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INTERACTIONS BETWEEN NITROGEN FERTILIZATION AND APPLE FRUIT THINNING WITH NAA

Biserka Milić[1✉], Ranko Čabilovski[2], Zoran Keserović[1], Nenad Magazin[1], Jelena Tarlanović[1]

[1] University of Novi Sad, Faculty of Agriculture, Department of Fruit Growing, Viticulture, Horticulture and Landscape Architecture, 21000 Novi Sad, Serbia

[2] University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops, 21000 Novi Sad, Serbia

ABSTRACT

The objective of this research was to determine the effects of chemical thinning with α-naphthaleneacetic acid (NAA) and nitrogen (N) fertilization, as well as their interaction in relation to fruit set and physical properties of apple cv. Golden Delicious Reinders®. Nitrogen rates used were 30 and 60 $kg \cdot ha^{-1}$ N applied at the pink bud stage. NAA for fruit thinning was applied at the rates of 10, 12 and 14 $\mu l \cdot l^{-1}$. Fruit set was increased by increasing the amount of N applied while the efficacy of NAA for fruit thinning was not linearly correlated to the concentration of the chemical. The total number of fruits per tree was inconsistently reduced in NAA treatments at 30 and 60 $kg \cdot ha^{-1}$ N, while total yield was reduced by thinning at 60 $kg \cdot ha^{-1}$ N, meaning that apple trees are more responsive to NAA application at higher N supply. At sufficient N supply, fruits responded more intensively to thinning with NAA by increasing their growth. Thinning with NAA caused a significant decrease in flesh firmness only in fruits from unfertilized plot.

Key words: α-naphthaleneacetic acid, flesh firmness, fruit set, fruit weight, *Malus domestica* Borkh., yield

INTRODUCTION

Chemical fruit thinning with plant growth regulators stimulates flower bud formation, prevents alternate bearing and improves fruit quality of apple [Milić et al. 2012b]. The efficacy of fruit thinning chemicals largely depends on the environmental conditions at the moment and during days following the application [Stover and Greene 2005]. The recent studies are focused on the attempts to predict the thinning response of apple trees by considering factors such as the concentration of the chemical used for thinning, timing of the spray application, varietal and rootstock differences, climate factors, pruning, spraying technique, dosage, age of tree, vigor, crop in the previous year, the required size of fruits, and interaction between two or more plant growth regulators as sources of variation [Pellerin et al. 2012, Greene et al. 2013]. Milić et al. [2012a] reported that the same thinning treatment with benzyladenine (BA) in conditions of excess N supply might lead to a weak thinning response, smaller fruit weight, some advanced maturity and poorer storage ability. Therefore, when deciding on the BA rate for thinning apple fruitlets, N regime should be taken into consideration.

Apple trees grown on vegetative rootstocks of low vigor, such as M.9, with 3300 trees per hectare, have low annual requirements for N supply, ranging from 8.8 to 44 $kg \cdot ha^{-1}$ during the first six years after planting [Neilsen et al. 2009]. Reserve N and current sea-

✉biserka@polj.uns.ac.rs

son's N uptake each contribute about 50% to the total N in the whole tree at harvest. Nitrogen uptake from bud break to bloom is very limited, and the most of the N demand by new growth at bloom was provided by tree reserve N remobilized from the perennial parts of the tree [Cheng and Raba 2009]. The most rapid N uptake from the fertilizer occurred from bloom to the end of shoot growth, corresponding to the highest tree N demand. Early spring N application recompenses for the use of N reserves [Neilsen et al. 2006]. Nitrogen application in summer time leads to the accumulation of N in the perennial parts of the tree, while N from fertilizer applied at bloom ends in leaves and fruits in the current season. Increasing N supply improves leaf N status, leaf and whole tree photosynthetic capacity, and leaf area to fruit ratio, leading to more cells per fruit, larger fruit, and higher soluble solids [Xia et al. 2009]. The increased average fruit weight at the excessive N supply increases the total yield of apples [Wargo et al. 2003, Raese et al. 2007, Xia et al. 2009].

Nitrogen supply is a significant factor influencing fruit set and size, which may further affect the result of thinning with bioregulators. Considering previously found interaction between N fertilization and chemical fruit thinning with BA, it was assumed that N fertilization might affect the thinning effectiveness of α-naphthaleneacetic acid (NAA), as well. The objective of this research was to determine the effects of chemical thinning with NAA and N fertilization, as well as their interaction in relation to fruit set and physical properties of apple cv. Golden Delicious Reinders®.

