Germany). Fruit dimensions for oblate spheroid Idared fruits (length [L] and diameter [D], both in mm) were measured using a Starrett 727 digital caliper gauge (Athol, USA). Length/diameter ratio (L/D) was calculated. Flesh firmness was measured with a Bertuzzi FT-327 hand penetrometer (Facchini, Italy) with a scale in kg $cm^{-2}$ and with a piston diameter of 10 mm.

#### 2.3.3. Chemical properties of the fruit

For the fruit chemical analysis performed in 2016 and 2017 two months after storage in NA (normal atmosphere) at 2 °C and 90% relative humidity, samples of 30 fruits per fertilizer treatment were collected and divided into 3 subsamples, each consisting of 3 whole ripe fruits. They were chopped and squeezed using a Nutri Power BN1000W commercial blender (Gorenje, Slovenia). The extracted juices (3 subsamples per fertilizer treatment) were filtered under vacuum and centrifuged at 15,000 rpm for 20 min, and the apple juice filtrate was used for the chemical analysis.

##### 2.3.3.1. Determination of primary metabolites

The soluble solids content (SSC, °Brix) was determined with a Carl Zeiss 32-G hand refractometer (Carl Zeiss, Germany) at 20 °C. Titratable acidity (TA, % of malic acid) was determined in 30 mL of the filtrate by titration with 0.1 mol $L^{-1}$ NaOH up to pH 8.1 using a Metrohm 719S titrimeter (Titrino, Switzerland). The ripening index (RI) was calculated as the ratio of SSC/TA.

Total sugars (TS), invert sugars, and sucrose were measured by the Luff–Schoorl method proposed by the AOAC (1995). Results were expressed as % of fresh weight (FW). The sweetness index was calculated as the TS/TA ratio.

##### 2.3.3.2. Determination of secondary metabolites

The apple juice filtrate was used to determine the total phenolic content (TPC), total flavonoid content (TFC), total nonflavonoids (TNFs), and total antioxidant capacity (TAC). The extracted TPC was determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965). TFC was estimated by the $AlCl_3$ colorimetric method proposed by Lamaison and Carnat (1990). TNFs were calculated as the difference between TPC and TFC. The TPC, TFC, and TNFs were expressed as mg gallic acid equivalents per 100 g of dry weight (DW) (mg GAE 100 $g^{-1}$ DW) of edible apple. TAC was evaluated by the phosphomolybdenum method described by Prieto et al. (1999). Ascorbic acid (AA) was used as a standard, and TAC was expressed as mg of AA per g of DW (mg AA $g^{-1}$ DW).

Spectrophotometric measurements for TPC, TFC, and TAC were performed using a MA9523-SPEKOL 211 UV–vis spectrophotometer (Iskra, Slovenia). Data for TPC, TFC, and TAC were measured in triplicate and expressed as the mean ± standard error (SE).

### 2.4. Statistical evaluation

All data, except TCSA and YE, are average values for both 2016 and 2017, as differences between years were minor and nonsignificant. Differences between data were separately determined by one-way analysis of variance (ANOVA) using the Microsoft Office Excel software package (Microsoft Corporation, Redmond, WA, USA). The means were compared with the LSD test at $P \leq 0.05$.

## 3. Results and discussion

### 3.1. Tree growth habit and productivity

The present study clearly indicates that factors such as organic, organomineral, and mineral fertilizers may affect tree growth and productivity-related properties of apple cultivar Idared (Table 1). The TCSA values for all combinations of fertilizers applied in 2017 were on average only 4% higher than at the beginning of the trial in 2016 (data not shown). Treatment with CAN and urea produced similar results and more vigorous Idared trees than the control, whereas no significant difference was found among the other fertilizers and control. These results were expected, given that both of these fertilizers contained higher amounts of N compared to the other fertilizers and thus improved tree growth (Hill-Cottingham and Williams, 1967; Pole et al., 2017), which is in accordance with the physiological role of N. Differences between the effect of CAN (27% N) and urea (46% N) on tree growth were random. These data are in agreement with the results of Wrona (2004, 2011), who reported that neither N application rate nor N fertilization mode had any effect

