REGULAR ARTICLE

Timing and rates of NAA as blossom and fruitlet chemical thinner of apple cv. Braeburn

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ABSTRACT

Three concentrations of NAA (0, 10, 15 and 20 μ l·l⁻¹) were studied at three application timings (flower, fruit and the combination of each other) in order to evaluate the thinning effect on physico-chemical attributes of apple cv. Braeburn. NAA concentration was more significant factor than timing in terms of fruit production. The number and yield of fruits over 70 mm in diameter significantly increased in all application timings within all concentrations of thinner as compared to control. Thinning at 15 μ l·l⁻¹ in flower application appeared the best. The highest share of fruits over 70 mm was acquired using 20 μ l·l⁻¹ NAA in fruit application timing. The untreated trees yielded highest total number of fruits with maximum total yield efficiency and total yield efficiency in terms of number of fruits over 70 mm in diameter. Significant differences were found regarding the effects of NAA concentration and combination of both timing and concentration while timing alone showed non-significant results for SSC, TA, SSC/TA and starch. NAA at 20 μ l·l⁻¹ nesulted in highest fruit firmness, SSC and TA in fruit application time. The highest levels of SSC/TA and starch degradation level were recorded with NAA at 15 μ l·l⁻¹ sprayed in combination of flower and fruit applications. Comparatively, application of 10 μ l·l⁻¹ NAA at both flower and fruit application timing is recommended to achieve best results.

Keywords: Apple cv. Braeburn; NAA; Thinning; Productivity; Fruit quality

INTRODUCTION

Fruit thinning has a paramount importance in producing good sized fruit of high and uniform quality with regular yield in apple fruit crop (Milić et al., 2016; McArtney et al., 2013, McArtney et al., 2007; Greene and Costa, 2013). Thinning can be done manually, mechanically and by using chemical thinners to save on labour cost and make better economic return (López et al., 2011; Stopar and Lokar, 2003; Greene and Costa, 2013). The chemical thinners may be consisted of various chemical compounds, but the application of plant growth regulators and some insecticides are of preference in this regard (Reyes et al., 2008). The mode of action of the chemical thinners may be varied like ethephon and carbaryl chemicals normally effect and alter the hormonal status of the fruitlets while some chemicals like auxin and cytokinin (PGRs) exhibit their effect on whole plant not just limited to flower or fruitlets (Bangerth, 2000; Cin et al., 2007; Wertheim, 2000). However, the use of chemical thinners in inappropriate application time, dosage or without knowing the cultivar response, often resulted in yield reduction (Stopar and Lokar, 2003; Esitken et al., 2009; Milić et al., 2012), decreased fruit growth and fruit russeting (Keserović et al., 2016; Weibel et al., 2012; Stopar et al., 2007a; Milić et al. 2012), poor colouration of fruits (Byers and Carbaugh, 1991; Wertheim, 2000) and sometimes resulted in lowering the Ca concentrations in fruits (Elfving and Cline, 1993).

In apple, thinning may be done on flower and fruitlet alone or in combination as it maximizes the thinning effect. There are a number of chemical thinners available but with limitation of either blossom thinner (ammonium thiosulphate (ATS), sodium chloride, ethephon, armothin etc.) or fruitlet thinner (usually done by hand thinning as very few chemical thinners are effective such as: NAA, benzyladenine (BA), naphthalene acetamide (NAD) etc.) and only few works in both action. Other compounds, such as potassium-bicarbonate (KHCO₃) was successfully tested as blossom thinner for organic apple cultivation

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on 11 different cultivars of apple, including cv. Braeburn (Weibel et al., 2012). Cv. Braeburn is a newly introduced cultivar of apple in Croatia. It is highly productive but is highly prone to alternate bearing (McArtney et al., 2007). The thinning is required for obtaining regular bearing in cv. Braeburn as it produces flowers abundantly and has a high fruit set as well. Since, high crop load leads in decreasing fruit size especially in young apple trees (Milić et al., 2011).

NAA is a synthetic plant growth regulator of auxin group and widely applied as chemical thinner of apple. However, its efficacy significantly differs among apple cultivars due to some environmental factors as well as the amount of dosages (Greene, 2002; Wertheim, 2000; Stopar et al., 2007b). NAA lowers the efflux of diffusible auxin, especially from weaker fruitlets (Wertheim, 2000; Schröder and Bangerth, 2006; Bangerth, 2000) and reduce the availability of carbohydrates to the developing fruits by interfering with photosynthesis (Zhu et al., 2011; Untiedt and Blanke, 2001) which promotes abscission in young fruitlets.