MATERIALS AND METHODS

Experimental site

The experiment was conducted from 2009 to 2011, in a commercial apple orchard located in Mala Remeta, Irig, Serbia (45°05'N and 19°44'E, 215 m a.s.l.). Different trees were used in each year of the experiment, on different experimental plots, providing that the treatments were applied on three-years-old Golden Delicious Reinders® trees. The trees were on M.9 T337 rootstock, planted at a 3.2 × 0.8 m distance. The soil on which the field experiment was set up was degraded chernozem, medium deep form, calcareous, gleyed. Basic chemical characteristics of the soils on which the experiment was set up are presented in Table 1. The experiment was performed in conditions of drip irrigation, without any additional N fertilization during the growing season. Meteorological data were monitored and downloaded from the weather station iMetos AG/CP/DD, installed at the experimental site.

Experimental design and treatments

The experiment was conducted using a 2-factorial split-plot completely randomized design, with N fertilization as whole-plot factor and fruit thinning treatments in split-plots (tab. 2). Each split-plot consisted from six uniform trees, with one tree per replicate. Nitrogen rates used were 30 and 60 $kg \cdot ha^{-1}$ N, determined in accordance with Guidelines for Integrated Pome Cultivation [AGRIOS 2011] and the content of mineral N in the soil. The fertilizer, ammonium nitrate (NH_4NO_3), was applied in a stripe of 50 cm at each side of the row, before flowering, at the pink bud stage (57 BBCH) (tab. 3). NAA was applied at the rates of 10, 12 and 14 $\mu l \cdot l^{-1}$. Thinning chemical used was Dirager (L. Gobbi, Italy), containing 3.3% NAA. The moment of NAA application was determined by measuring the king fruit diameter, which ranged in average from 8.1 mm in 2009 to 12.1 mm in 2010. To each treatment a surfactant, Trend® 90 (DuPont, USA), was added at the rate of 1 $ml \cdot l^{-1}$. The trees were sprayed with a mistblower (STIHL SR-420) until run-off, at spray volume of 1000 $l \cdot ha^{-1}$. For both factors (fertilization and thinning), untreated controls were included.

Measurements and analytical determination

The analyses of the basic soil properties were done using common methods. The pH value of the soil was determined in the suspension of soil and H_2O (1 : 2.5) and soil and 1 M KCl [ISO 10390] by METREL, MA 3657 pH meter. $CaCO_3$ content was determined volumetrically, by Scheibler calcimeter (HEDAS, Serbia). Total N content was determined by CHNS analyzer (ELEMENTAR, Vario EL, Ele-

Table 1. Basic chemical characteristics of the soils on which the experiments were set up

Year	Depth (cm)	pH		$CaCO_3$ (%)	Humus (%)	N total (%)	Al-P_2O_5 (mg· 100 g^{-1})	Al-K_2O (mg· 100 g^{-1})
		KCl	H_2O					
2009	0–30	7.30	8.24	5.61	1.90	0.16	25.26	16.1
	30–60	7.50	8.40	8.52	1.47	0.12	15.49	10.3
2010	0–30	6.73	7.97	2.55	1.97	0.15	20.72	23.14
	30–60	6.97	8.08	3.40	1.70	0.13	14.53	17.10
2011	0–30	6.39	7.50	3.35	1.91	0.10	18.18	31.01
	30–60	6.83	7.77	6.49	0.85	0.04	4.41	19.67

Table 2. Experimental design. (1) Fertilization treatments, where N_0 is the control, N_1 30 kg·ha^{-1} N and N_2 60 kg·ha^{-1} N. (2) Thinning treatments, where NAA_0 is unthinned control, NAA_1 10 µl·l^{-1}, NAA_2 12 µl·l^{-1} and NAA_3 14 µl·l^{-1}

N_0[1]				N_1				N_2			
NAA_0	NAA_1	NAA_2	NAA_3	NAA_0	NAA_1	NAA_2	NAA_3	NAA_0	NAA_1	NAA_2	NAA_3

Table 3. The time of fertilization and thinning treatments application and the weather conditions at the moment of application

Year	2009	2010	2011
The date of nitrogen fertilization	9th April	7th April	7th April
The date of chemical thinning	3rd May	7th May	3rd May
Growth stage of central fruits at the moment of thinning (mm)	8.1	12.1	10.2
Air temperature at the moment of thinning (°C)	15.1	17.0	14.8
Relative humidity at the moment of thinning	68.9	63.6	96.2
Harvest date	21st Sep.	6th Sep.	9th Sep.

mentar Analysensysteme GmbH, Hanau, Germany). Plant available P and K were extracted with AL solution (0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75) at a soil to solution ratio of 1 : 20 (w/v) [Enger et al. 1960]. The concentration of P was measured by spectrophotometer (Shimadzu UV 2600, Japan), while concentration of K was measured by flame photometer (JENWAY, PFP 7, UK). The concentration of mineral N (NO_3–N and NH_4–N) in the soil was measured three times in the course of the growing season, by taking samples (four repetitions per plot) from each whole plot: I – before flowering (beginning of April), II – intensive shoots growth (mid-June), and III – at harvest (end of September). Mineral N in soil layers 0–30 cm and 30–60 cm was determined by Wehrmann and Scharpf [1979] method.

