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INFLUENCE OF NITROGEN FERTILIZER ON N CONTENTS IN SOIL LEAVES AND K, Ca CONTENT IN APPLE FRUIT

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Abstract

In modern farming systems, effective fertilization provides plants with nutrients in appropriate quantities, according to plant requirements, which affected by plant age and weather conditions, and creates favorable conditions for developing fruit quality and improving production efficiency. An experiment was performed to determine the changes in the N contents in soil and apple leaves, as well as its correlation coefficients and regression equations on yield parameters, and K, Ca contents in apple fruit. The experiment was conducted over two years (2018 - 2019) on apple trees cv (Cross-validation for choosing Tree complexity). 'Cultivar 'Yona Gold'were M9 T337 rootstock, in the experimental orchard located in the fields of Maradik region in Serbia. The three types of fertilizers (ammonium nitrate, monoammonium phosphate, potassium nitrate)that used in this study. Different doses were applied to prepared trees by a drip irrigation system and the traditional method of fertilization.

The results concluded that the application of ammonium nitrate led to an even distribution of mineral N forms in the root zone, as well as had not led to a significant increase in the total N in the apple leaf at the first year, but in the second year had a positive impact on the content of N in the leaves. However, the addition of N fertilizer with both ways was negatively affected the Ca content and the K / Ca ratio in apple fruit.

Key words: drip irrigation, fertigation, nitrogen fertilizer**,** apple 'Yona Gold'

الملخص

في أنظمة الزراعة الحديثة، يزود التسميد الفعال النباتات بالمغذيات بكميات ونسب مناسبة، وفقًا لمتطلبات النبات المتأثرة بعمر النبات والظروف البيئية، مما يخلق ظروفً التنمية المحاصيل بجودة كبيرة. أجريت تجربة لتحديد التغيرات في محتويات النيتروجين في التربة وأوراق التفاح وكذلك معاملات الارتباط ومعادلات الانحدار على معاملات المحصول ومحتويات البوتاسيوم والكالسيوم في ثمار التفاح. أجريت التجربة على مدار عامين (2018 – 2019) على صنف التفاح " يونا جولد Yona Gold "كان الأصل M9 T337، في البستان التجريبي الواقع في حقول منطقة ماراديك في صربيا. تم استخدام ثلاث انوع من الاسمدة (نترات الامونيوم، احادي فوسفات الامونيوم، نترات البوتاسيوم) المستخدمة في هذه الدراسة بجرعات مختلفة تم تطبيقها على الأشجار عن طريق نظام الري بالتنقيط والطريقة التقليدية للبث.

خلصت النتائج إلى أن استخدام نترات الأمونيوم أدى إلى توزيع متساوٍ لأشكال النيتروجين المعدنية في منطقة الجذر، وكذلك لم يؤد إلى زيادة معنوية في إجمالي نيتروجين في ورقة التفاح في السنة الأولى، ولكن فيا لسنة الثانية. كان لهذا العام تأثير إيجابي على محتوى النيتروجين في الأوراق. وضح أيضًا أن استخدام السماد النيتروجيني مع كل من طريقتي تطبيق النيتروجين قد أثرت سلبا على محتوى الكالسيوم ونسبة الكالسيوم والبوتاسيوم في ثمار التفاح.

Introduction

Apple production in the world during the last 60 years has intensified by virtue of new cultivation systems, which allow for higher yields and better fruit quality (Robinson et al., 2007&Perry et al., 2008). Where in the modern cultivation systems the efficient fertilization supplies plants with nutrients in appropriate quantities and ratios, which creates conditions for crop development with significantquality. Waraich et al., (2011).

The nitrogen is a nutrient essential for a plant's metabolism Santos et al., (2016). Apple trees use stored nitrogen reserves to support vegetative growth, and fruit set Cheng and Raba (2009). For the remainder of the growing season, apple trees acquire nitrogen from the soil or from nitrogen applied to the leaf surface .Lailiang Cheng, and Jim Schupp (2004).