The NAA thinning efficacy is inconsistent and it is hard to predict the outcome since it depends on environmental conditions (Schröder and Bangerth, 2006; Keserović et al., 2016) which can lead towards extensive fruit dropping or insufficient thinning that can cause higher production costs for growers. Such unpredictable problematic issues shows the inadequate comprehension regarding its mode of action in promoting the fruit abscission. However, multiple post-bloom application of NAA have positive florigenic activity in apple that contribute to regular cropping and decrease biennial bearing (McArtney et al., 2013), making NAA useful tool in maintaining regular yield of apple crop. Significant florigenic activity has been achieved even with single application of Iow NAA concentrations (Milić et al., 2012)

Therefore, the aim of current study was to investigate the effect of different application timings and dosages of NAA in order to achieve the proper thinning in apple cv. Braeburn.

MATERIALS AND METHODS

The trials on fruit thinning experiments were conducted on cv. Braeburn during 2012 in a commercial apple orchard near Krapina, Croatia (latitude 46° 09' N, longitude15° 53' E) using commercial chemical thinner Diriger[®] (NAA) with three different NAA concentrations (0, 10, 15 and 20 μ l·l⁻¹) applied in following timings: thinning in full bloom (FBT), fruit thinning (FT) when king fruitlets were 6-8 mm in diameter either alone, and their combination (FBT + FT). Thinner concentration and application timing were considered as individual factors and experiment was set up according to two-factorial experimental design with completed randomized block design.

Experiments were carried out on adult trees (8 years old) of the apple cv. Braeburn, planted with 3 m \times 1 m distances and grafted onto M9 rootstock. The trees were trained in spindle bush training form. Experiment was performed as complete randomized block with three replicates consisting of five trees per replicate. Trees were not irrigated, and other cultural practices (pest control, pruning, fertilization, weed control) were performed as per recommendations form Agricultural Extension Service of Croatia.

The parameters recorded were the trunk cross sectional area measured at 25 cm above grafting place (TCSA), crop density (number of fruits cm⁻² TCSA) of fruits over 70 mm and all fruits, mean fruit weight of fruit over 70 mm in diameter, mean fruit weight of all fruits, share of fruit over 70 mm (%), yield of fruits over 70 mm in diameter (marketable yield) and total yield per tree ((kg·tree⁻¹), yield efficiency of fruits over 70 mm in diameter (kg·cm⁻² TCSA) and total yield efficiency (kg·cm⁻² TCSA). The fruit quality parameters studied were fruit firmness (kg·cm⁻²), soluble solids concentration (SSC) expressed as °Brix, titratable acidity (TA) expressed as % of malic acid, SSC/TA ratio and starch level degradation. The starch level degradation was scored using a 10 point CTIFL scale (Centre Technique Interprofessionnel des fruits et Legumes, Paris, France). Chemical analyses performed on 15 randomly selected fruits from each treatment in three replicates.

Weather data were taken from Croatian Meteorological Service from weather station located about 500 m from experimental orchard.

Data analysis

Data were statistically analysed using SAS statistical software ver. 9.4 (SAS Institute, NC) using ANOVA, regression analysis and LSD test ($P \le 0.05$). Regression analysis was performed with thinner concentration (C) as constant on combined data from all three application times (T) for traits with significant effect of C, and nonsignificant C × T interaction (TCSA, marketable yield, total yield, total yield efficiency and fruit weight). Since thinning was performed twice in FBT+FT timing, actual thinner concentrations in this treatment were 20, 30 and 40 µl·l⁻¹ and this fact was taken into account when performing regression analysis.

RESULTS AND DISCUSSION

Thinner concentration (C) was more important factor for thinning than timing since it significantly affected ($P \le 0.05$) all studied parameters except average weight of fruits over

70 mm in diameter. Application timing (T) significantly affected only total yield efficiency. Interaction $C \times T$ was significant for share of fruits over 70 mm in diameter in the tree as well as their yield efficiency (Table 1). Regression analysis showed the better fit with quadratic model than with linear and exponential models (data not shown).