Fruit set was presented with three parameters: the number of fruits harvested per cm^2 of trunk cross-sectional area (TCSA), per 100 flower clusters and as a total number per tree. Both flower clusters and fruits at harvest were counted on whole trees. Apples from the treatments were harvested at a commercial harvest time, on 21st September in 2009, 6th September in 2010 and 9th September in 2011 (tab. 3). A mean sample of 30 fruits per replicate (tree) was randomly picked to assess fruit physical properties which included fruit weight, diameter and flesh firmness. Fruit flesh firmness was measured using a FT 327 penetrometer (Winopal Forshchungsbedarf

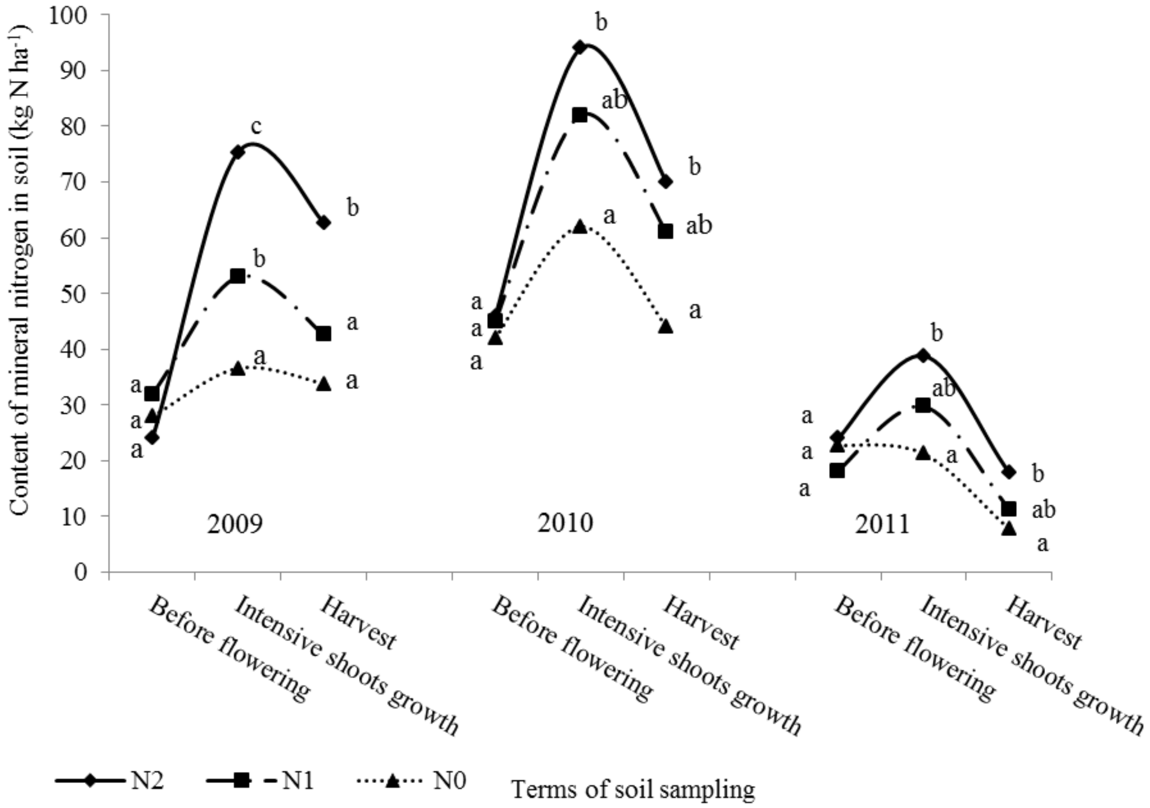

Fig. 1. Content of mineral N (NH_4–N + NO_3–N) in the soil (0–60 cm depth) during apple growing season (2009–2011)

Table 4. p values for ANOVA of the experimental factors year, N fertilization and chemical thinning with NAA, and their interactions affecting fruit set, yield and fruit physical properties

