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NITROGEN CONTENT IN APPLE LEAVES UNDER VARIOUS FERTIGATION TREATMENTS IN YOUNG, SEMI-YOUNG AND OLD LEAVES

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Abstract

Nitrogen (N) deficiency in apple (Malus domestica Borkh.) leads to typical physiological changes (discoloration of leaves and fruits). Fertigation can significantly increase apple growth, quality and optimize nutrient uptake. The aim of this study was to evaluate how various fertigation treatments affect the N content in apple leaves. The hydroponic cultivation of apple, variety 'Braeburn' was carried out on low vigorous rootstock (M9) in the substrate perlite. The experiment included four fertigation treatments: 1) Hoagland's solution (HS) full solution-control; 2) HS nitrogen excluded; 3) HS iron excluded; 4) HS magnesium excluded. Plant sampling was carried out in June 2024 on three positions of the shoots, based on the leaves age (young, semi-young and old leaves) followed by chemical analyses. Statistically the lowest leaves N content was detected in the young, semi-young and old leaves (1.69, 1.81 and 1.80% N, respectively) in the treatment without N, while the highest N content was detected in the treatments without iron and magnesium (2.07-2.49% N). The N content in the apple leaves varies depending on the type of fertigation treatment.

Key words*: fruit, hydroponic system, macroelement, mineral, rootstock.*

INTRODUCTION

The apple (*Malus domestica* Borkh.) is a woody perennial plant that has been cultivated for hundreds of years. Its fruit is one of the most widely cultivated and consumed fruits in the world (Cornille et al., 2012; Spengler, 2019).
Grown primarily for its fruit, the apple is known for its refreshing flavor and crisp texture, making it a popular choice for both fresh consumption and processed forms such as juice, cider, and dried snacks (Hyson, 2011).
Apples come in many varieties, each with their own balance of sweetness and tartness (Harker et al., 2002). Aside from their flavor, apples are highly nutritious and offer numerous health benefits (Boyer & Liu, 2004; USDA, 2023).
The origin of the apple can be traced back to the wild apple *Malus sieversii*, which is believed to have originated in the Caucasus region, which includes present day Turkey, Georgia, Armenia and Iran. These wild ancestors are considered to be the ancestors of today's apple varieties (Peil et al., 2011; Volk et al., 2021).
In 2023, apples were grown worldwide on an area of 46,182,85 hectares (ha) and a yield of over 973,393,38 tons (t) was achieved (FAO, 2023).
Nutrient deficiencies in apple trees can have a negative impact on yield, quality and resistance to pests and diseases, reducing the overall productivity and profitability of orchards. Apple trees often suffer from deficiencies in key macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (Licina et al., 2021). These deficiencies often manifest themselves through visible symptoms such as leaf discoloration, stunted growth, reduced fruit size and increased susceptibility to pathogens. Identifying and correcting nutrient deficiencies in apple orchards is critical to maintaining optimal tree health, maximizing fruit yield and ensuring the

production of high-quality apples (Kowalczyk et al., 2022).

Nitrogen is a crucial element in plant metabolism, involved in the formation of amino acids, nucleic acids, proteins, biochemistry of coenzymes, photosynthetic pigments and polyamines (Lešić et al., 2016; Javornik et al., 2023). Although it makes 78.1% of the Earth's atmosphere in the form of a molecule (N_2), most plants cannot utilize N directly due to the stability of the triple bond between two N atoms. The conversion of molecular N into forms of ammonium and nitrate ions (NH_4^+ and NO_3^-, respectively) which are accessible to plants in the soil, requires a considerable amount of energy (Vukadinović & Vukadinović, 2011; Kabange et al., 2022).

The dry matter of plants usually contains between 2 and 5% N. Plants are important stores of N and continuously convert it from mineral to organic forms during vegetation and incorporate it into their tissues. However, due to limitations in mobilization from organic reserves, rapid mobility of NO_3^- and N losses, availability of N often becomes a key factor limiting plant growth and yield (Vukadinović & Vukadinović, 2011; Grzyb et al., 2021; Postma et al., 2021).

It is noteworthy that about 70% of all cations and anions taken up by the roots are in the form of NH_4^+ or NO_3^- ions. This fact has a strong influence on the ratio of uptake of cations and anions of other elements in plant nutrition (Vukadinović & Vukadinović, 2011; Zhu et al., 2021).

