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Comparison of metamitron efficiency for postbloom thinning of young 'Gala' and 'Golden Delicious' apple trees

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Abstract: The aim of this study was to evaluate the thinning efficacy of metamitron applied either alone (single or double application) or in combination with 6-benzyladenine (BA) or naphthalene acetic acid (NAA) in young 'Gala' and 'Golden Delicious' apple trees. The experiment was carried out during the first three crop seasons, 2014, 2015, and 2016, at the commercial orchard located on the mountain Fruska Gora, Serbia. Application of chemical thinners was carried out at two fruit diameter stages (6–9 and 12–15 mm). The number of fruit and yield per tree, fruit size distribution, parameters of fruit ripeness, and return bloom were assessed. Single metamitron treatments were effective when the weather conditions were favourable on the day of application and 3 days after (minimum night temperature >10 °C, maximum daily temperature >20 °C) regardless of fruit diameter stages. A reduction in fruit number per tree was followed by an increase in mean fruit weight. Double metamitron application at lower concentration caused relatively stable thinning activity during the whole experimental period in both cultivars tested. Metamitron combined with either BA or NAA at standard rates significantly reduced the negative influence of low temperature on metamitron thinning activity in the first experimental year. These treatments slightly influenced overthinning in 'Gala' trees if applied when the air temperature was too high (minimum night temperature >15 °C, maximum daily temperature >25 °C). Repeated metamitron treatment and treatments in which metamitron was combined with BA and NAA resulted in an increase in fruit size and share of large fruit in the total yield during the whole experimental period in both cultivars tested. A reduction in fruit number per tree affected by these treatments was followed by increasing flowering intensity in the subsequent year, with the exception of double metamitron application to 'Golden Delicious' trees in the last experimental year. Fruit maturity parameters were slightly different under the influence of metamitron treatments.

Key words: Metamitron, alpha-naphthylacetic acid, 6-benzyladenine, crop load, fruit quality, return bloom

1. Introduction

Establishing new apple orchards using well-feathered apple trees has recently become common practice. In favourable growing conditions, these trees can produce high yield in the second leaf, achieving full productivity in the fourth leaf (Radivojevic et al., 2014). High-quality trees of the cultivars 'Gala' and 'Golden Delicious' during the establishing period frequently produce an excessive number of fruitlets but this can be insufficient to guarantee fruit of good marketable size at harvest (Bregoli et al., 2007). Furthermore, an unwanted excessive number of fruit per tree during that period can reduce the growth of a young tree and delay the achievement of maximum tree size. Because of this, trees require adequate thinning to reach marketable fruit sizes and regular yields by preventing alternate bearing (Maas, 2006). In mature orchards chemical fruit thinning is a major cultural practice (Bound, 2006; Dorigoni and Lezzer, 2007), but in young apple orchards hand fruit thinning is usually done. However, due to the increasing lack of seasonal workers and high labour costs, this job is becoming unrealisable, especially in large areas (Radivojević et al., 2011). Moreover, hand thinning is usually carried out after June-drop, by which time only increases in fruit size are achieved and there is no reduction in alternate bearing (Maas, 2006). Chemical thinning, as compared to hand thinning, is a quick operation and allows thinning fruit at the right moment, guaranteeing better fruit quality and significantly reducing labour costs (Costa et al., 2006). In young apple orchards chemical thinning has to be regularly performed and start in the second leaf, whereby different chemicals can be applied either alone or in combination in order to improve reliability and thinning efficacy (Verjans et al., 2018). Plant growth regulators such as auxins and cytokinins have become essential in commercial apple production (Brunner, 2014; Stern, 2015). However,

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when benzyladenine (BA) is used alone it is considered a relatively mild thinner (Fallahi et al., 2014). The effect of chemical thinners is dependent on the interaction between genotype and climatic conditions (Petri et al., 2016), and in some years naphthalene acetic acid (NAA) and BA, alone or in combination, fail to thin apples when applied at a fruit diameter of 5–16 mm due to low temperature after applications (Yuan, 2007). The climatic variability observed in past years in Serbia makes chemical thinning difficult at the appropriate fruit size. Therefore, new chemical thinners with another mode of action should be tested during certain stage of trees development. One of the new chemical thinners is the triazinone herbicide metamitron, which at a low dosage reduces photosynthesis by blocking electron transport in photosystem 2 and consequently enhances fruit drop (Dorigoni and Lezzer, 2007; Lafer, 2010; Basak, 2011; Stern, 2014). Metamitron exhibited thinning activity when applied to apple fruitlets 10 to 12 mm in diameter (Lafer, 2010) or even later at 20 mm (McArtney et al., 2012). As noted by Maas and Meland (2016), metamitron usually achieves much stronger thinning activity than the other thinning agents, whereby its activity also depends on the climatological conditions before and after application. However, there is a lack of information about the possibility of using metamitron as a postbloom chemical thinner in young apple trees, as well as the effects of postbloom metamitron application in combination with BA and NAA chemicals, which are commonly used for fruitlet thinning.

The objective of the present study was to evaluate the thinning efficacy of metamitron applied either alone (single or double application) or in combination with BA or NAA in young 'Gala' and 'Golden Delicious' apple trees during the first three crop seasons.

2. Materials and methods

2.1. Experimental design and treatments

The experiment was conducted on young 'Gala' and 'Golden Delicious' apple trees in the commercial plantation "Zlatni Jazak" located on the mountain Fruska Gora (45°06′N and 19°46′E, 260 m a.s.l.). Well-feathered nursery trees with more than 7 lateral branches of the cultivars 'Gala' 'Schniga® Schnitzer(s)' and 'Golden Delicious' 'Reinders®', grafted on a M9 rootstock, were planted in 2013 in single rows at a distance of 3.2 × 0.8 m (3906 trees per hectare). Planted trees were minimally pruned and a slender spindle tree shape was formed according to the standards of integrated fruit production used in Serbia. For the purpose of pollination 'Professor Sprenger' and 'Golden Gem' trees were planted alternately within every second row, at every 20th position. The irrigation system consisted of a lateral line (Eurodrip S.A., Greece), with 2 L h^{-1} pressure-compensated in-line drippers, spaced 0.8 m apart.

