

# Induction of lateral shoots of sweet cherry on the Gisela 6 rootstock

Indukcija lateralnih izboja trešnje na podlozi Gisela 6

**Stanisavljević, A., Bošnjak, D., Todorčić Vekić, T., Teklić, T., Špoljarević, M., Štolfa, I., Tomaš, V., Dugalić, K., Lisjak, M.**

**Poljoprivreda/Agriculture**

ISSN: 1848-8080 (Online)

ISSN: 1330-7142 (Print)

<http://dx.doi.org/10.18047/poljo.21.1.10>



**Poljoprivredni fakultet u Osijeku, Poljoprivredni institut Osijek**

Faculty of Agriculture in Osijek, Agricultural Institute Osijek

## INDUCTION OF LATERAL SHOOTS OF SWEET CHERRY ON THE GISELA 6 ROOTSTOCK

Stanisavljević, A.<sup>(1)</sup>, Bošnjak, D.<sup>(1)</sup>, Todorčić Vekić, T.<sup>(1)</sup>, Teklić, T.<sup>(1)</sup>, Špoljarević, M.<sup>(1)</sup>, Štolfa, I.<sup>(2)</sup>, Tomaš, V.<sup>(3)</sup>, Dugalić, K.<sup>(3)</sup>, Lisjak, M.<sup>(1)</sup>

Original scientific paper  
Izvorni znanstveni članak

### SUMMARY

*Number of lateral branches and overall development of seedlings is a crucial parameter for achieving the required quality standards dedicated to modern intensive production systems. Branched seedlings have formed the structure of the canopy, which is defined and cultivation model for future plantations. In addition to the uniformity of the plantation better and earlier productivity is achieved. The research, in order to standardize the techniques for induction of lateral branching, was conducted in 2013 in the nursery (Koprivna, Eastern Croatia) on 3 cherry cultivars grafted on Gisela 6 rootstock. The treatments included the application of different combinations of Bioregulators 6-Benzyladenine and gibberellic acid 4 and 7 (BA + GA<sub>4+7</sub>) as well as cyclanilide on cultivars 'Carmen', 'Grace Star' and 'Black Star'. All applied treatments resulted in a significant increase in the number of new branches compared to the control. Cultivar Black Star induced the development of the highest number of branches. Treatment BA + GA<sub>4+7</sub> increased the number of lateral shoots compared to the control in all the investigated cultivars. The most significant induction in the number of branches has been achieved with Cyclanilide treatment which improved more correct distribution and resulted in a more uniform length of induced shoots. Combination of the treatment cyclanilide and BA + GA<sub>4+7</sub> has not resulted in synergistic increase in the number of branches in response to treatment with cyclanilide. The applied treatments also significantly influenced the length of the branches and the branching angle. Results of this study indicate the need for further testing of cyclanilide as a potential regulator of growth in nursery production.*

**Key-words:** cyclanilide, lateral branch, sweet cherry

### INTRODUCTION

The quality of the seedling plants has a big impact on the earlier production and profitability of high-intensity systems in orchards. Plants should have the proper diameter, many ramifications, good position of lateral branches with wide angle. Very branched plants allow earlier formation of the crown structure and the growing shape, especially slender spindle modification.

Early and rapid development of the lateral branches of the fruit plants encourages earlier fruiting with the exponential increase in the yield (Preston, 1968; Quinlan and Preston, 1978; Wertheim 1978). Accordingly, it

is preferred that saplings are branched and have the desired amount of lateral shoots spirally distributed having appropriate length and branch angle in relation to the trunk. When using unbranched sapling full productivity