**Table 1.** Influence of different fertilizers on tree vigor and yield attributes of apple cultivar Idared.

| Fertilizer | Final TCSA ($cm^2$) Year 2017 | Yield per tree (kg) Year 2016/2017 | Yield per hectare (t) Year 2016/2017 | Yield efficiency ($kg\ cm^{-2}$) Year 2017 |
|---|---|---|---|---|
| CAN | 103.47 ± 3.12 a | 15.21 ± 0.25 cd | 42.26 ± 0.68 cd | 0.150 ± 0.00 b |
| NPK | 91.95 ± 4.29 b | 15.47 ± 0.50 cd | 42.98 ± 1.39 cd | 0.177 ± 0.01 a |
| Urea | 104.56 ± 2.51 a | 18.95 ± 0.29 a | 52.64 ± 0.82 a | 0.183 ± 0.00 a |
| Italpolina | 95.19 ± 2.35 b | 14.84 ± 0.29 d | 41.22 ± 0.89 d | 0.162 ± 0.00 b |
| Manure | 91.86 ± 2.28 b | 16.53 ± 0.28 b | 45.91 ± 0.78 b | 0.182 ± 0.00 a |
| Control | 91.66 ± 2.23 b | 15.78 ± 0.24 bc | 43.85 ± 0.68 bc | 0.176 ± 0.00 a |

Values followed by different letters in the same column are significantly different (LSD test).

on apple tree growth in soil rich in humus. Nitrogen application rate and N fertilization mode were associated with the soil organic matter because of it being closely correlated with the N content. It is well known that soil N is transformed into a plant-available form by organic matter mineralization (Ernani et al., 2008). Herbicide strips were additionally enriched by organic matter with grass mown in alleyways (Wrona and Sadowski, 1999). Fallahi et al. (2010) recommended 250 kg N $ha^{-1}$; however, in this trial, the soil contained only 0.5% organic matter. Some other authors recommended higher rates of N in nutrient-poor soils having very low levels of organic matter (Teravest et al., 2010), but high N application rates in poor soils may be associated with N loss through leaching. Importantly, nitrogen fertilizer rates should be strictly dependent on the soil organic matter content, as well as on the tree nutrient status and the rootstock used (Milošević et al., 2013, 2017; Pole et al., 2017). In the present study, the soil contained 1.91% organic matter (optimal level for apple ranged from 1.74 to 2.91; authors' observation), and the rootstock used was MM 106, which is less susceptible to competition for nutrients and water than dwarf rootstocks such as M 9 and/or M 26 due to a more extensive tree root system developing on it (Scudellari et al., 1993).

Urea applied to the soil led to the highest yield per tree and per hectare, whereas the lowest yield was obtained under ITP treatment. The effect of manure on yield was also different from urea and ITP effects but was similar to the control. Treatments with CAN and NPK produced similar effects with no significant differences when compared to the control. Scudellari et al. (1993) found that N fertilization improved the yield of cultivar Heavy Stripe, but its effect on the yield of Hi Early was not significant, indicating cultivar variation (Milošević and Milošević, 2015). In other studies, neither N application rate nor N fertilization mode had any effect on apple yield (Wrona, 2004; Ernani et al., 2008). As opposed to previous studies and similar to our results, apple yield increased after successive N fertilization over several years under southern Brazilian conditions (Nava and Dechen, 2009).

As seen in Table 1, YE was similar and statistically higher in the untreated control and under compound NPK, urea, and manure treatments in comparison with CAN and ITP treatments, which showed no significant difference. Wrona (2011) found that different rates of N did not change the YE value as compared with the unfertilized control.

Data from the relevant literature are contradictory regarding the effect of N fertilizers on apple tree growth and productivity, as these properties are highly complex categories dependent on genetics, biotic and abiotic factors, and applied cultural practices, including fertilization (Milošević and Milošević, 2017). In the present trial, the roots of MM 106 under herbicide strips grew under luxurious conditions of nutrient supply (Wrona, 2011). In addition, N fertilization enhanced root development, which improved the supply of nutrients and water to the growing parts of the plants, resulting in an increased photosynthetic area and hence greater vegetative growth and better productivity of Idared apples. In our previous study on the same cultivar grafted on M 9 rootstock, compound mineral fertilizer NPK (15:15:15) resulted in better production capacity compared to the other mixture fertilizers (Milošević and Milošević, 2017). However, treatment of acidic soils with urea, which is also acidic, had negative effects. For example, urea applied to an acidic soil during one season resulted in a pH decrease and nutrient imbalances, which were potentially and sufficiently severe to inhibit fruit tree growth (Belton and Goh, 1992). Also, acidification of orchard soils increases the solubility of toxic elements such as Mn and Al (Ross et al., 1985). Therefore, fertilization with urea under these soil conditions may have severe consequences. Hence, due to the alkaline reaction of the soil, treatment with CAN to improve apple tree growth, in combination with foliar N fertilizers, is probably a better option (Milošević

et al., 2013). In addition, a current trend in fruit tree management is to use mineral nutrition as a major tool for optimizing tree growth, yield, and fruit quality in South Tyrol (Tagliavini and Marangoni, 2002). These authors recommend an application rate of less than 60 kg $ha^{-1}$ of N for pome fruits.