The trials were conducted during three consecutive years (2014–2016), starting from the second leaf. Trees that were uniform according to size and the first crop load were selected in the first year of the experiment. In the next two experimental years all treatments were replicated and applied to the same experimental trees. The experimental design was randomised block with four replicates, whereby every replication consisted of two trees. The following commercial products containing a different active ingredient were applied: 1) "Brevis" (Adama Agan Ltd., Israel) containing 150 g kg^{-1} metamitron (MM) as the active ingredient; 2) "Dirager" (L. Gobbi s.r.l., Italy) containing 33 g L^{-1} alpha-naphthylacetic acid (NAA) as the active ingredient, 3) Globaryll 100 (Globachem NV, Belgium) containing 100 g L^{-1} 6-benzyladinine (BA) as the active ingredient. In treatments in which more than one thinner was applied, the thinners were tank mixed and then applied together. Application was performed by knapsack sprayer as diluted solutions until runoff, equivalent to a spray volume of 400 L ha^{-1} in the second leaf, 700 L ha^{-1} in the third leaf, and 1000 L ha^{-1} in the fourth leaf. No wetting agent was added. Unsprayed trees served as a control treatment, while tank mix solution of BA and NAA served as a commercial control treatment. Depending on the cultivar, different concentrations were applied (Tables 1 and 2).

Weather conditions during the spray application period were very variable and depended on the year. Daily temperatures and rainfall per each experimental year (2014–2016), starting from 3 days before the first application and finishing 10 days after the last application, are presented in Figure 1.

The apples were harvested in August ('Gala') and September ('Golden Delicious') in a single pass-through and afterwards, based on their diameter, they were graded into five classes by hand calibrator at intervals of 5 mm ('Gala' from <65 mm to >80 mm, 'Golden Delicious' from <70 mm to >85 mm). The apples of each class were counted and weighed by a digital scale with accuracy of ±0.001 kg. Total yield per tree was calculated as a total of the mass of all classes. Average fruit weight (g) was obtained from a ratio of total yield and total number of harvested fruit. In 2016, pygmy fruit (apples <50 mm in diameter) appeared in 'Golden Delicious'. They were not included in the fruit weight and were recorded separately in the size results. Samples of 20 randomly selected fruit from each replication were examined for fruit firmness (kg cm^{-2}), iodine-starch index (rating scale of 1–5), and total soluble solids content (°Brix). Additionally, percentage of the surface that was red was visually evaluated on all sampled fruit of 'Gala' in the two years (2015 and 2016). Fruit flesh firmness was measured with a penetrometer using a tip with a diameter of 11 mm on two opposite sides of the

Table 1. Treatments in the thinning trial with the cultivar 'Gala'.

1. 200 mg L^{-1} MM + 200 mg L^{-1} MM. First application was at 6–9 mm and second at 12–15 mm fruitlet diameter (MM 200 I + II)
2. 250 mg L^{-1} MM, applied at 6–9 mm fruitlet diameter (MM 250 I)
3. 250 mg L^{-1} MM, applied at 12–15 mm fruitlet diameter (MM 250 II)
4. 250 mg L^{-1} MM + 10 mg L^{-1} NAA, applied as tank mix solution at 12–15 mm fruitlet diameter (MM 250 II + NAA II)
5. 250 mg L^{-1} MM + 75 mg L^{-1} BA, applied as tank mix solution at 12–15 mm fruitlet diameter (MM 250 II + BA II)
6. The commercial control treatment was 100 mg L^{-1} BA + 10 mg L^{-1} NAA, applied as tank mix solution at 12–15 mm fruitlet diameter (CCT)
7. Untreated control treatment (UCT)

Table 2. Treatments in the thinning trial with the cultivar 'Golden Delicious'.

1. 225 mg L^{-1} MM + 225 mg L^{-1} MM. First application was at 6–9 mm and second at 12–15 mm fruitlet diameter (MM 225 I + II)
2. 300 mg L^{-1} MM, applied at 6–9 mm fruitlet diameter (MM 300 I)
3. 300 mg L^{-1} MM, applied at 12–15 mm fruitlet diameter (MM 300 II)
4. 300 mg L^{-1} MM + 15 mg L^{-1} NAA, applied as tank mix solution at 12–15 mm fruitlet diameter (MM 300 II + NAA II)
5. 300 mg L^{-1} MM + 100 mg L^{-1} BA, applied as tank mix solution at 12–15 mm fruitlet diameter (MM 300 II + BA II)
6. The commercial control treatment was 150 mg L^{-1} BA + 10 mg L^{-1} NAA, applied as tank mix solution at 12–15 mm fruitlet diameter (CCT)
7. Untreated control treatment (UCT)

fruit. Juice squeezed from the apples during the firmness measurements was collected to analyse total soluble solids concentration using a digital refractometer (Pocket PAL-1, Atago, Japan). The apples were then cut and half-fruit were dipped for 5 s in a solution of 1 g of potassium iodide plus 0.25 g of iodine in 100 mL of water. The starch patterns, which indicated the relative amounts of starch, were scored on a scale of 1–5 (Werth, 2009), where 1 represents maximum starch content and 5 complete degradation of the starch. Return bloom was estimated as the flowering index at full bloom in the year after the treatment using an arbitrary scale (1 = no flowers, 9 = abundant flowering) (Lafer, 2010).

2.2. Statistical analysis

The data were analysed using analysis of variance (ANOVA) and the software Statistica 8 (StatSoft Inc., Tulsa, OK, USA). The data are presented as mean values for each treatment. Fisher's least significant difference (LSD) test was used to assess the significance of differences between the treatment means at $P \leq 0.05$.