Xia et al., (2009)found that reduced yields and declining fruit quality when nitrogen is limited in apple trees. While Wang and Cheng (2011)stated that the negative impact of high doses of nitrogen on fruit yields and quality leads to a reduced amount of dissolved solids, flesh firmness and fruit color. It is assumed that the applied fertilizers with N, depending on the method of application, and dose, will have different effects on yield of apple fruit and will have an impact on the translocation of elements between soil, leaves and fruit.

The aim of this research was to examine the effects of different methods and doses of N fertilizer on distribution of N in soil profile, and leaves as well as its impact on K, Ca content in an apple fruit.

Material and methods

The experiment was carried out at the experimental fields of Maradik in Serbia (45°06'N; 19°59"E)over two years 2018- 2019. Three and four years old'Yona Gold' variety were used in the experiment, apple trees of the cultivar'Yona Gold' were M9 T337 rootstock, planted at distance 3.1 × 0.8 m. The experiment was conducted in a two-factor split-plot design in order to determine the changes in the N content in soil and apple leaves as well as its effect onK and Ca content in apple fruit.

The effect of N application was studied involving three fertilization doses of 50, 100, 150 kg N ha^{-1} in the form of ammonium nitrate (NH4NO3, 34.4% N), with doses of phosphorus fertilizers were 30 kg P_2O_5/ha, 60 kgP_2O_5/ha and 90 kgP_2O_5/ha, in the form of monoammonium phosphate (MAP), while potassium application doses were 60 kg K_2O / ha, 100 kgK_2O / ha and 140 kgK_2O / ha, in the form of potassium nitrate (these doses from a trial study).All these fertilizers were applied by a drip irrigation system and broadcasting on the soil surface, in the first half of vegetative growth

of apples, and were applied twice a month and periodically during the period from April (blossom stage) to September (harvest stage)in eachyear(Table 1) as well as the drip irrigation system was operated periodically (from April to September) to maintain soil moisture tension between 15 and 25 kPa, in all experimental plots.

Table 1. Time and doses of NPK fertilizer application through fertigation and broadcasting during apple vegetation in 2018 and 2019.

Fertilization periods	Treatments (kg / ha)								
	NPK1			NPK2			NPK3		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
April	4	15	2	8	30	3	12	45	5
May I	4	2	2	8	4	3	12	6	5
May II	5	1	2	10	2	3	15	3	5
June I	6	1	6	12	2	10	18	3	14
June II	7	1	7	14	2	12	21	3	17
Jul I	7	1	10	14	2	17	21	3	22
July II	5	2	10	10	4	17	15	6	22
August I	3	3	9	6	6	15	9	9	20
August II	4	3	9	8	6	14	12	9	20
September	5	1	4	10	2	6	15	3	10
Total (kg ha^{-1})	50	30	60	100	60	100	150	90	140

The orchard was planted on the Chernozem soil (Chernozem is a soil of the semiarid steppe areas, located in Northern Serbia on an area of about 1,000,000 ha. Parent material is calcareous loess, aeolian sediment with 20–30% calcium carbonate ($CaCO_3$),with chemical properties shows in table 2.

Table 2. The chemical properties of Chernozem soil.

Soil type	pH (KCl)	pH (H_2O)	N mg kg^{-1}	P_2O_5 mg kg^{-1}	K_2O mg kg^{-1}	$CaCO_3$	organic carbon (C)
a light sandy loam soil	7.4	8.5	9.2	69.30	232.82	4.2%;	2.01%

The pH value of the soil was determinated in 1 : 2.5 suspension of soil : water and soil : 1 M KCl (Met¬tler Toledo, Five Easy FE 20). The content of $CaCO_3$ was measured by a Scheibler calcimeter (Hedas, Serbia) (JUS ISO 10693:2005). Organic carbon was determined by Tyurin's method [ISO 14235:2005]. Extractable potassium (K_2O) and phosphorus (P_2O_5) were extracted with a solution of 0.1 M ammonium lactate and 0.4 M acetic acid, at a soil : solution ratio of 1 :20. The concentration of K_2O was measured by flame photometrically (Jenway 6105, USA), while the concentration of $P_2O_5$was measured by usingAL methodEnger et al.,(1960).