Regression model for TCSA (TCSA = $0.002C^2$ +0.283C+16.586) was significant (*P*=0.025) but both C² and C coefficients were nonsignificant with low R² (0.201). This means that vegetative growth was not affected by thinning since only low proportion of TCSA variability could be explained by regression model and is additional confirmation of mostly nonsignificant effect of thinner concentration in individual application timings on TCSA shown in Table 2. However, FT with 10 µl·l⁻¹ NAA and FBT + FT with 20 µl·l⁻¹ significantly increased TCSA as compared to other concentrations (Table 2) due to the decreased crop load (Fig. 1) that can significantly affect vegetative growth (Radivojević et al. 2014).

The data presented in Table 2 and Fig. 1 show that the effect of thinner concentration was different depending on timing. NAA applied at 15 μ l·l⁻¹ in FBT appeared best for important productivity parameters (share of fruits

over 70 mm in diameter, marketable yield and marketable yield efficiency and crop density of fruits over 70 mm in diameter). For FT, the same effect was achieved with

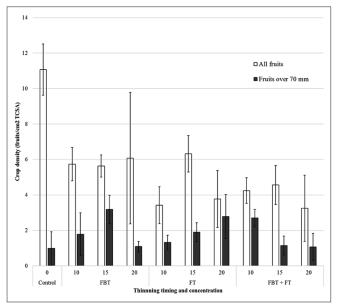


Fig 1. The effect of NAA thinning time (FBT, FT and FBT+FT) and concentration (10, 15, and $20 \ \mu l \cdot l^{-1}$) on crop density of cv. Braeburn apple (error bars represent standard deviation). FBT: Full bloom thinning; FT: Fruit thinning; FBT + FT: Full bloom and fruit thinning.

Table 1: ANOVA table of fruit production parameters as affected by timing of thinning with NAA, concentration of thinner and combination of both in apple cv. Braeburn

Source of variability	TCSA (cm²)	Fruit weight over 70 mm (g)	Total fruit weight (g)	Share of fruits over 70 mm (%)	Yield of fruits over 70 mm (kg·tree ⁻¹)	Total yield (kg·tree⁻¹)	Total yield efficiency (kg·cm ⁻²)	Yield efficiency over 70 mm (kg·cm ⁻²)
Timing	0.78 n.s.	0.96 n.s.	0.91 n.s.	0.66 n.s.	0.68 n.s.	0.29 n.s.	0.05*	0.68 n.s.
Concentration	0.03*	0.11 n.s.	0.004**	0.0003***	0.01*	0.06*	0.0001***	0.01*
T×C	0.16 n.s.	0.48 n.s.	0.22 n.s.	0.02*	0.07 n.s.	0.54 n.s.	0.17 n.s.	0.002**

n.s. *,***: Nonsignificant or significant at $P \le 0.05$ or 0.001, respectively

Table 2: The effect of NAA thinning time (FBT, FT, FBT+FT) and concentration (10, 15, 20 µl·l⁻¹) on productivity of apple cv. Braeburn

Treatment	Thinner concentration (μl·l⁻¹)	TCSA (cm²)	Fruit weight over 70 mm (g)	Total fruit weight (g)	Share of fruits over 70 mm (%)	Yield of fruits over 70 mm (kg·tree ⁻¹)	Total yield (kg·tree ⁻¹)	Total yield efficiency (kg·cm ⁻²)	Yield efficiency over 70 mm (kg⋅cm ⁻²)
Flower	0	14.15a	162.56a	120.06a	12.19b	2.33b	18.63a	1.31a	0.15b
thinning	10	25.55a	165.97a	136.42a	36.29ab	6.43ab	19.37a	0.79b	0.30ab
	15	22.16a	183.22a	165.20a	63.25a	13.73a	20.57a	0.92b	0.59a
	20	18.73a	207.50a	160.10a	37.54ab	5.23ab	12.40a	0.83b	0.23b
Fruit	0	14.15b	162.56b	120.06b	12.19c	2.33b	18.63a	1.31a	0.15b
thinning	10	31.16a	172.29b	149.87b	48.20b	7.23ab	14.87a	0.50c	0.23 b
	15	21.20ab	170.38b	141.54b	35.78bc	7.07ab	19.37a	0.89b	0.32ab
	20	20.99ab	202.43a	187.50a	81.84a	11.53a	14.13a	0.68bc	0.56a
Flower	0	14.15b	162.56c	120.06b	12.19b	2.33b	18.63a	1.31a	0.15b
and fruit	10	18.44b	196.15a	172.83a	74.13a	9.98a	13.30b	0.72b	0.53a
thinning	15	21.98ab	182.94b	146.26ab	31.37b	4.63ab	14.20b	0.66bc	0.21b
	20	34.61a	173.22b	148.49ab	44.14ab	6.10ab	14.00b	0.46c	0.19b