Nitrogen deficiency leads to slower overall plant growth and a smaller assimilation area, resulting in shorter, narrower and pale green leaves. Chlorophyll deficiency reduces the intensity of photosynthesis, accelerates plant aging and reduces yield. Nitrogen deficiency often limits plant growth as it easily returns to its molecular state and is thus rapidly lost from the soil (Bergmann, 1992; Vukadinović & Vukadinović, 2011).

An excess of N leads to the formation of a large leaf mass of dark green color. The plants become susceptible to diseases, pests, low temperatures and drought. The structure of the plants becomes brittle, which can lead to encrustation of the vegetation due to the increased formation of parenchyma tissue. The quality and taste of the vegetables are low due to reduced sugar synthesis, and it becomes more difficult to maintain freshness (Lešić et al., 2016; Fathi, 2022). On light and permeable soils, excess of N can lead to leaching of nitrates and pollution of groundwater (Vukadinović & Vukadinović, 2011).

According to Resh (2022), fertigation is a system used in hydroponics that maximizes nutrient uptake by applying vital nutrients such as N, P and K directly into the root zone of plants. Fertigation reduces the risk of nutrient deficiencies, making it an environmentally friendly method for growing apples. To avoid imbalances and ensure healthy growth of apple trees, fertigation schedules and nutrient solutions must be properly managed (Dominguez & Robinson, 2024).

The aim of this work is to evaluate the effects of different fertigation treatments on the N content in apple leaves depending on their age.

MATERIALS AND METHODS

The plant material for this study was collected from the apple orchard located at the University of Zagreb Faculty of Agriculture, in Zagreb, Croatia (longitude 45°49'36" S and latitude 16°01'47" E).

The apple variety 'Braeburn' was grown in hydroponics on a low vigorous rootstock – M9 in a substrate perlite. The cultivation of apples was carried out under four fertigation treatments: 1. Hoagland solution, full solution (control); 2. Hoagland solution, nitrogen excluded; 3. Hoagland solution, iron excluded; 4. Hoagland solution, magnesium excluded.

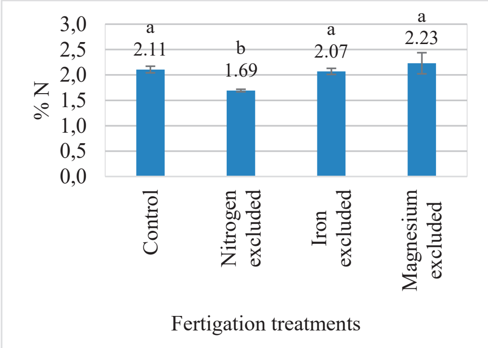
Hoagland solution is a nutrient-rich liquid used for the hydroponics of plants. It is prepared by mixing concentrated stock solutions of essential macro (such as KNO_3, $Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4) and micronutrients (including boron, manganese, zinc, copper and molybdenum) and a source of iron (Fe-EDTA). For the preparation, small amounts of the individual components (stock) are added to distilled water and the pH is adjusted to around 5.8-6.2. The solution provides all the nutrients that plants need for healthy growth in soil-free systems (Trejo et al. 2012).

The apple leaves were sampled in three replicates, during the 2024 growing season and depending on the different leaf position on the shoot (young, semi-young and old leaves).

The samples were submitted to the Analytical Laboratory of the Department of Plant Nutrition at the University of Zagreb Faculty of Agriculture. Homogenized samples were dried at 105 °C and grounded. Nitrogen (N) in the apple leaves was determined using the Modified Kjeldahl method (HRN ISO 11261:2004) and expressed as % N in dry weight.
The statistical software SPSS (version 22.0; SPSS Inc., Chicago, IL) was used for the statistical analysis. One-way ANOVA was used to determine the effect of the different fertigation treatments on N content in young, semi-young and old leaves. When significant differences between means, were observed, additional post hoc assessment was performed using Tukey's Test ($p < 0.05$).