The total nitrogen content in the leaf and fruit was determined by the Kjeldahl method Arsenijević at al., (2002). The assessment of P content in leaves of apple and dry fruit was by using ammonium vanadate-molybdate method (MAFF, 1986). The content of total potassium in the leaf and dry fruit was determined by direct measurement of K concentration in the solution obtained after mineralization of the sample by dry method (650 ° C, 2-3 h) and evaporation with 25% HCl, using a flame photometer (JENWAY), Arsenijević et al., (2002).

The content of microelements and macroelements in the leaves and dry mass of apple fruit were determined by the method of digestion with a mixture of nitric (HNO3) and hydrochloric acid (HCI) with a ratio 1: 3. After digestion, the concentration of total Ca, Mg, Fe, Mn, copper (Cu) and Zn were measured by atomic adsorption spectrophotometry (Shimadzu 6300), flame technique Arsenijević et al., (2002).

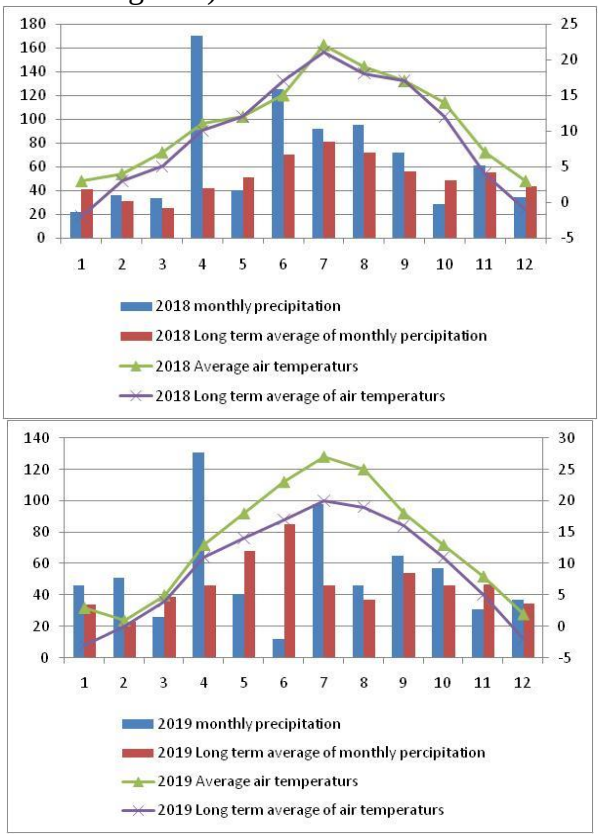
The experiment was carried out in a split block design with four replicates of ten trees per experimental plot that were fertilized in an identical way. The data were elaborated using an analysis of variance by STATISTICS 10 (StatSoft Inc, Tulsa, USA)

Results and discussion

Weather conditions

The weather was mostly hot during the year 2018. On the other hand, the year 2019 was characterized by very high temperatures during July and August, while the other months were at the multi-

year average level (Graph 1). The year 2018 is characterized by a significantly higher rainfall compared to the long-term average (LTA), especially in the first half of apple vegetation (Graph 1). The precipitation rate in Aprilwas almost four times higher (by 142 mm) than LTA, so total amount of water used for drip irrigation was 80 l m^{-2}, while in 2019 the annual irrigation norm was 170 l m^{-2}. (http://www.hidmet.gov.rs).