Experimental factor	Number of fruits per			Yield (kg·tree^{-1})	Fruit weight (g)	Fruit diameter (mm)	Flesh firmness (kg·cm^{-2})	Number of seeds
	cm^2 TCSA	100 flower clusters	tree					
Year	0.0000	0.0000	0.0074	0.0000	0.0000	0.0000	0.0000	0.0363
Fertilization	0.0062	0.0000	0.4093	0.3374	0.9308	0.8456	0.0246	0.0003
Thinning	0.0000	0.0000	0.0000	0.0032	0.0000	0.0000	0.0046	0.0084
Year × fertilization	0.0014	0.0000	0.0002	0.0002	0.0333	0.0008	0.0183	0.0032
Year × thinning	0.0000	0.0001	0.6866	0.4898	0.0003	0.0000	0.0014	0.9170
Fertilization × thinning	0.0070	0.0032	0.1522	0.1535	0.0000	0.0000	0.0045	0.0000

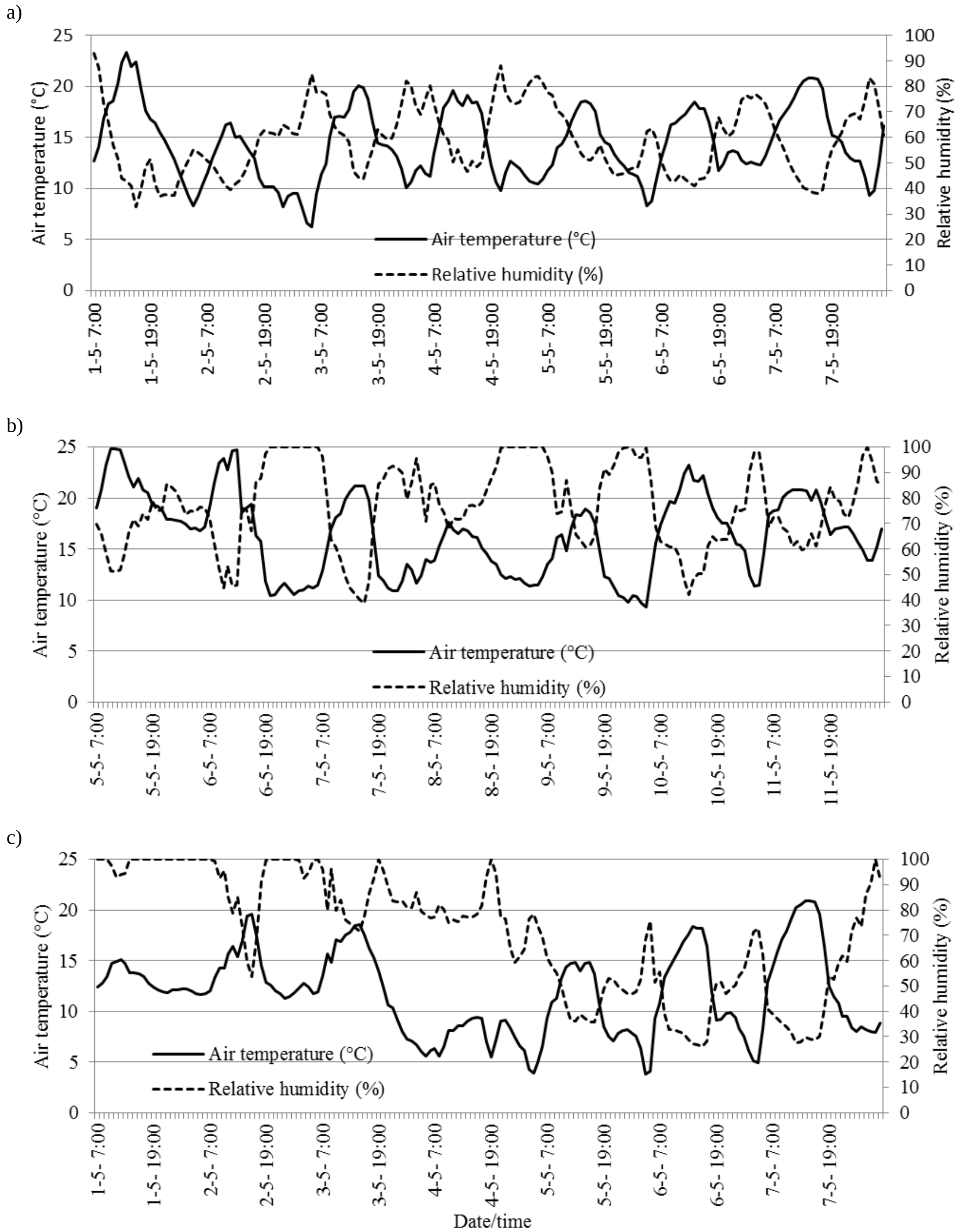

Fig. 2. Air temperature (°C) and relative humidity (%) during the period two days before up to four days after the treatment in a) 2009, b) 2010 and c) 2011. The moment of the treatment is marked with vertical line

GmbH, Ahnsbeck, Germany), with an 11 mm probe. Two measurements were made on the opposite sides of each fruit.