3. Results

3.1. 'Gala'

In the first experimental year (the second leaf, 2014) metamitron treatments resulted in thinning activity in 'Gala' trees (Table 3). All treatments, with the exception of metamitron treatment applied alone when fruitlet diameter was 12–15 mm, significantly reduced final fruit number compared with the untreated control. There was a visible trend for more thinning in the treatments in which metamitron was applied twice or with simultaneous application with BA or NAA. Although postbloom application of metamitron caused thinning, total yield per tree did not differ significantly among the treatments due to an increase in fruit size as a consequence of reduced crop load. In these treatments, the yield of small fruit (<70 mm) was significantly reduced and the quantity of large fruit (75–80 mm) almost doubled compared to a single metamitron application at 12–15 mm fruitlet diameter and the control treatment (Figure 2A). The highest average fruit weight was achieved by treatments with double metamitron application and in the treatment in which metamitron was combined with BA. Metamitron also had an effect on return bloom. All metamitron treatments significantly increased flower index compared to an unthinned control treatment (Figure 3). In the second experimental year (the third leaf, 2015), there were no statistically significant differences among the studied treatments for the number of fruit or the yield per tree (Table 3). However, significantly higher fruit weight was recorded both in the treatment with double metamitron application and in the combined treatment with BA compared to the untreated control treatment. It was influenced by a significantly higher yield of larger fruit classes (75–80 mm) and a lower yield of small fruit (<70 mm) in these treatments (Figure 2B). Return bloom for the next year was abundant without significant differences among the treatments (Figure 3).

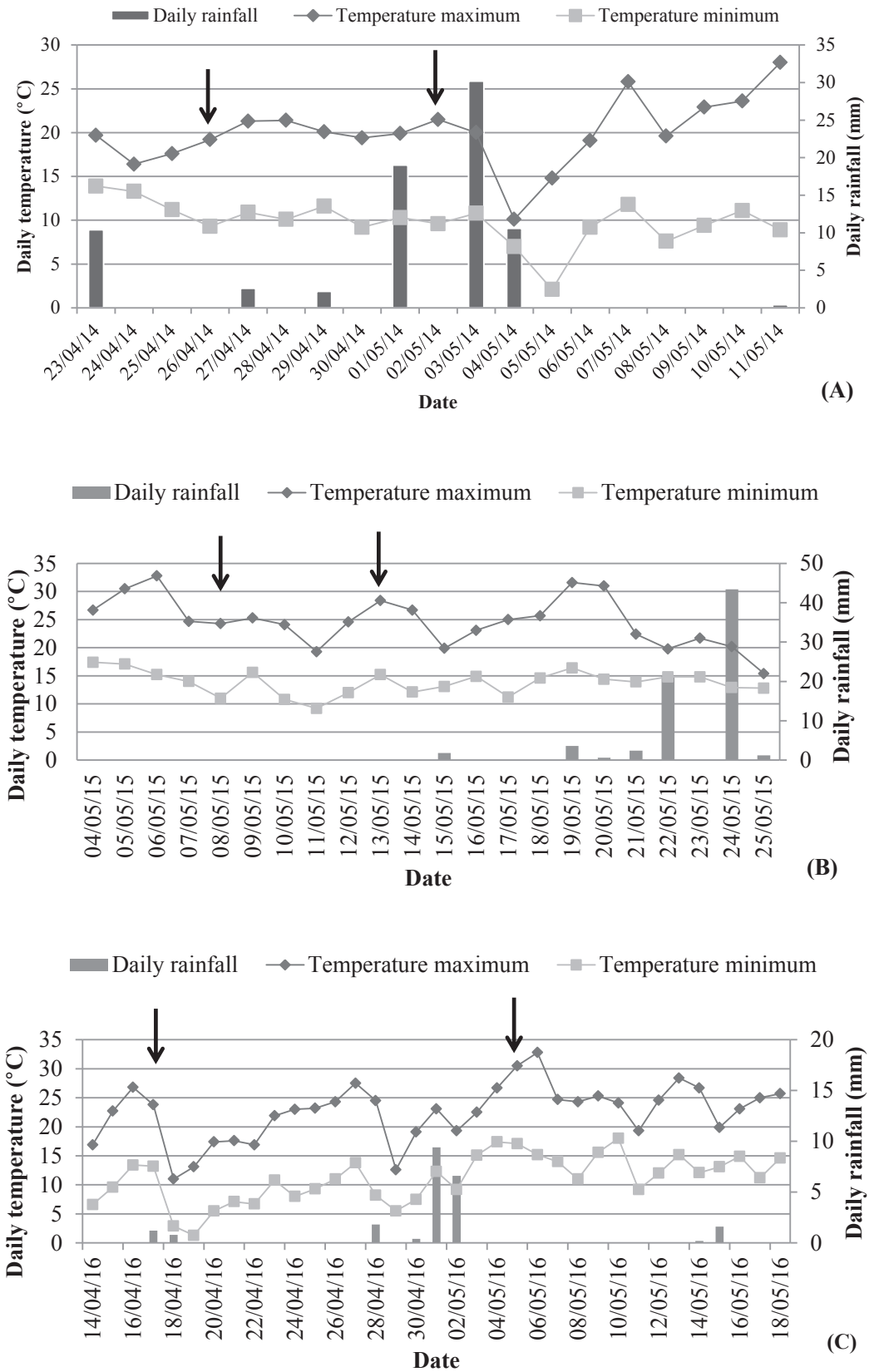

Figure 1. Maximum and minimum daily temperatures and daily rainfall at spray application in 'Gala' and 'Golden Delicious' apples during the three experimental years (A - 2014; B - 2015; C - 2016). Arrows indicate the dates of treatment application.

Table 3. The effect of metamitron used alone or in tank mix with BA or NAA on number of fruit per tree, yield, and fruit weight of 'Gala' apples in three experimental years[1].