Means followed by the same letter within the same timing are not significant according to the LSD test at P≤0.05 level

NAA at 20 μ l·l⁻¹. This treatment also achieved significantly higher mean fruit weight of fruits over 70 mm in diameter as compared to other concentration within this timing. FBT + FT achieved the best results when NAA was applied at 10 μ l·l⁻¹. FBT + FT with 15 and 20 μ l·l⁻¹ NAA significantly reduced average fruit weight of fruits over 70 mm (Table 2) and crop density of fruits over 70 mm in diameter (Fig. 1) compared to thinning with 10 μ l·l⁻¹ NAA. This indicates negative effects of high dosages of NAA since in this treatment actual NAA concentrations were 20, 30 and 40 μ l·l⁻¹ (see Material and Methods).

The average fruit weight in thinned trees was significantly higher compared to the control, but difference was not always significant (Table 2). Regression model for average fruit weight (Fruit weight = $-0.080C^2 + 3.839C + 118.71$) was significant (P < 0.001) together with both C² and C coefficients (P < 0.001 and P = 0.002, respectively) and constant (P < 0.001). However, R^2 was 0.377, meaning that low proportion fruit weight variability could be explained by thinner concentration alone and additionally supports importance of application timing as discussed previously. Crop load significantly affected fruit weight in apple cv. Braeburn as the fruits with diameter of less than 70 mm were only found on the trees carrying the heaviest crop (Radivojević et al. 2014). In FBT + FT, there was no significant difference in total number of fruits in trees thinned with 10 and 15 µl·l⁻¹ NAA, but average fruit weight was lower at 15 µl•l-1 (Fig. 1). This means that crop load cannot be the only factor responsible for decreasing fruit weight. Possible explanation for reduced fruit weight might be the negative effect of high NAA concentration on fruit growth (Milić et al., 2016). No significant differences among thinned and un-thinned apples cv. Braeburn in terms of fruit weight in Bosnian agro-climatic conditions (Misimović et al., 2012). Higher concentrations were needed to achieve increase of crop density of fruits over 70 mm in FT (20 μ l·l⁻¹) than in FBT (15 μ l·l⁻¹), but in FT the share of fruit was higher (81.84%) than in FBT (63.25%) (Table 2). This confirms NAA as an effective post-bloom (fruit) thinner (Greene and Costa, 2013). Although NAA can be similar to BA in thinning efficacy of cv. Braeburn considering regulation of fruit set and fruit weight increase (Milić et al., 2012), our results show that concentrations higher than 10 µl·l⁻¹ are needed to achieve significant effect. This might be due to favourable climatic conditions (Fig. 2) after thinning which enabled good carbohydrate supply of fruits and more resistance of fruits to NAA (Lakso and Robinson, 2015; Lakso et al., 2006; Robinson et al., 2016; Schröder and Bangerth, 2006).

Regression model for marketable yield (Marketable yield= $-0.014C^2+0.599C+2.590$) was significant (*P*=0.003) together with both C² and C coefficients (*P*=0.01 and 0.003,

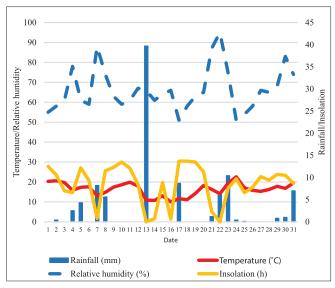


Fig 2. Air temperature, relative humidity and solar insolation after thinning treatments (May 2012). Thinning treatments were performed on 1. 05. 2012. (FBT: Full bloom thinning) and 15. 05. 2012 (FT: Fruit thinning).

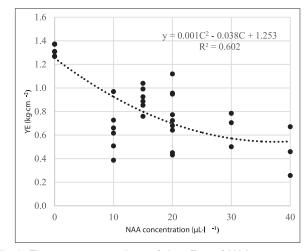


Fig 3. The regression analysis of the effect of NAA concentration (10, 15, 20, 30 and 40 μ l·l⁻¹) on yield efficiency of apple cv. Braeburn

respectively) and constant was close to P=0.05 significance level (P=0.063). However, R² was only 0.291, meaning that low proportion of marketable yield variability could be explained by thinner concentration alone and additionally supports importance of application time (T) as discussed previously.