The data were analyzed using analysis of variance (ANOVA). Duncan's multiple range test was used to compare the means at $P < 0.05$ with STATISTICA 9 (StatSoft Inc, Tulsa, USA).

RESULTS

Mineral N content in the soil during the growing season is the result of the mineralization of organic matter and the application of N fertiliz ers on one hand, and on the other, of mineral N uptake in the soil by apple roots and groundcover grass, as well as the activity of microorganisms in the soil. Mineral N content varied between experimental years due to the different climatic conditions. However, the highest N content was measured in the middle of the vegetation period (mid-June), at the time of the most rapid N uptake by the roots. In each experimental year, the mineral N content was higher at the plots where 60 kg·ha^{-1} N was added to the soil compared to unfertilized control plots. The differences in mineral N content between 30 kg·ha^{-1} N and the control plots were not statistically significant.

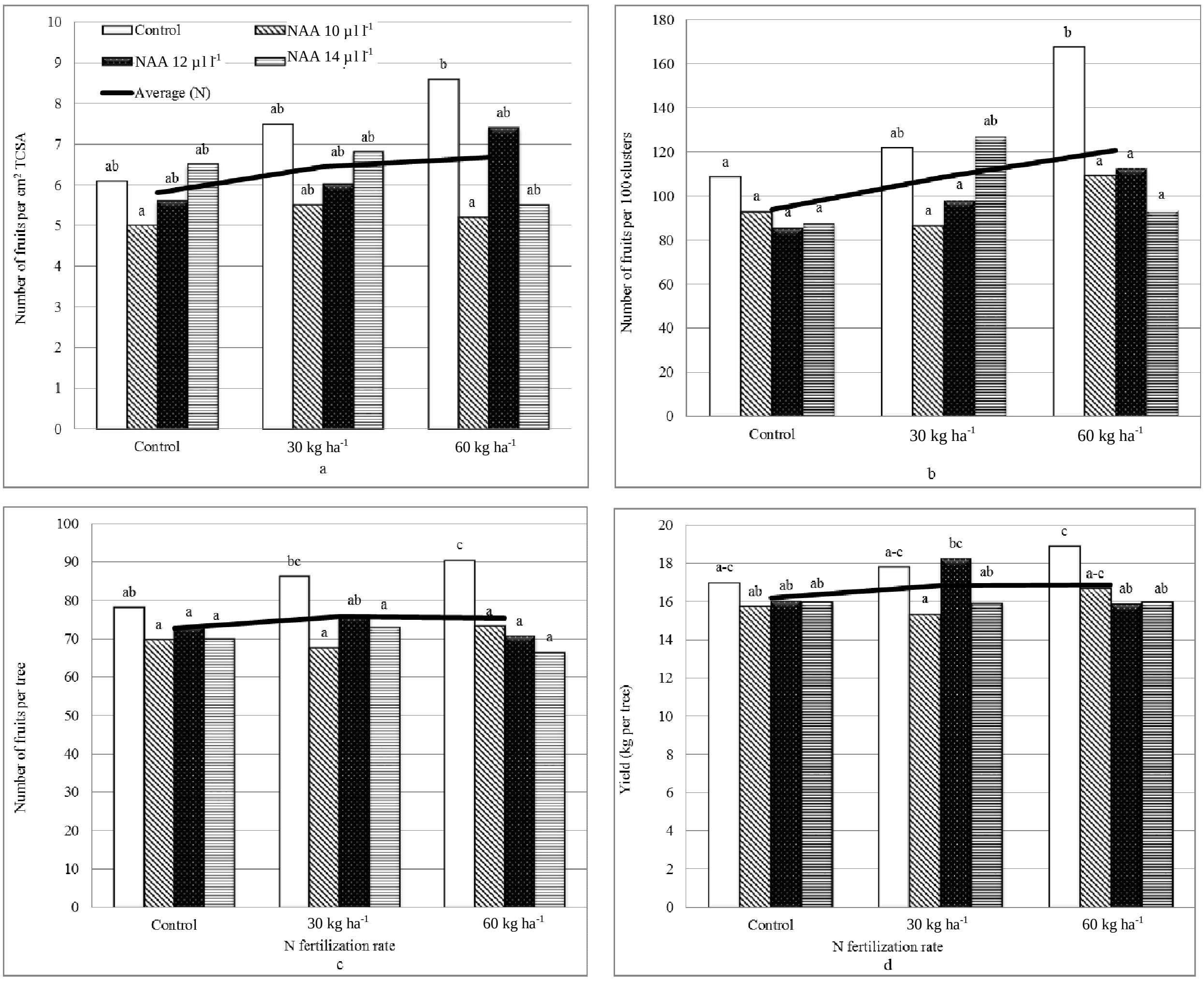

Fig. 3. The effects of the interaction between nitrogen fertilization and thinning with NAA on apple fruit set and yield; a) number of fruits per cm^2 TCSA; b) number of fruits per 100 clusters; c) number of fruits per tree; d) yield

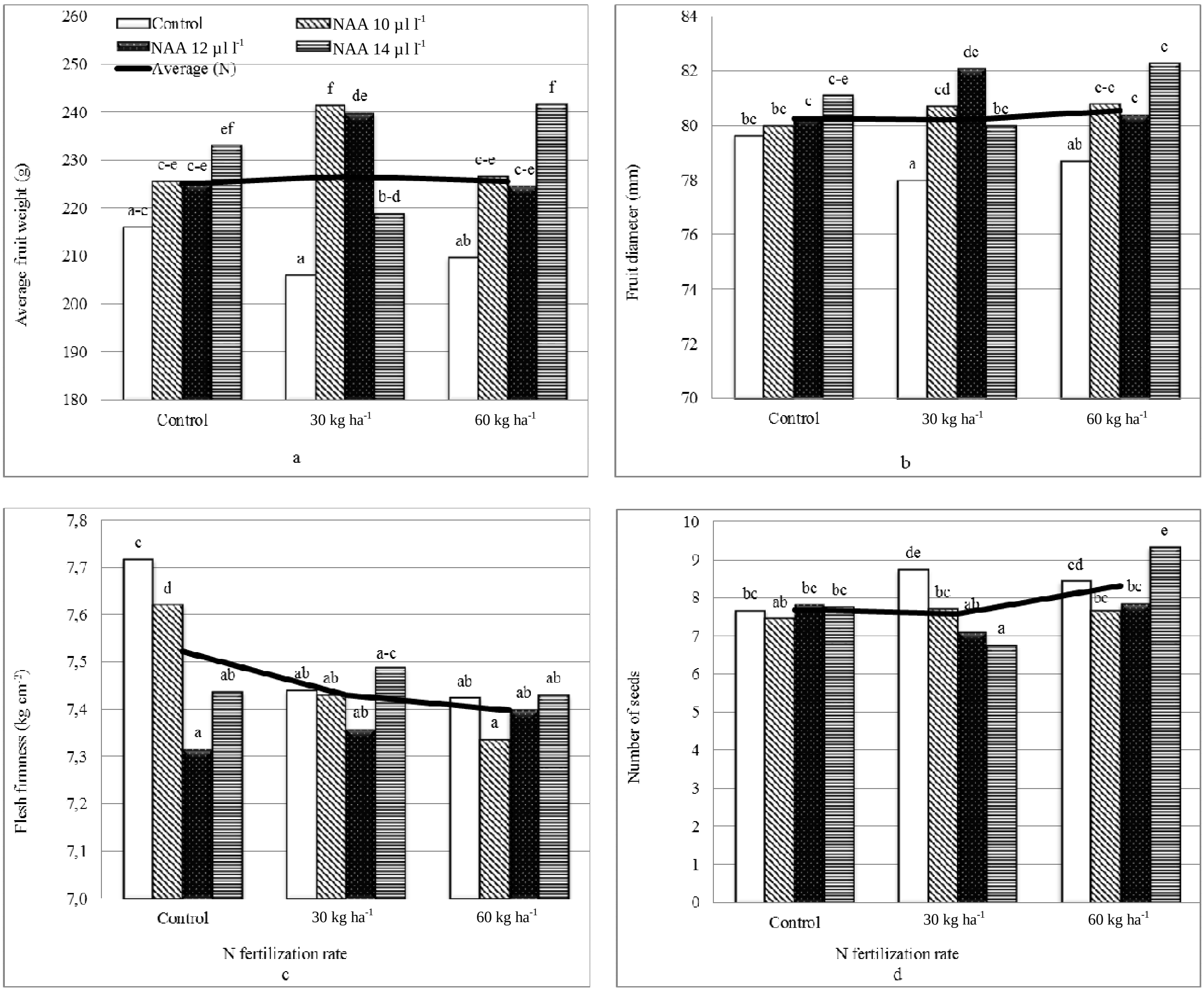

Fig. 4. The effects of the interaction between nitrogen fertilization and thinning with NAA on apple fruit physical properties; a) fruit weight; b) fruit diameter; c) flesh firmness; d) number of seeds.