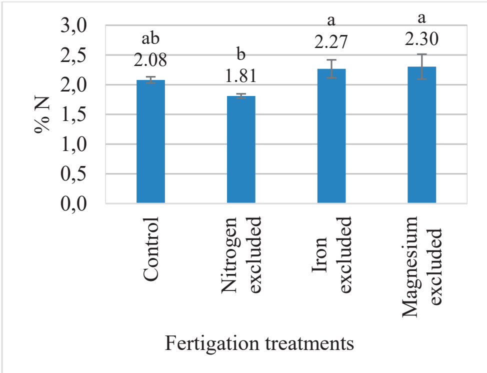
RESULTS AND DISCUSSIONS

Figure 1 shows the N content in young leaves under different fertigation treatments, determined in a range from 1.69 to 2.23 % N. The statistically significant highest N contents were found in the treatments: iron excluded, control and magnesium excluded (2.07, 2.11 and 2.23% N, respectively). Overall, the statistically lowest N content was found in treatment where N was excluded (1.69% N).
The N content in semi-young leaves under various fertigation treatments is displayed in Figure 2 and ranges from 1.81 to 2.30% N. Iron excluded and magnesium excluded treatments had the statistically significant highest N contents (2.27 and 2.30% N, respectively). Furthermore, the treatment where N was excluded had the statistically lowest N content (1.81% N).
The N content of old leaves under various fertigation treatments ranges from 1.80 to 2.49 % N, as shown in Figure 3. The treatment with iron excluded had the statistically highest N content (2.49% N), while the treatment with N excluded had the statistically lowest N content (1.80% N).
Figure 4 shows dynamics of N content in young, semi-young and old leaves under the fertigation treatment in which N was excluded. The N content was determined in a range from 1.69 to 1.81% N. The statistically significant highest N contents were determined in the semi-young and old leaves (1.81 and 1.80% N, respectively), while the statistically lowest was found in the young leaves (1.69% N).



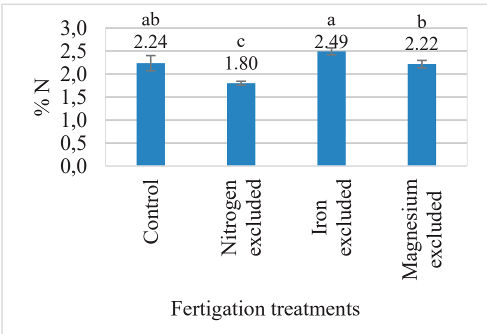
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

Figure 1. Nitrogen content (% N) in young leaves under different fertigation treatments



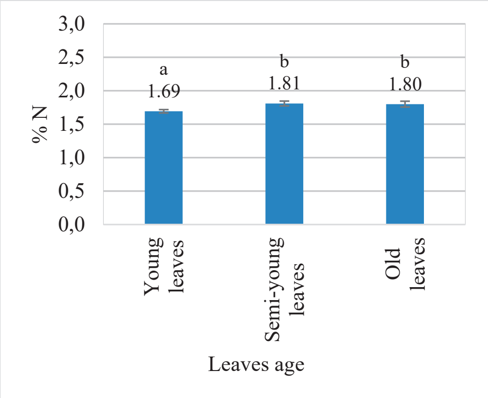
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 2. Nitrogen content (% N) in semi-young leaves under different fertigation treatments



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 3. Nitrogen content (% N) in old leaves under different fertigation treatments



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 4. Nitrogen content (% N) in young, semi-young and old leaves in treatment with nitrogen excluded

Apple production worldwide can be limited by a number of causes, but water and N deficiency are particularly important (Haotian et al., 2023). The N content in plant leaves depends on the interaction of different macro and micronutrients which are crucial for the assimilation and utilization of N as they are contained in fertilizers. Nutrients influence N uptake (Marschner, 2012) and metabolism (Song et ač., 2025) depending on their concentration and type of application Therefore, balanced fertilization is crucial for optimizing N assimilation and establishing normal concentration in plants (Anas et al., 2020; Kowalczyk et al., 2022; Song et al., 2025). The distribution of N in apple leaves changes with age, with N gradually being transported to younger leaves, fruit or storage tissue. Transport leads to a gradual decrease in N content in older leaves (Marschner, 2012). Additionally, changes in N concentration in leaves can be influenced by season, vegetation stage, stress conditions, correct/incorrect fertilization, irrigation and the influence of other micro and macroelements (Marschner, 2012; Treder, 2022).