There was no significant reduction in total yield as compared to control, except for FBT + FT (Table 2) where yield was significantly lower in thinned trees than in control tress, as result of significantly decreased total number of fruits per tree (Fig. 1). Regression model for total yield (Total yield = $0.002C^2$ -0.204C+19.015) was nonsignificant (data not shown) with lowest R² (0.163), which additionally supports the results presented in Table 2.

Thinning resulted with increase fruit weight which can compensate the reduction of fruit number per tree (Milić et al., 2012; Keserović et al., 2016), although some authors reported opposite effects (Stopar and Lokar, 2003; Esitken et al., 2009) and even increase of yield as result of thinning (Stopar et al., 2007b). Combined flower and fruit thinning showed some detrimental effect on fruit productivity at the NAA concentration above 10 µl·l⁻¹. The possible explanation for this can be accumulation of NAA which caused reduced fruit growth (Milić et al., 2016), which is additionally supported by the fact that yield efficiency of fruits over 70 mm was significantly lower in these treatments (Table 2). Furthermore, regression model for total yield efficiency (YE) (Total YE = 0.001C-0.038C+1.253) was significant (P < 0.001) together with both C² and C coefficients (P=0.025 and P<0.001, respectively) and constant (P < 0.001). R² was 0.602, meaning that total YE variation can be well described as the result of thinner concentration. This additionally confirms significant effect of thinner concentration in individual thinning applications (Table 2). The highest YE was achieved with 15 and 20 μ l·l⁻¹ NAA (Fig. 3).

Milić et al. (2012) evaluated the NAA and BA efficiency regarding the fruit set, quality and the bearing potential of the apple cultivars Braeburn and Camspur in Serbia. They used NAA at 6, 8, 10 μ l·l⁻¹, BA at 50, 100 and 150 μ l·l⁻¹

Table 3: ANOVA table for the effect of NAA thinning time (FBT, FT, FBT+FT) and concentration (10, 15, and 20 μ l·l⁻¹) on fruit quality of apple cv. Braeburn

Source of variability	Firmness (kg·cm ⁻²)	SSC (°Brix)	TA (% as mallic)	SSC/TA	Starch (1-10 CTIFL)
Timing	0.0001***	0.059 n.s	0.10 n.s	0.19 n.s.	0.46 n.s.
Concentration	0.14 n.s.	0.001***	0.001***	0.001***	0.08 n.s.
T×C	0.0006***	0.001***	0.001***	0.04*	0.001***

n.s. *,***; Nonsignificant or significant at P≤0.05 or 0.001, respectively

dosages to cv. Braeburn and 8, 10, 12 μ l·l⁻¹ NAA, 50, 100 and 200 μ l·l⁻¹ BA to cv. Camspur. They found that both thinners efficiently reduced the fruit set in cv. Braeburn while showed inconsistent results in terms of fruit quality parameters in cv. Braeburn. NAA application significantly affected the fruit quality in cv. Braeburn (Tables 3 and 4). Significant differences ($P \le 0.05$) were found regarding the effects of NAA concentration (except on starch conversion level) and combination of both timing and concentrations while timing alone showed non-significant results for SSC, TA, SSC/TA ratio and starch degradation level (Table 3).

Fruit firmness increases as direct result of the reduction in fruit number and yield (Johnson 1994), but in some cases decrease is recorded (Misimović et al. 2012). In our study, thinning with NAA exhibited decrease in case of FBT and increase in FT and mix response in case of FTB + FT as compared to untreated trees. Inconsistent effect of NAA on firmness of cv. Braeburn was also found by other authors (Milić et al., 2012; Keserović et al., 2016). The variations in results might be occurred according to type of cultivar & thinner, time of application and climatic conditions. Thinning in FT application timing resulted with higher firmness as compared with other application timings. Values were above level (8.19 kg·cm⁻²) required for long CA storage, while firmness of fruits thinned with 10 and $20 \,\mu l \cdot l^{-1}$ NAA in FBT, and with $15 \,\mu l \cdot l^{-1}$ NAA in FBT + FT were more appropriate for middle term CA storage (Belding, 2006). Only fruits thinned with 20 µl·l⁻¹ NAA in FBT and 15 µl·l⁻¹ NAA in FBT+FT were softer than minimum value (8.0 kg·cm⁻²) required for start of harvest in South Africa (Harker, personal communication). Increased flesh firmness and dry matter with decreasing crop load was found in cv. Braeburn in New Zealand (Wünsche et al., 2005). Furthermore, authors stated that typically, advanced maturity in light-cropping trees is indicated by