Treatment	Number of fruit per tree			Yield (kg tree^{-1})			Fruit weight (g)		
	2014	2015	2016	2014	2015	2016	2014	2015	2016
MM 200 I + II	62.8c[2]	61.4	99.0c	10.9	10.5	16.0b	178a	173a	161b
MM 250 I	73.8bc	65.1	186.8a	12.0	10.1	22.2a	162abc	158bc	119cd
MM 250 II	84.8ab	66.1	84.1c	12.5	10.1	13.6b	150bc	155bc	168ab
MM 250 II + NAA II	65.9c	69.6	78.0c	10.6	10.6	12.7b	165abc	154bc	169ab
MM 250 II + BA II	69.0c	65.1	72.8c	11.7	10.6	13.0b	175a	163ab	182a
CCT	71.6bc	72.5	155.4b	11.5	10.9	20.6a	165ab	153bc	132c
UCT	88.0a	70.4	193.1a	12.8	9.8	21.7a	148c	148c	114d
Significance	**[3]	ns	***	ns	ns	***	**	**	***

[1]Data represent the means of 4 replicates.
[2]Means within a column followed by different letters are significantly different at $P \leq 0.05$ by LSD test;
[3]ns, **, *** indicate no significant or significant differences at $P \leq 0.01$ or 0.001, respectively.

In the third experimental year (the fourth leaf, 2016) chemical thinning expressed a strong influence on reducing fruit number per tree. Single metamitron application at fruitlet diameter 12–15 mm and double metamitron application as well as simultaneous metamitron application with BA and NAA caused significant decreases in the number of fruit per tree compared to the other treatments (Table 3). Even slighter overthinning in the single metamitron application at 12–15 mm of mean fruitlet diameter and in combined treatments with BA and NAA was also recorded. A lower total yield appeared as a consequence of reduced number of fruit per tree, but the yield of large fruit (>75 mm in diameter) was significantly higher than that in the treatment with single metamitron application when fruitlet diameter was from 6 to 9 mm and the commercial control and unthinned control treatments (Figure 2C). All of the applied metamitron treatments improved return bloom compared to the unthinned control treatment, except for the earlier single application of metamitron at fruitlet diameter stage 6–9 mm (Figure 3).

Differences among the treatments in internal fruit quality parameters (total soluble solids, flesh firmness, and iodine-starch index) were not recorded in the first or second experimental years (Table 4). Moreover, a different thinning strategy had no significant influence on the percentage of red colour in the second experimental year (Table 4).

In the third experimental year fruit soluble solids were significantly increased on the trees with a reduced number of fruit compared to the untreated control. However, there was no treatment effect on other fruit ripeness parameters (flesh firmness and starch content). Chemical thinning improved fruit coloration, but the percentage of red colour on the fruit significantly increased only in the treatment with double metamitron application and in the treatments when metamitron was combined with BA and NAA in comparison to the unthinned control treatment.

3.2. 'Golden Delicious'

A strong thinning effect of metamitron was observed on 'Golden Delicious' in the first experimental year (the second leaf, 2014), reducing significantly the number of fruit compared with the control treatment, with the exception of a single metamitron application at the stage 12–15 mm of fruitlet diameter (Table 5). However, only the metamitron treatment in combination with BA reduced the number of fruit per tree as strongly as in the commercial control treatment. Average fruit weight in the double metamitron treatment, single metamitron treatment at 6–9 mm fruitlet diameter, and combined treatments with NAA and BA was increased as in the commercial control treatment, whereby double metamitron treatment and combined metamitron treatment with BA significantly increased the share of the largest fruit compared to the other metamitron treatments (Figure 4A). Total yield per tree was decreased in these four metamitron treatments as compared to the untreated control treatment, but the strongest yield reduction was observed in the commercial control treatment. Return bloom per tree in the metamitron treatments was significantly lower than in the commercial control treatment, but it was acceptable for a regular crop in the next year (Figure 5).

In the second experimental year (the third leaf, 2015), number of fruit and yield per tree were not significantly different among the treatments (Table 5). Treatments