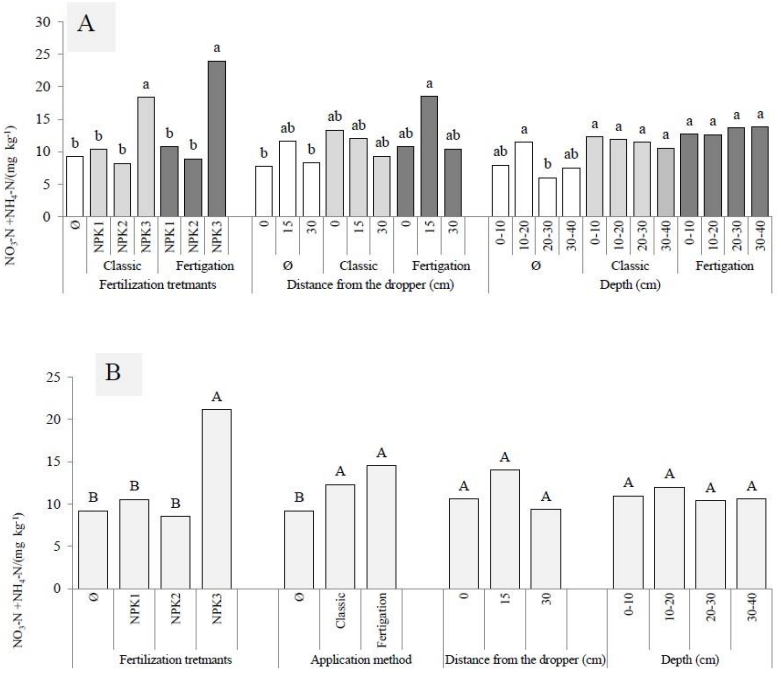


Graph 1. Total monthly precipitation and average air temperature(2018 - 2019) (Republic hydrometeorological service of Serbia)

Content of N in soil

The content of N in the soil did not differ significantly depending on the method of application, layer depth and distance from the dropper, but significant differences existed only between doses of N

(Graph **2**). These results indicate that the application of ammonium nitrate led to an even distribution of mineral N forms in the root zone system, primarily due to the high mobility of the N ion, as the predominant mineral form of N after the application of ammonium nitrate.



Graph 2. Content of mineral N in the zone of the root system of apples in 2018 (A-interactions, B-average of treatment).

* Values marked with different letters are statistically significantly different ($p<0.05$).

* Letters represent a difference at 95% between data in the column.

Ab in same column is significantly different, only a or bin the set of columns means no significant differences found andif A values higher than B in the same set of columns are statistically significantly high.

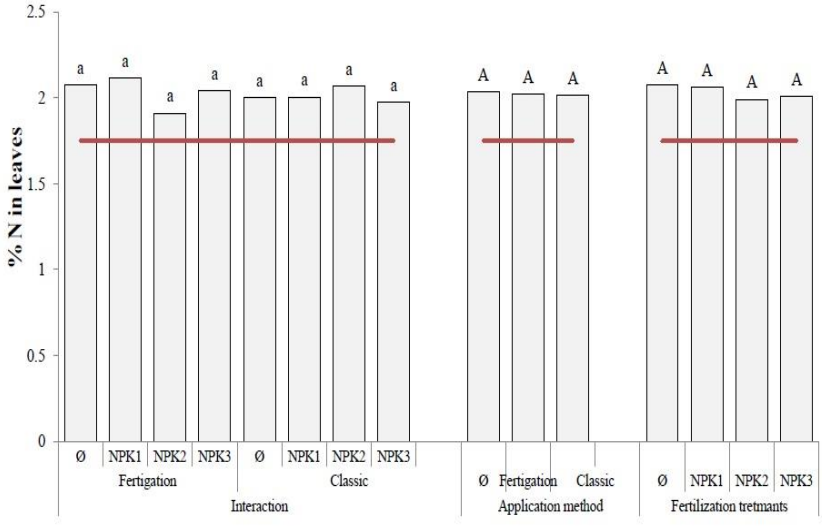
As the experiment was set on a soil of weakly alkaline reaction, therefore the favorable conditions for the process of nitrification and transformation of ammonia N from fertilizer to nitrate, it is assumed that most of N fertilizer is in nitrate form, as a dominant form in neutral and alkaline soils (Rorison 1980). In our research, the N concentration in the surface layer of soil did not differ significantly for the deeper layers, including the layer from 30 to 40 cm. Previous studies shows that nitrate nitrogen (NO_3-N) as the only mobile form of N in the soil is mostly exposed to migration and leaching processes Ligang Xuet al,. (2013).Numerous researches related to mobility of N, it leachesof NO_3-N from the soil, have been conducted in Serbia. (Uddin 1977)used labeled N15 in his research on chernozem soil to monitor the movement of N from applied fertilizers, and found that NO_3-N moved the deepest (140 cm), with most of the N being in the upper part of profile up until 90 cm, and only minimal amounts were measured at a depth of 140 cm, also states that NO3-N, in chernozem soil, migrates up to 140 cm in agroecological conditions ofVojvodina(Stevanović 1976).