### 3.2. Physical properties of the fruit

The attractiveness of fruit to consumers is determined by visual attributes that include shape, size, appearance, uniformity, color, and freshness. These external quality attributes of fruit are affected by inheritance, in addition to environmental growing conditions and cultural practices (Nour et al., 2010). In the present study, fruit weight and flesh firmness were significantly affected by fertilizer treatment, whereas differences in the effect of fertilizers on fruit dimensions and L/D ratio were not significant (Table 2).

The highest fruit weight was observed under treatment with compound NPK, as well as under urea, and the obtained value was similar to that of the unfertilized control. These data are in agreement with the results of Scudellari et al. (1993), who reported higher values for fruit weight as the result of N-P-K, N-K and split-N inputs, and lower values under N-only fertilization. Other studies also revealed that N-only fertilization did not affect fruit weight in apples (Wrona, 2004, 2011; Pole et al., 2017). Moreover, apple fruit weight and size were more affected by K than by N fertilization (Nava and Dechen, 2009), as confirmed by our data. Usually, K has been associated with fruit quality in general. The organomineral ITP fertilizer resulted in good fruit weight, while CAN led to the smallest fruits in the present study. Apples have low N requirements compared to other plants (Scudellari et al., 1993). In addition, maintaining soil in a weed-free state by means of herbicides undoubtedly favors an abundant availability of N and other nutrients (Wrona, 2011). The fruit diameter in our study was >75 mm under all fertilization regimes, which is a preferable commercial trait (Dobrzañski et al., 2006). Otherwise, Idared is classified as a large-fruited cultivar under several protocols. However, fruits that are too large suffer from physiological disorders such as bitter pit in Idared, especially during storage, and must be harvested and consumed first (Pole et al., 2017).

The analysis of flesh firmness (Table 2) showed that manure led to the firmest fruits, whereas compound NPK gave softer fruits. Moreover, there were significant differences among fertilizer treatments, in the decreasing order of flesh firmness: manure > ITP > CAN > urea > control > NPK. Since the content of available K in the soil was high, the additional amount of K incorporated through NPK severely decreased flesh firmness, as flesh firmness of apples receiving Ca was higher than under treatment with N and/or compound NPK (Raese, 1998). Skendrović Babojelić et al. (2007) reported much lower values for Idared apples stored for 4 weeks under conditions similar to those of the present experiment. Differences between the present results and those of the above authors can be explained by the fact that the storability of the same cultivar depends on factors such as maturity stage and storage conditions. In addition, smaller apple fruits were firmer as flesh firmness negatively correlated with mean fruit weight and crop load (Johnson, 1994), as was the case in the present trial (Table 1). Postharvest softening of apple fruit is a serious problem for growers worldwide and is generally considered an undesirable ripening process, as firmer apples tend to be juicier, crisper, crunchier, and less mealy than softer fruit (Johnston et al., 2002).

### 3.3. Chemical properties of the fruit

#### 3.3.1. Primary metabolites

Soluble solids, which include mainly sugars (approximately 20%–70%) and smaller amounts of organic acids, vitamins, proteins, free amino acids, essential oils, salts, and glucosides (Wills et al., 1983), are good indicators of the sugar content of apples and presumably of sweetness.

**Table 2.** Influence of different fertilizers on fruit physical properties of apple cultivar Idared. Values are the mean ± standard error for 2016 and 2017.

| Fertilizer | Fruit weight (g) | Fruit length (mm) | Fruit diameter (mm) | L/D ratio | Flesh firmness (kg $cm^{-2}$) |
|---|---|---|---|---|---|
| CAN | 204.10 ± 4.93 d | 67.81 ± 0.75 a | 79.67 ± 0.71 a | 0.85 ± 0.00 a | 7.53 ± 0.17 c |
| NPK | 237.05 ± 9.45 a | 72.50 ± 1.29 a | 84.94 ± 1.32 a | 0.86 ± 0.01 a | 7.26 ± 0.16 f |
| Urea | 230.25 ± 4.90 ab | 69.74 ± 0.94 a | 83.39 ± 0.77 a | 0.84 ± 0.01 a | 7.46 ± 0.16 d |
| Italpolina | 226.50 ± 4.74 b | 69.30 ± 0.91 a | 84.33 ± 0.72 a | 0.82 ± 0.01 a | 7.66 ± 0.13 b |
| Manure | 215.65 ± 4.94 c | 69.22 ± 0.95 a | 80.48 ± 0.73 a | 0.86 ± 0.01 a | 7.86 ± 0.14 a |
| Control | 231.15 ± 5.14 ab | 70.45 ± 1.00 a | 82.28 ± 0.77 a | 0.86 ± 0.01 a | 7.33 ± 0.14 e |

Values followed by different letters in the same column are significantly different (LSD test).