(1) Assoc. Prof. Aleksandar Stanisavljević (astanis@pfos.hr); Dejan Bošnjak, M. Eng. Agr., Teodora Todorčić Vekić, BSc student; Prof. Dr. Tihana Teklić, Assist. Prof. Miroslav Lisjak, Marija Špoljarević, BSc. - Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Ulica kralja Petra Svačića 1d, 31000 Osijek, Croatia, (2) Assist. Prof. Ivna Štolfa - Josip Juraj Strossmayer University of Osijek, Department of Biology, Cara Hadrijana 8/A, 31000 Osijek, Croatia, (3) Krunoslav Dugalić, M. Eng. Agr., Vesna Tomaš, M. Eng. Agr. - Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia

is achieved later and there is a greater risk of biennial bearing (Čmelik et al., 2005). Sapling of cherry shows strong apical dominance, naturally inducing a strong and upright trunk with limited lateral branches (Miller, 1983). Standard practice in nurseries include control apical dominance or disruption and / or interruption of domination peak bud stopping the auxin creation, which inhibits the formation of lateral buds (branches). For this purpose, various common exogenous treatments were applied such as the application of physical (pinching, undeveloped leaves removal, removal of peak lateral buds and scoring) or chemical treatment (treatment with cytokinin) (Hoying et al., 2001; Čmelik, et al., 2005). Physical measures are time-consuming and laborious task that is often not sufficiently effective, and requires additional manpower and expenses (Sazo and Robinson, 2011).

The use of plant growth regulators today is more consistent and cheaper versions unlike traditional hand leaves pinching. So far, plenty of research has been conducted on the possibility of use plant growth regulators in the induction of branching (Wertheim, 1978; Elfving, 1984; Cody et al., 1985; Elfving et al., 2005, 2006, 2010; Sazo et al., 2011; Jacyna et al., 2008; Čmelik et al., 2005) but their application in practice is not very spread. The reasons for this are numerous and important unknown of the term of application, the concentration of preparation, methods of application, the interaction of environmental and technological factors that often attain uncertain and unreliable results (Čmelik et al., 2005). Furthermore, very little research has been conducted on the induction of cherry lateral branching. Most growth regulator preparations that induce lateral branching on apple or pear, are ineffective in cherry (Quinlan and Preston, 1973; Plich and Basak, 1978). Products containing cytokinin (6-benzyladenine BA) with or without the isomers of gibberellic acid ( $GA_{4+7}$ ) proved to be very effective in the nursery (Elfving, 2010). It is important to note that there is a big difference in the response to the applied concentration or treatments between different cultivars of cherry (Wustenberghs and Keulemans, 1999).

The development of lateral shoots directly correlates with the apical dominance (Martin, 1987; Cline, 1997; Čmelik et al., 2005). Apical dominance in apple is associated with the amount of auxin in the branches, and its transport to the base (Baldini et al., 1973; Abbas, 1978), assuming that the same mechanism with cherries. Young leaves in the apical top (vegetative tip) produces the hormone auxin, which is translocated basipetally realizing it as the correlative growth inhibition lower ranking lateral buds (Čmelik et al., 2005; Sazo and Robinson, 2011). Application of the cytokinin ie. 6-benzyladenine (BA) with or without gibberellic acid isomers  $GA_4$  and  $GA_7$  ( $GA_{4+7}$ ) lead to an improved branching cherry sapling (Cody et al., 1985; Hrotkó et al., 1999). By studying the molecular structure plant growth regulators, Don Elfving 2000 notes that the

structure of cyclanilide has similarities to the structure of the synthetic auxin 2,4-D and 2,4,5-T (Warner, 2012). Plant growth regulator cyclanilide (Cyclanilide, CYC) binding to cell receptors of auxin obstructed the transportation and thereby inhibited its activity on the apical dominance which allowed a correlative increase in lateral buds (Pederson et al., 1997; Elfving 2006). Bayer in 2009 in the United States registered Cyclanilide® (CYC, Bayer Environmental Science, Research Triangle Park, NC) as the new bioregulator called "Tiberon™ SC" for the induction of lateral branching apple trees (*Malus domestica* ×), pears (*Pyrus communis*), cherry (*Prunus avium*) and woody ornamental plants (Sazo and Robinson, 2011). Cyclanilide proved to be very effective in inducing branching of some cultivars of cherry that developed 6 times better and properly distributed premature lateral branches, with minor and insignificant effect on the trunk, Elfving (2010).