For example:
If A and B marked in the same set of columns are statistically significantly different.
If only A or B marked in the same set of columns is no significant differences.
If A values higher than B in the same set of columns are statistically significantlyhigh.
In our agroecological conditions, with an average rainfall of 650 mm per year, it is 54% total amount of N is lost during autumn and winter, 36% in spring and 22% during summer, with the total amount of N that is washed away depends on the soil type and ranges from 5.1 kg N ha-1 to 33.4 kg N ha-1 (Resulović and Savić 1980&Šestić et al., 1989).

Content of N in apple leaves

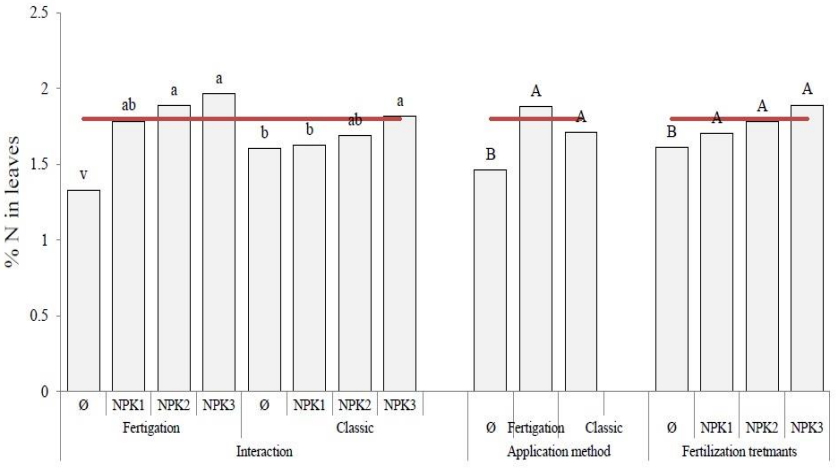
The content of total N in the apple leaves during the two years of experimentrangedfrom 1.33% to 2.12%. In the first year of the

study, the N content was above the lower limit of optimal state by Hoying et al.,(2004), while in the second year on some fertilization treatments it was somewhat lower in relation to the limit values stated by these authors (Graphs 3, 4).In 2018, fertilization had no effect on the content of total N in the apple leaves, (Graph 4). While in the second year, a significantly higher N content was measured compared to the control, regardless of the method of fertilizer application (Graph 4).Based on the analysis of the leaves, the results showed that in the first year with a large amount of precipitation, neither of the two methods of application led to a significant increase in the total N in the apple leaves. And in years with less precipitation (closer to average values), it was shown that both methods of application of N had a positive and equal impact on the content of N in the leaves. It can be concluded that the efficiency and effect of NPK fertilizers is largely influenced by weather conditions during the growing season.



Graph 3. Content of N in apple leaves in 2018. (lower case-interactions, upper-average treatment.