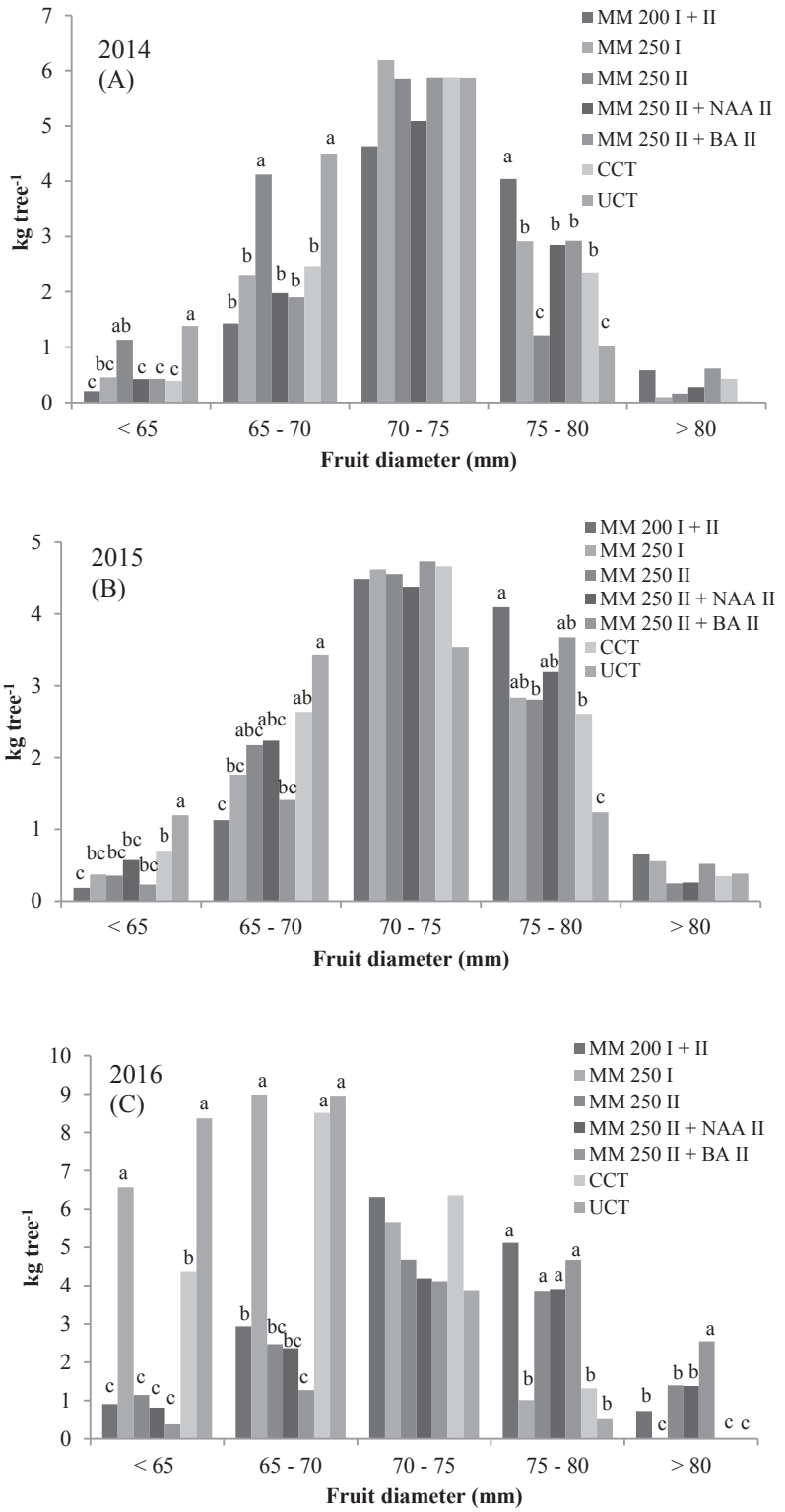

Figure 2. The effect of metamitron used alone or in tank mix with BA or NAA on fruit size distribution of young apple 'Gala' trees in 2014 (A), 2015 (B), and 2016 (C). Different letters within each size group denote significant differences between the means of kg tree^{-1} according to the LSD test at $P \leq 0.05$.

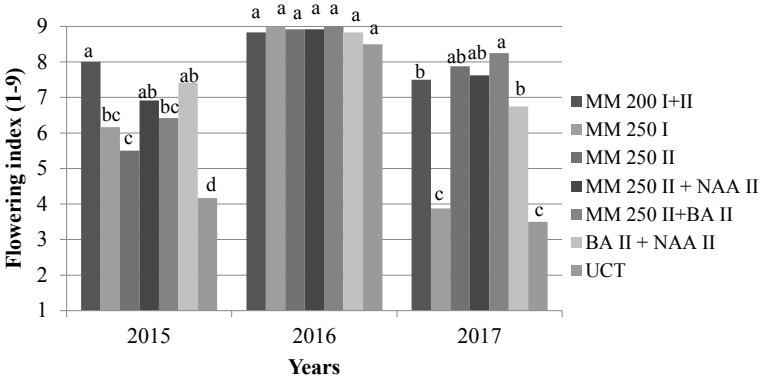

Figure 3. The effect of metamitron, used alone or in tank mix with BA or NAA, on return bloom (flowering index 1–9) of 'Gala' apples in years after the thinner applications (2015–2017). Different letters within each year denote significant differences between the means of flowering index according to the LSD test at $P \leq 0.05$.

Table 4. The effect of metamitron used alone or in tank mix with BA or NAA on fruit maturity parameters of young 'Gala' apples in three experimental years.

Treatment	TSS (Brix°)			Flesh firmness (kg cm^{-2})			Iodine starch index (1–5)			Red colour (%)	
	2014	2015	2016	2014	2015	2016	2014	2015	2016	2015	2016
MM 200 I + II	12.7	15.3	12.2bc	6.87	7.49	9.32	3.38	3.9	2.21	99.1	90.6ab
MM 250 I	12.6	15.4	11.5c	6.17	7.36	9.05	3.52	4.1	2.09	98.8	83.0bc
MM 250 II	12.4	15.1	12.7ab	7.29	7.63	9.32	3.52	3.8	2.19	97.3	83.3bc
MM 250 II + NAA II	12.6	15.1	13.2a	6.28	7.38	9.05	3.67	3.8	2.57	94.7	94.3ab
MM 250 + BA II	12.2	15.1	13.0a	7.00	7.29	9.32	3.47	4.2	2.44	98.0	96.7a
CCT	12.4	15.0	12.0bc	7.26	7.87	9.05	3.33	3.8	2.48	96.2	84.5bc
UCT	12.2	14.5	11.8c	7.25	7.66	9.05	3.32	4.1	2.11	94.5	73.0c
Significance	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	**

[1]Data represent the means of 4 replicates.
[2]Means within a column followed by different letters are significantly different at $P \leq 0.05$ by LSD test; [3]ns, **, *** indicate no significant or significant differences at $P \leq 0.01$ or 0.001, respectively.

when metamitron was applied twice and in combination with BA resulted in larger fruit weight than with the commercial control and unthinned control treatments. In these two treatments a significant difference was observed in the distribution of fruit in greater size classes (>80 mm) in relation to the other treatments (Figure 4B). All metamitron treatments increased the return bloom compared to the commercial control treatment (Figure 5).