In this study, all fertilizers significantly changed the SSC, which was the highest under manure and the lowest in the untreated control (Table 3). Additionally, N fertilizers alone and/or as part of compound NPK decreased the SSC. These results were consistent with those reported by other researchers (Crisosto et al., 1997; Wrona, 2004). Contrary to the present experiment, the split-N application increased the content of soluble solids in apple fruit (Scudellari et al., 1993) in soil with a high pH (8.2) and a low $N_{TOT}$ content (0.105%). The favorable effect of manure on SSC might be attributed to its effect in supplying the trees with various nutrients, improving soil pH, encouraging microbial activity, and producing natural auxins (Mansour et al., 2007). In a study by Skendrović Babojelić et al. (2007), the SSC of Idared was much higher than the present data. This phenomenon has been explained by differences in fruit maturity stage, fruit position in the canopy, daily temperatures before and during harvest, crop load, ecological conditions, and cultural practices applied (Radivojević et al., 2014).

TA may be an important tool in predicting the taste of apples (Moor et al., 2008). This may also be important during the assessment of fruit quality, since consumers often have distinct preferences for acid or sweet-tasting apples (Nour et al., 2010). Malic acid is the characteristic acid of apple and hence is mainly responsible for the sour taste of the apple. The data summarized in Table 3 show that all treatments tended to change the acidity content; the highest and statistically similar values were produced by CAN treatment and no fertilization (control), whereas the lowest value was obtained with urea. Contradictory results are reported in the available literature regarding the effect of fertilizers on the acid content in apple fruit (Ernani et al., 2008). Fertigation with K in four apple cultivars increased fruit acidity (Fallahi et al., 2010), whereas N fertilization had no effect on titratable acidity (Drake et al., 2002). Discrepancies between the present results and those of other researchers may be associated with differences in acid metabolism caused by the specific nature of fertilizers, as well as other reasons such as diverse agroclimatic conditions, the cultivar factor per se (genotype), cropping method, and tree age (Milošević and Milošević, 2017). In general, our values for TA are similar to the data previously found for the same cultivar (Skendrović Babojelić et al., 2007), but slightly lower than our previous results under different fertilization regimes (Milošević and Milošević, 2017).

The relationship between SSC and TA or the ripening index (RI) plays an important role in consumer acceptance. However, evidence for the relationship between SSC or SSC/TA ratio and consumer acceptability and perception of sweetness is often unreliable in the case of apples (Skendrović Babojelić et al., 2007). In some studies, the relationship has probably been clouded by the influence of fruit maturity and starch index (Yuen et al., 1995), while other studies show a good relationship between SSC alone or SSC/TA and acceptability (Thiault, 1970). In the present study, urea promoted the best RI value, whereas the lowest and similar values were found in the control and CAN treatment (Table 3). As the above treatments led to the lowest or highest acidity, RI values were the highest or lowest. In addition, less pronounced acids in Idared apples (Yoon et. al., 2005) and less harmonious and incomplete taste, which makes the fruits rather unacceptable for consumers (Gliha, 1978), were also confirmed in the present experiment.

Differences between the fertilizer treatments and the control in fruit sugar content were not statistically significant (Table 4). Other authors also reported an inconsistent effect of fertilization on the accumulation of sugars in apple fruit. John et al. (1942) found that the percentages of sucrose and total sugars were highest in Jonathan apples from trees receiving P and K without N, whereas Hopkins and Greve (1931) determined that N fertilization had

**Table 3.** Influence of different fertilizers on soluble solids content, acidity, and ripening index of apple cultivar Idared. Values are the mean ± standard error for 2016 and 2017.