This study was conducted to evaluate cyclanilide (Cyclanilide) in inducing lateral branching cherry (Carmen, Grace Star and Black Star) grafted on Gisela 6 in comparison with 6-benzyladenine (BA, cytokinin) and gibberellins  $GA_4 + 7$  (Promalin®, PR).

## MATERIAL AND METHODS

This research, with the aim of standardizing techniques of lateral branching induction was conducted in 2013 in the fruit trees nursery (Koprivna, Eastern Croatia) with the annual cherry inoculant (rootstock Gisela 6). The treatments have included the application of various combinations of the BA +  $GA_{4+7}$  (Promalin®) and cyclanilide. The experiment was set up in a randomized block design that included three currently very actual cultivars (‘Carmen’, ‘Grace Star’ and ‘Black Star’) and 4 treatment + control (untreated sapling). As the surfactant Silwet® L-77 was used in a concentration of 0.1% (v / v). The experiment was set up in three replications with 5 saplings per replication for a total of 15 saplings per treatment. Dates of application of treatments and pinching are shown in Table 1. Treatment CYC150 contained 150 ppm cyclanilide, CYC250 - 250 ppm cyclanilide, PR250 - 250 ppm BA +  $GA_{4+7}$ , treatment 2 x 250PR - 250 ppm BA +  $GA_{4+7}$  cycles twice and treatment MIX with a combination of 100 ppm cyclanilide + 250 ppm BA +  $GA_{4+7}$ . The application treatment of CYC150, CYC250 and PR250 was conducted on 10 June 2013. The top undeveloped leaves were manually pulled off (pinched June 8, 2013 at the height of the peak shoots at about  $50 \pm 0.2$  cm above the graft union, i.e. around 70 cm from the ground). All the treatments were applied to tip portion of sapling (30 - 40 cm above the graft sites) using a hand-pressure sprayers. Application of 2x250PR treatment was performed twice on the 2nd and 30th June (first pinching was carried out on June 1 at the height of the apical tip of  $42 \pm 0.6$  cm, and the second on June 28 at a peak height of  $91 \pm 0.3$  cm). Additional treatment was performed only on the cv. ‘Black Star’ with combination of cyclanilide and BA +

GA<sub>4</sub>+7 (MIX) on June 20, 2013 (pinching conducted on June 18 at the height of the terminal bud of about 63 ± 0.4 cm above the graft union). Promalin® N- (phenylmethyl) -1H-purine-6-amine + Gibberellins A4 and A7 represents a mixture of two natural plant growth regulators, gibberellic acid (G<sub>4</sub> + G<sub>7</sub> - 1.8% (v / v)), which stimulates an increase in cells and 6-benzyladenine (BA, cytokinin 1.8% (v / v)) that promotes cell division. Plant

growth regulator cyclanilide 1- (2,4-dichlorophenylaminocarbonyl) -cyclopropane carboxylic acid (cyclanilide, CYC) was used as a technical standard. Plants were treated in the late afternoon at 20 to 23°C. At the time of application climatic conditions were stable and the effect of the applied preparation was not compromised with a possible flush of rain within 48 hours after the moment of application.

**Table 1. Treatments in the research, concentration of used bioregulators, date of application and leaf pinching (\*\*Black Star only)**

Tablica 1. *Tretmani u istraživanju, korištene koncentracije, datum aplikacije i pinciranja*

Treatments <i>Tretmani</i>	Concentration <i>Koncentracija</i>	Wetting agents <i>Surfaktant</i>	Application date <i>Datum aplikacije</i>	Pinching date <i>Datum pinciranja</i>
K – Control	0		10 June 2013	8 June 2013
CYC150	150 ppm	Silwet® L-77		
CYC250	250 ppm	Silwet® L-77		
PR250	250 ppm	Silwet® L-77		
2x250PR	250 + 250 ppm	Silwet® L-77	2 and 30 June 2013	1 and 28 June 2013
**100CYC + 250 PR (MIX)	**100 + 250 ppm	**Silwet® L-77	**20 June 2013	**18 June 2013

\*\*Treatment was applied only at cv. 'Black Star' - \*\*Aplikacija izvršena samo na sorti Black Star