In the third experimental year (the fourth leaf, 2016), all metamitron treatments reduced the fruit number considerably in comparison to the untreated control treatment, except for metamitron treatment applied at the stage 6–9 mm of fruitlet diameter. A decrease in the fruit number per tree resulted in an increase in average fruit weight in comparison to the untreated control treatment, without a reduction in total yield per tree (Table 5). In these treatments, the share of large fruit with diameter >75 mm was significantly increased (Figure 4C). In particular, in the treatment when metamitron was combined with BA, fruit size and yield of fruit with diameter >80 mm were significantly greater than when metamitron was used by itself. Pygmy fruit were observed on the trees in 2016 for the first time (Table 5). All metamitron treatments reduced the yield of these fruit compared to the unthinned control treatment, but only double metamitron application and combined metamitron treatments with NAA and BA decreased the yield of pygmy fruit as compared to the commercial control treatment. Combined application

Table 5. The effect of metamitron used alone or in tank mix with BA or NAA on number of fruit per tree, yield, and fruit weight of 'Golden Delicious' apples in three experimental years[1].

Treatment	Number of fruit per tree			Yield (kg tree^{-1})			Fruit weight (g)			Pygmy fruit (%)
	2014	2015	2016	2014	2015	2016	2014	2015	2016	2016
MM 225 I + II	67.8bc[2]	69.9	148.1cd	13.7bc	13.3	26.5	208ab	201a	180b	0.47c
MM 300 I	72.8b	85.7	203.0ab	14.2ab	14.5	27.5	196b	176c	137d	5.85b
MM 300 II	88.7a	70.4	137.6cd	15.0ab	11.8	23.7	170c	182bc	174b	1.75bc
MM 300 II + NAA II	65.8bc	88.4	142.4cd	13.5bc	15.3	25.2	208ab	186abc	180b	0.40c
MM 300 II + BA II	56.7cd	85.4	122.6d	12.4cd	15.7	24.1	222a	197ab	203a	0.62c
CCT	51.5d	96.9	155.9cd	10.9d	15.6	24.7	214ab	177c	163bc	3.87b
UCT	98.4a	75.7	222.3a	15.7a	11.8	25.4	162c	170c	114e	10.96a
Significance	***[3]	ns	***	***	ns	ns	***	*	***	***

[1]Data represent the means of 4 replicates.
[2]Means within a column followed by different letters are significantly different at $P \leq 0.05$ by LSD test;
[3]ns, *, *** indicate no significant or significant differences at $P \leq 0.05$ or 0.001, respectively.

of metamitron with NAA or BA significantly enhanced return bloom in comparison to the unthinned control treatment (Figure 5).

In the first and second experimental years there was no significant effect of the applied treatments on the total soluble solids, starch content, or flesh firmness (Table 6). In the third experimental year metamitron had minimal influence on the fruit maturity parameters. Total soluble solids increased in the treatments with reduced number of fruit per tree, while the content of starch was decreased (Table 6). None of the treatments had a significant influence on flesh firmness.

4. Discussion

The present trial demonstrates that chemical fruit thinning in young 'Gala' and 'Golden Delicious' apple orchards must be started in the second leaf. For this purpose metamitron can be used alone or in combination with the existing thinners. Its application to apple trees can result in transient carbohydrate stress that may increase the sensitivity of the fruit to a chemical thinner application (McArtney et al., 2012). In our study, metamitron expressed a similar mode of action in both cultivars tested by reducing the number of fruit per tree. Intensity of thinning was dependent on the weather conditions, number of applications, and combinations with other chemical thinners. The normal time for application of thinning chemicals in apples extends from bloom until 3 weeks after bloom, when the fruit reach a mean diameter of ≈16 mm (McArtney and Obermiller, 2012). During that period single metamitron treatments, regardless of application time, when the fruit diameter reached 6–9 mm or 12–15 mm, showed inconsistent action in the different experimental years. The results of our study confirmed the previous findings reported by Brunner (2014) indicating that the same amounts of metamitron applied in different years might not always reduce fruit set to the same extent. It seems that thinning efficiency was much more dependent on temperature during the day of application and several days after than on the fruit diameter, which was previously stated by Greene (2014). In our experiment single metamitron application had a positive influence on reducing fruit number only when minimum night temperature was above 10 °C and maximum daily temperature was above 20 °C, showing similar thinning activity when the fruitlet diameter was either 6–9 or 12–15 mm (Figure 1). During the periods with low temperatures (2014 and 2016) metamitron had no additional thinning effect compared to the untreated control in either cultivar tested, without an increase in fruit weight or influence on fruit size distribution. The results obtained confirm Robinson and Lakso's (2004) hypothesis that the temperature regime during treatment has a dramatic influence on the efficiency of chemical thinners. We can conclude that single metamitron application can be reliable for thinning only when the temperature regime is favourable for its action. Stern (2015) emphasised that, in Israel's growing conditions, relatively low doses of metamitron are effective for 'Golden Delicious' fruit thinning at the 6-mm fruitlet diameter stage due to the high night temperatures (above 12.8 °C) for 3 weeks following the application. Similar results for 'Elstar' were obtained by Lafer (2010), who indicated that metamitron applications in early fruit development stages (6–8 mm in diameter) significantly reduced the fruit number, but not in later application when fruitlet diameter was 12–14 mm in three consecutive years. In our study,