| Fertilizer | Soluble solids content (°Brix) | Titratable acidity (%) | Ripening index |
|---|---|---|---|
| CAN | 12.85 ± 0.18 d | 0.58 ± 0.01 a | 22.30 ± 0.51 d |
| NPK | 12.35 ± 0.16 e | 0.48 ± 0.01 bc | 26.14 ± 0.85 c |
| Urea | 12.95 ± 0.20 c | 0.42 ± 0.01 d | 31.38 ± 1.15 a |
| Italpolina | 13.11 ± 0.20 b | 0.45 ± 0.01 cd | 29.29 ± 1.14 b |
| Manure | 13.25 ± 0.20 a | 0.51 ± 0.01 b | 26.47 ± 1.19 c |
| Control | 12.28 ± 0.19 f | 0.55 ± 0.01 a | 22.90 ± 1.12 d |

Values followed by different letters in the same column are significantly different (LSD test).

little effect on the percentage of carbohydrates in Stayman Winesap apples. Improvement in apple fruit quality due to N and/or K nutrition was also observed by Verma and Chauhan (2013). Namely, the increase in total sugars in apple fruits due to the accumulation of photosynthates might be ascribed to the increased uptake of N and K in the plant system. Differences between these results and the present experiment may be due to different agronomic conditions.

Fertilization also changed the taste of apple fruit, which was assessed on the basis of the sugar/acid ratio (TS/TA). This ratio was highest under urea treatment and lowest under CAN treatment and in the control (without fertilization) (Table 4). This index is used by some authors for the classification of apple cultivars (Lea, 1995). Specifically, apple cultivars with TS/TA ratios below 20 are acidic and suitable for processing and cider production, while cultivars with TS/TA ratios above this value are sweet and suitable for direct consumption. In the present study, fruits from trees fertilized with CAN and control trees were not suitable for fresh consumption.

**3.3.2. Secondary metabolites**

Apple fruits are commonly used in human nutrition, but they are not the richest source of phenols and do not have the greatest antioxidant capacity. For example, the contents of phenolics and flavonoids are much lower in Idared apples than in strawberries, blueberries, tart cherries, oranges, and tangerines, but much higher than in Red Haven peaches and Hungarian Best apricots (Dragović Uzelac et al., 2009). Apart from the research on the beneficial effect of apples on human health, several studies have been conducted on the phenolic profile and contents of phenolic compounds in relation to infections induced by *Venturia inaequalis* (Cooke) G. Winter in the vegetative and reproductive organs of apple trees. Therefore, phenolic compounds accumulated in the fruit play an important role in the plant's defense mechanism against different fungal diseases and different stresses (Schovánková and Opatová, 2011).

In the present study, all secondary metabolites were significantly affected by fertilizer treatments (Table 5), which is in agreement with previous findings (Wang, 2006).

**Table 4.** Influence of different fertilizers on fruit sugar content and sweetness index of apple cultivar Idared. Values are the mean ± standard error for 2016 and 2017.

| Fertilizer | Invert sugars (% FW) | Sucrose (% FW) | Total sugars (% FW) | TS/TA ratio |
|---|---|---|---|---|
| CAN | 7.00 ± 0.16 a | 3.21 ± 0.33 a | 10.21 ± 0.30 a | 17.74 ± 0.64 d |
| NPK | 7.89 ± 0.14 a | 3.62 ± 0.25 a | 11.52 ± 0.28 a | 24.37 ± 0.90 b |
| Urea | 8.02 ± 0.15 a | 3.70 ± 0.32 a | 11.71 ± 0.34 a | 28.50 ± 1.42 a |
| Italpolina | 7.41 ± 0.15 a | 3.42 ± 0.33 a | 10.83 ± 0.34 a | 24.15 ± 1.42 b |
| Manure | 7.41 ± 0.14 a | 3.41 ± 0.33 a | 10.82 ± 0.35 a | 21.64 ± 1.46 bc |
| Control | 7.13 ± 0.14 a | 3.29 ± 0.34 a | 10.42 ± 0.36 a | 19.70 ± 1.42 cd |

Values followed by different letters in the same column are significantly different (LSD test).