Each year, the trials were set up early in the morning, obtaining the ideal environmental conditions for the application of plant growth regulators (tab. 3, fig. 2). The only exception was observed in 2011 (fig. 2c), where the relative humidity was very high at the moment and during the days that preceded the application, followed by the period with low air temperatures.

The main assumption for the research was that chemical fruit thinning and N fertilization affect fruit set, yield and physical fruit properties, whether in the same or in the opposite directions. All experimental factors assessed, the year of the study, N fertilization and thinning with NAA, as well as their interactions significantly affected fruit set presented as the number of fruits per cm^2 TCSA and in 100 flower clusters (tab. 4). However, the only significant decrease in the number of fruits per cm^2 TCSA compared to the unthinned control was achieved by lower concentration of NAA at 60 $kg \cdot ha^{-1}$ N added (fig. 3a). NAA thinning efficacy was not related to the concentration of the chemical applied, while the number of fruits per cm^2 TCSA increased by increasing the amount of N fertilizer. The number of fruits per 100 flower clusters was significantly reduced by thinning only at 60 kg·ha^{-1} N added (fig. 3b). The number of fruits per 100 flower clusters increases by increasing the amount of N.

Total number of fruits per tree was not affected by the amount of N added to the soil, neither by its interaction with chemical thinning. Such was the case with yield of fruits per tree which was calculated by multiplying the number of fruits per tree and the average fruit weight. Fruit thinning with NAA significantly decreased the number of fruits per tree at 30 and 60 kg·ha^{-1} N, while was inefficient when applied on unfertilized trees (fig. 3c). The yield was significantly decreased by NAA treatments at 60 kg·ha^{-1} N, but was independent on the N regime itself (fig. 3d). Fruit set and yield in Golden Delicious Reinders® were not negatively correlated with NAA concentration.

The significant effects on the average fruit weight and diameter were affected by the factors of the year and chemical thinning, as well as their interaction, while fruit size was not affected by N fertilization itself (tab. 4). However, changing N regime influenced variable effects of NAA to the fruit size (figs 4a and 4b). On unfertilized control plot, NAA significantly increased fruit weight only by the highest concentration of 14 µl·l^{-1}, while on fertilized plots all NAA treatments caused fruit weight and diameter to increase.

The sufficient N supply increases the fruit set of apple, while at the same time the thinning efficacy of NAA increases. At sufficient N supply, NAA becomes more efficient in increasing fruit size at the same thinning intensity.

Fruit flesh firmness was significantly affected by all factors assessed as well as by their interactions, therefore is the most complex trait concerning fruit quality (tab. 4). Flesh firmness decreases with the increase of the amount of N added to the soil (fig. 4c). Adding N to the soil caused a decrease in flesh firmness of fruits from unthinned trees only, while flesh firmness was unaffected by the N regime in thinning treatments with NAA. Thinning with NAA itself, caused flesh firmness to decrease only in fruits from unfertilized plot. Fruit thinning with NAA on plots fertilized either with 30 or 60 kg·ha^{-1} N did not influence flesh firmness.

The number of vital seeds in the apple fruits was significantly affected by all factors assessed except for the interaction between year and thinning (tab. 4). Seed set inside fruits was the highest at 60 kg·ha^{-1} N regime, counting about 8.3 seeds per fruit in average (fig. 4d). Fruit thinning with NAA did not affect the number of seeds on unfertilized plot, while at 30 kg·ha^{-1} N thinning decreased the number of seeds per fruit.

DISCUSSION

The final fruit set is the result of the interaction of chemical thinner applied, given N regime and environmental conditions of the year, meaning that the environmental conditions of the experimental year and N regime modify the effects of NAA applied for fruit thinning. The lower content of mineral N in the soil during the vegetation period in 2011 compared to the previous two years can be explained by weather conditions. The total amount of rainfall in 2011 (235 mm) was significantly lower than the long-term average (614 mm). The lower precipitation in 2011 could be a possible reason for lower solubility of N fertilizer which was applied in solid state in the form of ammonium nitrate and reduced mineralization of soil organic matter which finally resulted in a lower concentration of mineral N in the soil (fig. 1). Apple fruits are the active sink for N from their earliest growth stadium until 30 days before harvest [Tosseli et al. 2000]. Nitrogen applied at bloom increases the concentration of N in fruits, because fruits in the early stages of their growth successfully compete with other tree organs for N accumulation. The main source of N for pear flowers is N remobilized from storage, reaching 90% of N in flowers at the balloon stage. From petal fall, the amount of N taken up by the roots increases linearly to the beginning of fruit enlargement, still being less than N remobilized from storage [Tagliavini et al. 1997]. Fruit set was increased in N fertilizer treatments applied in spring and summer relative to unfertilized control in apple cv. Gold Rush, while yield was increased in the second year of the study [Wargo et al. 2003]. Such was the case in the present research, where fruit set was increased by increasing the amount of N applied to the plot at the pink bud stage in apple cv. Golden Delicious Reinders®. Curvilinear relationship between the rate of NAA applied and its thinning efficacy was previously reported [Robinson 2006] meaning that NAA can be more efficient in reducing fruit set when applied at lower than at higher rates. In accordance with previously published results, the efficacy of NAA for fruit thinning in Golden Delicious was not linearly correlated to the concentration of the chemical.