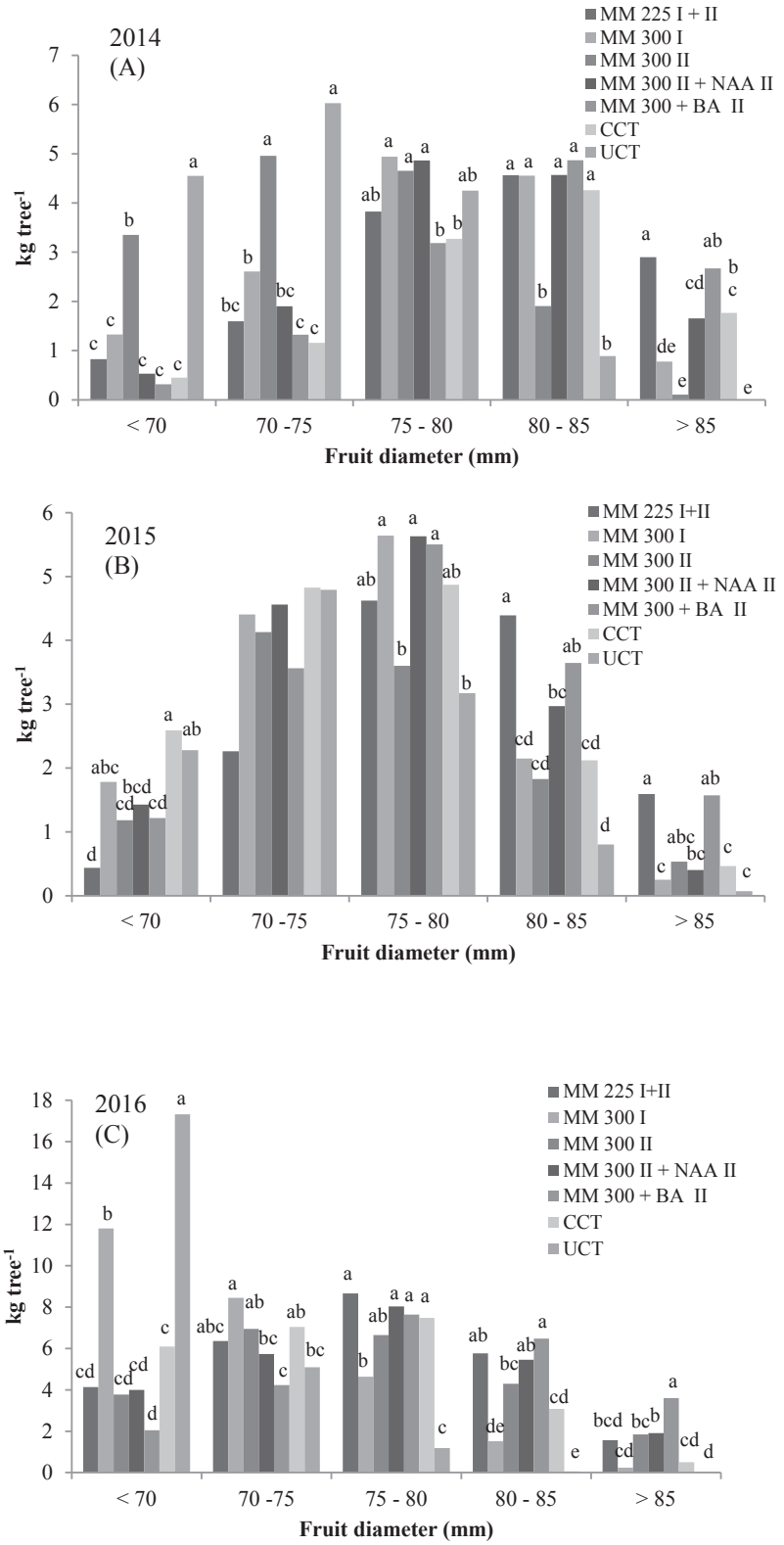

Figure 4. The effect of metamitron used alone or in tank mix with BA or NAA on fruit size distribution of young apple 'Golden Delicious' trees in 2014 (A), 2015 (B), and 2016 (C). Different letters within each size group denote significant differences between the means of kg tree^{-1} according to the LSD test at $P \leq 0.05$.

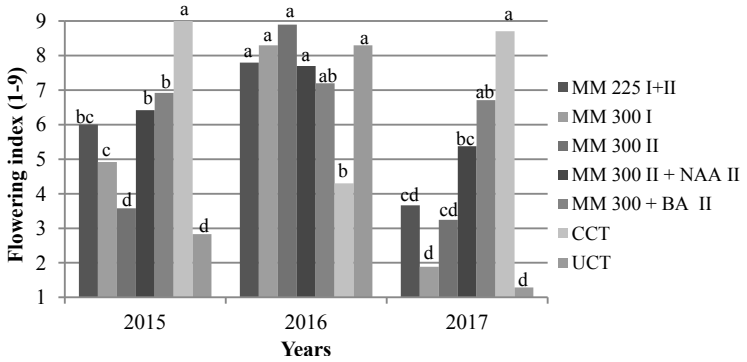

Figure 5. The effect of metamitron applied alone or in tank mix with BA or NAA on return bloom (flowering index 1–9) of 'Golden Delicious' apples in the years after thinner applications (2015–2017). Different letters within each year denote significant differences between the means of flowering index according to the LSD test at $P \leq 0.05$.

Table 6. The effect of metamitron applied alone or in tank mix with BA or NAA on fruit maturity parameters of young 'Golden Delicious' apples in three experimental years.

Treatment	TSS (Brix°)			Flesh firmness (kg cm^{-2})			Iodine starch index (1–5)		
	2014	2015	2016	2014	2015	2016	2014	2015	2016
MM 225 I + II	13.6	14.51	13.41ab	6.92	7.72	7.35c	3.52	2.52	3.90a
MM 300 I	13.0	13.51	12.91b	6.88	7.92	7.61abc	3.68	2.54	3.83ab
MM 300 II	12.1	14.26	13.64ab	6.84	8.22	7.38c	3.92	2.94	3.14bc
MM 300 II + NAA II	12.6	13.76	13.70ab	6.91	7.91	7.52bc	3.78	2.15	3.66abc
MM 300 + BA II	13.0	14.39	14.31a	6.85	7.84	7.38c	3.54	2.75	3.00c
CCT	13.4	13.81	14.03a	7.05	8.47	7.84a	3.53	3.00	3.49abc
UCT	12.4	14.79	12.83b	6.98	8.27	7.72ab	3.95	2.81	4.04a
Significance	ns	ns	*	ns	ns	*	ns	ns	*