According to Kowalczyk et al. (2017) and MSU (2025) over-fertilization can lead to an excess of N in young leaves, while under-fertilization can lead to a deficiency in older leaves. Therefore, proper fertilization is very important for maintaining optimal levels across all leaf ages.

Old leaves, from which samples are often taken for nutrient analysis, usually contain N concentration between 1.80 and 2.50% N, which is a reliable indicator of the overall N status of the tree (Tagliavini & Marangoni, 2002; Marschner, 2012).

In this study, the N content in old leaves was determined in a range from 1.80 to 2.49% N under different fertigation treatments. The data obtained in the literature on the N content in old leaves are consistent with the data obtained in this study.

Additionally, as leaves age, their N content can even drop below 1.50% N, especially during the fruit development period. In addition, older leaves may show visible signs of deficiency by yellowing (chlorosis) as N is remobilized to support new growth or fruit production (Zheng Tan et al., 2021). On the other hand, semi-young leaves that are in the middle of the current year's shoots typically contain higher N concentrations than old leaves, ranging from 1.80 to 3.0 % N (Tagliavini & Marangoni, 2002; Marschner, 2012). In this study, the N content in semi-young leaves was determined in a range from 1.81 to 2.30% N under different fertigation treatments. The results of this study corroborate the existing literature about the nitrogen concentration of semi-young leaves.

Younger leaves, which are at the tip of the current year's shoots, typically contain higher N concentrations than older leaves, which are between 1.80 to 3.50% N as well as stated by MSU. This is due to the plant's physiological processes in which N is mobilized to support the growth of new leaves. Cheng and Fuchigami (2002) conducted a study in which they found that about 50% of the tree's N content was remobilized to support the growth of new shoots and leaves, highlighting the importance of N in the development of young leaves.

One of the studies investigated how the timing of N application affects the uptake, distribution and remobilization of N in 'Gala' apples. Zheng Tan et al., (2021) found that a pre-harvest N application resulted in significantly higher N content in the leaves, especially between 7 and 20 weeks after full bloom, than a post-harvest application. This suggests that young leaves respond more actively to early fertilization, making the timing of nutrient application a key factor in optimizing N use.

In this study, the N content in young leaves was determined in a range from 1.69 to 2.23% N under different fertigation treatments. The data obtained in this study for the N content in the young leaves are within the range reported in the literature, except for the treatment in which no N fertilization was applied. In this treatment, a lower N content (1.69% N) was determined in the young leaves than reported in the literature.

Several explanations for the lower N content in young apple leaves compared to semi-young and old leaves can be found in the literature. Environmental factors such as drought (Xu & Zhou, 2006), excessive rainfall (Cameron et al., 2013) or extreme temperatures (Lawlor & Cornic, 2002) can disrupt N uptake and translocation which can lead to a decreased concentration of N in young leaves. These stress factors impair root function, limit nutrient uptake and alter the plants ability to distribute N efficiently, highlighting the complex interplay between environmental conditions and N dynamics in plants (Tagliavini & Marangoni, 2002; Fallahi et al., 2010).

Furthermore, Masclaux-Daubresse et al. (2010) discuss how N is remobilized from leaves into developing tissues, including young leaves and reproductive organs, during N deficiency. This process can lead to reduced N content in leaves as the plant redistributes resources to support critical growth areas.

In addition, imbalances in other nutrients, such as high levels of P or K, can interfere with N uptake and utilization, especially in young leaves, further affecting plant health and growth. These results emphasize the importance of maintaining a balanced nutrient content in plants to ensure optimal N supply (Tagliavini & Marangoni, 2002).

CONCLUSIONS

An analysis of apple leaves revealed that the lowest N content was observed in the young, semi-young, and old leaves (1.69, 1.81, and 1.80% N, respectively) in the fertigation treatment with Hoagland solution where N was excluded. In contrast, the highest concentration of N was found in treatments where iron and magnesium excluded were excluded, with values ranging from 2.07 to 2.49% N. The N content in apple leaves varies according to the type of fertigation treatment applied. It was found that the treatment where N was excluded had the statistically lowest concentration of N in the young leaves. Future research should examine how the dynamics of N distribution vary on leaf age over several growing seasons and under different fertigation treatments.

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