The total number of fruits per tree and yield were not affected by the N regime or by the interaction between N fertilization and thinning in the present research. The lack of effects of different amounts of N applied on yield and fruit weight was also recorded previously and explained by the low requirements of low-vigor apple trees for N, a good soil preparation before planting, maintaining herbicide strips (favors abundant N availability) along tree rows and increase of available N content in the soil during the vegetative period [Wrona 2011]. Ernani et al. [2008] calculated the maximum amount for N required for apples always less than 50 $kg \cdot ha^{-1}$, even during the years of high yields, which can be provided from organic matter decomposition in the soil. The total number of fruits per tree was inconsistently reduced in NAA treatments at 30 and 60 $kg \cdot ha^{-1}$ N, while total yield was reduced by thinning at 60 $kg \cdot ha^{-1}$ N, meaning that apple trees are more responsive to NAA application at higher N supply.

Excessive N supply leads to an increased fruit weight which further increases the total yield of apples [Raese et al. 2007]. As reported by Xia et al. [2009], the apple fruit size was increased by increasing N fertilization rate while maintaining the uniform fruit set on experimental trees of 6.5 $fruits \cdot cm^{-2}$. In consistence with Wargo et al. [2003], in the present research N fertilization did not cause an increase in fruit size because fruit size is negatively correlated with fruit set. As a consequence, there were no effects of N fertilization on total yield of apple cv. Golden Delicious Reinders® in the present research. According to Black et al. [2000], fruit size was negatively correlated with the number of fruits per spur, but not with total number of spurs. Therefore, the increased number of fruits per 100 flower clusters in N treatments might be the reason for unaffected fruit size. At sufficient N supply, fruits responded more intensively to thinning with NAA, by increasing their growth.

Carbohydrate supply might be a limiting factor for cell-wall formation in fruits from apple trees with high crop load [Zhu et al. 2011]. On the other hand, flesh firmness is negatively correlated with the average fruit weight which increases after thinning with bioregulators [Siddiqui and Bangerth 1995]. According to Neilsen et al. [2006], N from fertilizer applied at flowering period ends in leaves and fruits from the current season. An increase in N concentration in fruits may lead to relative decrease in Ca concentration [Nava and Dechen 2009], which can further cause the weakening of cell walls [Tahir et al. 2007] and decrease in flesh firmness at harvest [Wargo et al. 2003, Raese et al. 2007, Wang and Cheng, 2011]. In the present research, thinning with NAA caused a significant decrease in flesh firmness only in fruits from unfertilized plot, on which, also, the lowest fruit set was recorded compared to 30 and 60 $kg \cdot ha^{-1}$ N plots (fig. 4c). The reason for the decrease in flesh firmness of fruits from unfertilized trees might be redistribution of N available to the relatively smaller number of fruits. Although more N was available from fertilizer at 30 and 60 $kg \cdot ha^{-1}$ N plots, it was redistributed to relatively larger number of fruits which might prevent the relative decrease in Ca concentration and flesh firmness decline.

Seed set inside fruits was the highest at 60 $kg \cdot ha^{-1}$ which is in accordance with the research conducted by Duchêne et al. [2001] who observed that at high N supply, fruit set ratio and the number of seeds per berry of wine grape increases, concluding that seed number per berry is strongly affected by the late spring N supply.

CONCLUSIONS

The interaction between N fertilization and chemical thinning with NAA was found significant factor affecting fruit set, size, firmness and seed set of apple cv. Golden Delicious Reinders®. Fruit set increases with an increase in the amount of N applied in early spring, while NAA thinning efficacy increases at high N supply. Nitrogen fertilization did not cause an increase in fruit size. However, at sufficient N supply, fruits responded more intensively to thinning with NAA, by increasing their growth. Crop load and yield of fruits per tree were not affected by the interaction of investigated factors.

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