[1]Data represent the means of 4 replicates.
[2]Means within a column followed by different letters are significantly different at $P \leq 0.05$ by LSD test;
[3]ns, * indicate no significant or significant differences at $P \leq 0.05$, respectively.

single metamitron treatment in different years expressed inconsistent thinning activity in 'Gala', confirming the previous findings reported by Basak (2011). In 2016, single metamitron application (concentration of 250 mg L^{-1} at development stage 12–15 mm fruitlet diameter) in 'Gala' when the temperature was favourable for thinning (minimum night temperature >15 °C, maximum daily temperature >25 °C) significantly reduced crop load to a level that was slightly lower than commercially acceptable. According to previous findings reported by McArtney and Obermiller (2014) and Gabardo et al. (2017), excessive fruit drop in some apple cultivars may appear when metamitron is used as an individual thinner at higher concentration. Since the thinning efficacy of the single metamitron application is inconsistent, it seems that unfavourable weather conditions can be overcome by repeated metamitron application or by adding of BA or even NAA in commonly used concentrations. Our results showed a greater thinning effect of double metamitron application (2 × 200 mg L^{-1} for 'Gala' and 2 × 225 L^{-1} for 'Golden Delicious') in slightly lower concentrations than with single metamitron application. This treatment showed the most consistent thinning effect during the whole experimental period in both cultivars tested. It confirms the previous findings reported by Clever (2007), Lafer, (2010), and Robinson et al. (2017), who found that multiple thinning sprays give better results than just one spray with hard to thin cultivars. In contrast, Stern (2015) determined that two metamitron applications did not give better results than only one application applied earlier. In

some growing conditions double metamitron treatment in high concentration can cause overthinning (Dorigoni and Lezzer, 2007; Basak, 2011).

Combined application of metamitron and BA as well as the combination of metamitron and NAA in standard rates caused sufficient fruit abscission. These combined treatments, unlike single metamitron treatment applied at higher fruit diameter (12–15 mm), successfully overcame the negative influence of low temperature and thinned fruit more aggressively than metamitron applied alone in the first experimental year in both cultivars studied. This disagrees with findings reported by Greene (2014) and Gabardo et al. (2017), who demonstrated that metamitron at high concentration combined with the low rate of BA had an additional thinning effect but not greater than that of single metamitron application. These combinations increased the level of thinning to the point of overthinning only in 'Gala' trees and even flowering was abundant, when the temperature during the application and 3 days after was too high. Thus, combination of metamitron with BA or NAA, as chemical thinner sprays, can be recommended for difficult to thin cultivars like 'Golden Delicious', especially when the weather conditions are not favourable for metamitron activity.

Single metamitron treatment improved average fruit size and fruit size distribution only when it significantly reduced the number of fruit, regardless of the application time, as similarly noted by Stern (2015). When metamitron application was repeated and it was mixed with other chemical thinners, increments in fruit size and share of the larger fruit in the total yield were regularly noted compared to the unthinned control treatment. Additionally, metamitron combined with BA at the standard rate improved fruit size much more than single metamitron application, especially in 'Golden Delicious'. Pygmy fruit in 'Golden Delicious' were noted for the first time in the fourth leaf, whereby metamitron treatments reduced the number of pigmy fruit, confirming the previous finding reported by Basak (2011). In the present experiment, combined application of metamitron and NAA also reduced pigmy fruit formation, although it is well known that NAA can have a negative effect on fruit size (Black et al., 1995).

Regular formation of fruiting buds over the seasons is very important when chemical thinners are applied. Return bloom in this trial was increased consistently in both cultivars studied by the application of metamitron if it thinned fruit up to the commercially accepted level. However, single application did not always provide sufficient flowering in the subsequent season, especially in 'Golden Delicious'. This result partially agrees with the findings published by Gabardo et al. (2017), who found that different metamitron concentrations for thinning of 'Baronesa' apple trees did not change the flowering return in the season following application. Tank mixing of metamitron with other chemical thinners, especially with BA, always increased flowering formation at an acceptable level for fruit production in the subsequent year in both cultivars studied. Repeated metamitron application had various effects on return bloom depending on the cultivar, whereby the optimal effect was observed in 'Gala'. Similarly, Lafer (2010) reported that repeated metamitron treatments resulted in the most homogeneous flowering during the whole experimental period in 'Elstar'.

Red colour formation in 'Gala' was improved only in the treatments with significant reduction in fruit number in 2016, except for the two treatments with single metamitron application. This partially confirms the previous research by Clever (2007) and Dorigoni and Lezzer (2007), who concluded that successful fruit thinning enhances fruit pigmentation.

Thinning treatment had a minimal and unclear effect on fruit maturity parameters. Total soluble solids content was increased in all the treatments with a large reduction in fruit number in both cultivars tested during the last experimental year. None of the treatments influenced flesh firmness. Contrary to this, Greene (2014) found an increment in flesh firmness when metamitron was combined with BA.

The present study suggests that metamitron may be applied for chemical thinning of 'Golden Delicious' and 'Gala' apples already in the second leaf, when well feathered nursery trees are used. A successful thinning effect was achieved in single metamitron treatments during the normal application time (5–15 mm in fruit diameter) at concentrations of 250 mg L^{-1} ('Gala') and 300 mg L^{-1} ('Golden Delicious') only under favourable weather conditions (minimum night temperature >10 °C, maximum daily temperature >20 °C). However, double metamitron application at lower concentrations (200 mg L^{-1} for 'Gala' and 225 mg L^{-1} for 'Golden Delicious') resulted in optimal thinning activity during the whole experimental period. The results also demonstrate that combined (tank mix) application of metamitron in the mentioned concentrations with BA or NAA can successfully overcome the negative influence of low temperature on thinning of fruitlets. These three treatments achieved a good thinning effect, which resulted in regular increments in fruit size and share of the large fruit in the total yield. Formation of flower buds was also significantly increased in both cultivars tested, associated with lower numbers of fruit per tree in the previous experimental year.

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