* Values marked with different letters are statistically significantly different ($p < 0.05$).



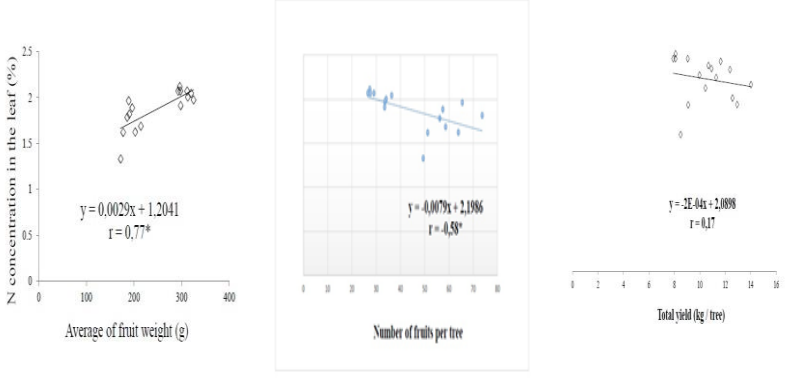
Graph 4. N content in apple leaves in 2019. (lowercase letters - interactions, uppercase letters - average treatment).
* Values marked with different letters are statistically significantly different ($p < 0.05$).

The absence of the influence of N fertilizer on the content of total N in the apple leaves, in the first year of research, can be explained by a large number of studies that show the apple plants needs a large part of N during vegetation period especially in the first half, through the process of remobilization of N from trees and branches, which was adopted by previous vegetation (Weinbaum et al., 1984&Millard and Neilsen 1989). The extent to which N fertilizer application during vegetation will affect on plant growth and N content in the leaves depends on soil fertility, fertilizer application time (Weinbaum et al. 1984&Sanchez et al., 1992) and plant age. (Miller and Miller., 1987). (Neilsen and Neilsen1997),concluded that in young apple plantsthe applied N during vegetation makes up only 13% of N found in leaves in late June, while the remaining N comes from reserves from previous vegetation. An identical conclusion can be reached on the basis of our research, because in the first year the applied N fertilizers, regardless of the method and dose of application, did not affect the content of N in the leaves, but

the following year, at all three doses and both methods application, the N content in the leaves was significantly higher than the control.

Apple yield parameters

It can be clearly seen in graph 5,thatthe regression equations and correlation coefficients between the yield parameters and the N content in the apple leaves. The total N content in the apple leaves was in a linear positive correlation with the average fruit weight, and a linear negative correlation with the total number of apple fruits per apple tree.



Graph 5. Correlation coefficients and regression equations between N content in apple leaves and total apple yield

* Correlation coefficients are statistically significant at the level ($p < 0.05$). r, Pearson correlation coefficient; rs, Spearman correlation coefficient.

K, Ca content and K / Ca ratio in apple fruit

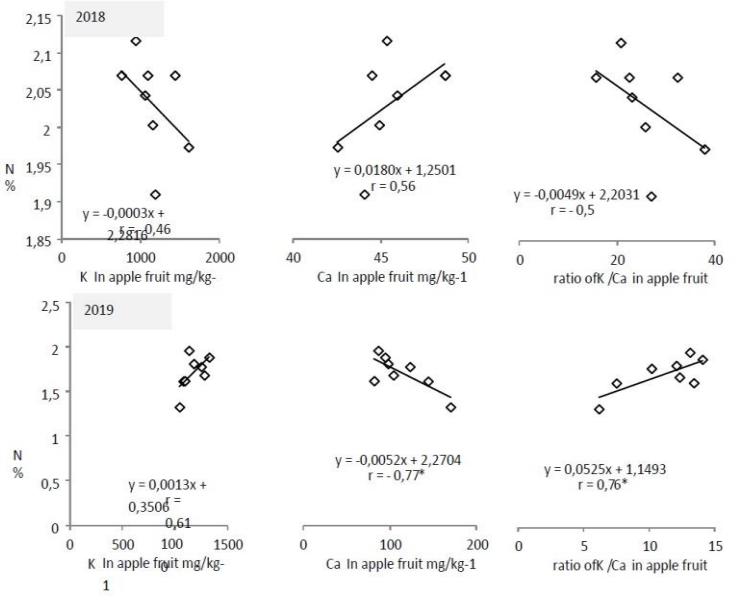
In 2018, no statistically significant correlation was found between the N content in the apple leaves and K, Ca contents in the apple fruit. In the second year of the study (2019), the total N content in the apple leaves was significantly negatively correlated with the Ca content in the fruit, and significantly positively correlated with the K / Ca ratio (Graph 6).