Table 4: The effect of NAA thinning time (FBT, FT, FBT+FT) and concentration (10, 15, 20 µl·l·1) on fruit quality of apple
cv. Braeburn

Treatments	Thinner concentration (µŀŀ¹)	Firmness (kg·cm ⁻²)	SSC (°Brix)	TA (% as mallic)	SSC/TA	Starch (1-10 CTIFL)
Flower thinning	0	8.50a	12.02a	0.50b	23.86b	5.88b
	10	8.07ab	11.53b	0.59a	20.08c	6.90a
	15	8.18ab	11.98a	0.46c	26.49a	6.10b
	20	7.91b	11.12c	0.44c	24.80ab	5.80b
Fruit thinning	0	8.48b	12.05b	0.52b	23.28ab	6.33ab
	10	8.52b	10.86d	0.55ab	20.53b	6.40ab
	15	8.58ab	11.40c	0.46c	25.20a	5.80b
	20	8.87a	12.77a	0.59a	21.79b	6.70a
Flower and fruit thinning	0	8.49ab	12.03b	0.51a	23.63b	6.06b
	10	8.74a	11.60c	0.51a	22.87bc	6.33b
	15	7.97c	12.52a	0.48a	27.08a	7.11a
	20	8.35b	11.22d	0.54a	21.07c	6.00b

Means followed by the same letter inside one treatment do not differ significantly according to LSD test at $P \le 0.05$ level

greater starch conversion and a higher percent of soluble solids compared with fruit on high-cropping trees. This is not in accordance with the results obtained in our study.

SSC level in our study was above 10.5° Brix (Table 4), the minimum value required for apples in EU Commission regulations (EC 460/2008 amending regulation (EC) No 85/2004), and within the harvest values (between 11.5° and 14.5° Brix) established for cv. Braeburn in south Africa (Harker, personal communication), except for fruits thinned in FBT with 20 µl·l⁻¹ NAA, FT with 10 and 15 µl·l⁻¹ NAA and FBT+FT with 20 µl·l⁻¹ NAA (Table 4). However, for the last two cases, the SSC values were close to the minimum values of 11.5° Brix. However, all fruits had fair (> 11.0° Brix) and good (higher >12° Brix) SSC values required for storage (Belding, 2006). TA levels were within the optimal values (0.4-0.6 %) (Harker, personal communication). No significant differences for total soluble solids (TSS) and total acidity (TA) in cvs. Gala and Braeburn apples were found as related to crop load (Radivojević et al., 2014), which is similar to our study. In cv. Golden Delicious increase of SSC and decrease of TA is found as result of thinning with NAA, but result was depended on concentration and was not consistent across the years (Keserović et al., 2016). The highest levels of SSC/TA were recorded with NAA at 15 μ l·l⁻¹ in FBT + FT (Table 4).

There were no much differences in starch conversion rates found among NAA concentrations in individual application timings. The highest levels of starch conversion rate were recorded with NAA at 15 μ l·l⁻¹ in FBT + FT (Table 4) and was significantly different from control and other thinning concentrations in that application time. The same was true for fruit from trees thinned with 10 μ l·l⁻¹ NAA in FBT. Other authors found increase or no effect on starch conversion rate as a result of thinning of cv. Braeburn with NAA (Milić et al., 2012). They used much smaller concentrations and thinned trees and applied only FT but their results show strong impact of climatic conditions on fruit quality parameters of chemically thinned trees of cv. Braeburn.

CONCLUSIONS

The best result was achieved when trees were thinned with 20 μ l·l⁻¹ of NAA when king fruitlet were 6-8 mm in diameter. The second best yield of marketable fruit of apple cv. Braeburn was achieved when trees were thinned with 10 μ l·l⁻¹ NAA in full bloom and again when king fruitlet were 6-8 mm in diameter. These results show that there is a cumulative effect of NAA concentration which is needed for achievement of high marketable yield apple cv. Braeburn. However, higher concentration can have even detrimental effect on both yield and fruit quality.

Author contributions

TJ is the research supervisor who designed the experimental plan and helped in data analysis. MF, MV, MJ, GF and JB participated in the experimental part of this study (field work and measurements). MJ interpreted the data and prepared the draft after which all other authors contributed towards the final shape of the manuscript. In addition, all authors were involved in the review and editing of the final manuscript. There is no any conflict of authors.

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