**Table 5.** Influence of fertilizer on non-nutritive compounds in apple cultivar Idared. Values are the mean ± standard error for 2016 and 2017.

| Fertilizer | Total phenolic content (mg GAE 100 $g^{-1}$ DW) | Total flavonoid content (mg GAE 100 $g^{-1}$ DW) | Total nonflavonoid content (mg GAE 100 $g^{-1}$ DW) | Total antioxidant capacity (mg AA $g^{-1}$ DW) |
|---|---|---|---|---|
| CAN | 177.74 ± 1.62 f | 105.01 ± 0.94 b | 72.79 ± 1.65 f | 42.85 ± 0.83 d |
| NPK | 188.22 ± 1.10 d | 107.95 ± 0.53 a | 80.27 ± 1.31d | 49.05 ± 0.40 c |
| Urea | 180.82 ± 0.73 e | 104.87 ± 0.54 b | 75.93 ± 0.82 e | 41.55 ± 0.49 d |
| Italpolina | 225.17 ± 0.73 a | 72.01 ± 0.53 c | 153.16 ± 0.81 a | 72.01 ± 0.48 a |
| Manure | 210.50 ± 0.73 b | 67.02 ± 0.55 d | 143.48 ± 0.82 b | 59.10 ± 0.46 b |
| Control | 203.75 ± 0.74 c | 67.32 ± 0.49 d | 136.42 ± 0.81 c | 57.75 ± 0.45 b |

Values followed by different letters in the same column are significantly different (LSD test).

The highest TPC, TNFs, and TAC were found under ITP treatment and the lowest under the CAN. The lowest TAC was also obtained by urea, with no significant differences in comparison with the effect of CAN treatment. The highest TFC was observed after fertilization with compound NPK, whereas the control and the manure treatment gave the lowest and similar contents. Interestingly, fertilizers with markedly high N content (CAN, urea, and complex NPK) led to a much higher TFC than ITP, manure, and control, and vice versa for TNFs. The relationship between TPC and TFC in this study was similar to the ratio previously determined by Dragović Uzelac et al. (2009) for the same cultivar.

A positive effect of fertilization on the flavonoid content in apples was previously reported by other researchers. Li et al. (2002) found an increase in flavonols in apples sprayed with Senifos (a mixture of P, K, and N), whereas Heimler et al. (2017) reported that soil N affected the flavonoid content and, generally, a higher polyphenolic content was observed when lower amounts of N fertilizer were added to the soil. In addition, fertigation and foliar fertilization with the mixture of N, P, and K and two application rates of CAN significantly increased TPC in Idared apples in comparison with the control (Murtić et al., 2013). Contrary to the above, Awad and Jager (2002) did not confirm any significant effect of Senifos on the content of flavonols in Jonagold apple. Similar findings were found by Štampar et al. (2015), who used the foliar Phostrade Ca fertilizer (a mixture of high P content and Ca) and determined no significant effect on flavanol content in Braeburn apples, although an increase was observed. It seems that a stimulating effect of N, P, and other nutrients on the flavonoid content in apples has been determined; however, the associated mechanism and function remain unknown (Štampar et al., 2015). In this trial, the organomineral ITP fertilizer and manure increased TPC, TNFs, and TAC to a greater extent than the other fertilizers did. These fertilizers probably caused changes in chemical and physical characteristics of the soil, increased beneficial microorganisms, and enhanced nutrient availability and uptake, thus favoring the growth and development of plants and fruits (Wang, 2006). In our earlier study on apricot, we also observed a positive influence of an organomineral (multinutrient) fertilizer added to the soil on total phenolic and total flavonoid contents in fruits (Milošević et al., 2013).

### 3.4. Conclusions

Treatments with organic, multinutrient, compound NPK, and two N-only mineral fertilizers led to changes in most fruit properties. However, the response of Idared trees grafted onto MM 106 rootstock to the various fertilizer treatments was not always the same. Although the soil was acidic and heavy, it was a good source of organic matter and available K, moderately supplied with N total and P, and without Ca problems. Herbicide strips were additionally enriched by organic matter with grass mown in alleyways and provided good conditions for optimal root growth. Both N fertilizers (urea and calcium ammonium nitrate) significantly stimulated tree growth, whereas urea used alone promoted the highest productivity, yield efficiency, ripening index, and sugar/acid ratio in general. Calcium ammonium nitrate as an alkaline fertilizer significantly increased fruit acidity, but decreased fruit weight, soluble solids/acidity, sugar/acidity ratios, phenolic compounds, and antioxidant power. The multinutrient fertilizer Italpolina, along with manure, increased the total phenolic and total nonflavonoid contents and antioxidant power, whereas compound NPK increased total flavonoids. The effect of all fertilizers on some key features of the external and internal quality of the fruit, such as fruit dimensions and the content of sugars, was not significant. Given some unexpected results, the nutrient supply of apple requires adjustment of fertilizer types and application rates for this soil type according to foliar and soil analyses. Liming, along with irrigation, is another operation required for this soil. Therefore, further studies on these and other fertilizers should be conducted in order to provide recommendations for acidic soils having similar physicochemical properties.

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