Graph 6. Correlation coefficients and regression equations between N content in apple leaves and K, Ca K / Ca ratio in apple fruit.

* Correlation coefficients are statistically significant at the level ($p < 0.05$). r, Pearson correlation coefficient; rs, Spearman correlation coefficient.

Regression equations and correlation coefficients between yield parameters and Ca content in apple fruit

In both years of research, there was a negative linear relationship between the Ca content in apple fruit and all three observed yield parameters (fruit weight, number of fruits and total yield). However, statistically significant correlation coefficients were found only in the second year of the study (Graph 7).



Graph 7. Correlation coefficients and regression equations between yield parameters of Ca content in apple fruit.

* Correlation coefficients are statistically significant at the level ($p < 0.05$). r, Pearson correlation coefficient; rs, Spearman correlation coefficient.

In our research, an agroecological conditions during the year had a great influence on the content of Ca and K / Ca ratio in apple fruit. The first year of research, the apple fruits had almost twice the K / Ca ratio, compared to the second year of fruiting. Different studies stated that agroecological conditions have a great influence on the mechanisms related to the uptake of Ca and its content in the membrane and wall of cell , on which it largely depends and resistance to physiological diseases such as bitter spots, which occur due to low Ca content, or a high K / Ca ratio in the apple fruit(Ho&White 2005; Amarante et al., 2006;De Freitas &Mitcham 2012,&Tonetto de Freitas 2015).It is well known that N fertilization can also have a negative effect on the Ca content in apple fruit, and thus on the sensitivity of fruits to the appearance of bitter spots.

N application leads to the growth of shoots and leaves formation further from the fruit, which practically leads to a shift of Ca in the direction to young leaves formation and further from the fruit, which directly reduces the accumulation of Ca in apple fruit (Ho & White, 2005). In addition, N application can lead to an intensive increase in fetus due to an increase in fetal cell volume leading to a decrease in Ca concentration due to the dilution effect (Bar-Tal et al., 2001&Saure, 2005). This practically means that larger fruits are characterized by a lower Ca content in compared to smaller fruits ones, which was confirmed in our research where in both years there was a negative correlation between the average fruit weight and the Ca content in apple fruit.

Conclusion

These results indicate that the application of ammonium nitrate led to an even distribution of mineral N forms in the root zone system, and the content of N in the soil did not differ significantly depending on the method of application, layer depth and distance from the dropper. While the results showed that the methods of application had not led to a significant increase in the total N in the apple leaf at the first year, but in the second year with less precipitation it was shown that both fertilization methodsof N had a positive impact on the content of N in the leaves.

The application of N fertilizer had an impact on the yield of apple fruit where the regression and correlation coefficients shows that the total of N content in leaves had negative correlation with the total number of apple fruits per apple tree.
Also the application of N fertilizer had an impact on the content of total Ca and K in apple fruit, and thus on the storage capacity, the potential danger of physiological diseases.
The study showed that theapplication of N fertilizer inboth years of research, especially higher doses had negatively affected on the Ca content and the K / Ca ratio in apple fruit compared to the control, where the classical application of fertilizer in the first year led to significantly lower Ca and higher K / Ca ratio not only in relation to the control, but also in relation to the application of N fertilizer by fertigation.

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