At the end of the growing season, after removing the sapling (16<sup>th</sup> December 2013) measurements were done following the observed parameters:

- A - Height from root to tip or total height (cm)
- B - Height of the roots to the first branch (cm)
- C - Height of the roots to the highest branches (cm)
- D - The average length of branches (cm)
- E - Number of branches
- F - Branching angle (deg°)

All determined results were analyzed using the SAS Software 9.3, software (2002 - 2010, SAS Institute Inc., Cary, USA) and Microsoft Office Excel, 2010. The following statistical methods were used: analysis of variance (ANOVA), statistical tests the significance of the

applied treatment - F-test and Fisher's LSD test (Least Significant Difference) ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

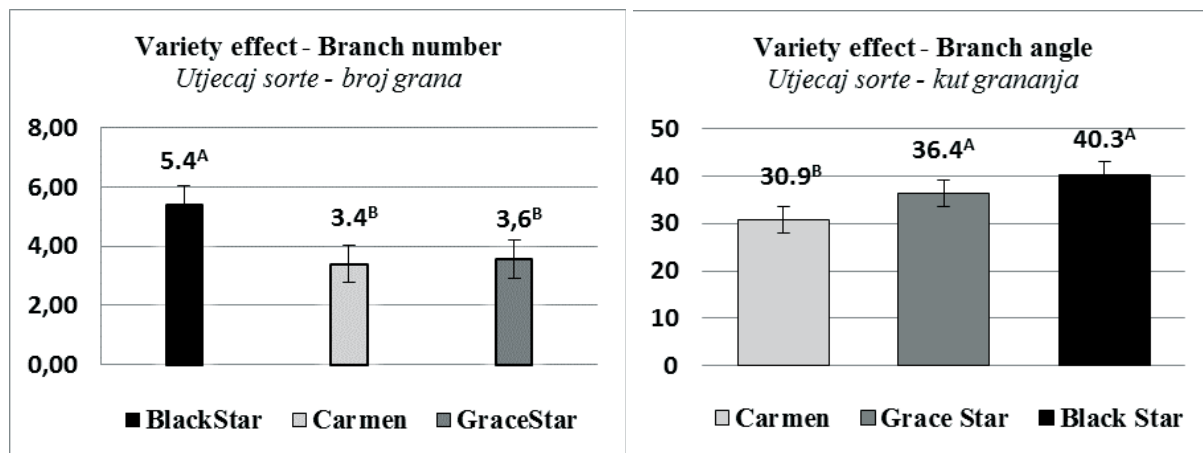
Very significant effect ( $p \leq 0.01$ ) of cultivars in overall height (F-test 6.73), the height of the last branch (F test 1.68), number of branches (F test 35.36), the angle of branching (F test 12.33) and significant impact ( $p \leq 0.05$ ) on the average length of branches (F test 3.31) (Table 2) were determined by the F test at the level of the whole experiment. Very significant effect of treatment on the number of branches (F-test 34.56), the length of the branch (F test 4.19), angle branching (F test 7.28) and a significant impact to the height the first branch (F test 2.56) were also found out. Interaction cultivar x treatment has a significant impact on the number of branches (F test 5.02).

**Table 2. Two-way ANOVA for observed parameters (A, B, C, D, E, F) under the influence of cultivar, treatments and interaction treatments x cultivar**

Tablica 2. *Dvofaktorijalna ANOVA za promatrane parametre (A, B, C, D, E, F) pod utjecajem sorte, tretmana i interakcija tretman x sorta*

Observations <i>Promatrani parametri</i>	Mean <i>Srednja vrijednost</i>	Cultivar <i>Sorta</i>	Treatments <i>Tretman</i>	Treatments x cultivar <i>Tretman x sorta</i>
Total height/Ukupna visina (A)	189.2	**	ns	ns
First branch height/Visina od korijena do prve grane (B)	63.74	Ns	*	ns
Last branch height/Visina od korijena do najviše grane (C)	80.97	**	ns	ns
Average branch length/Prosječna dužina grana (D)	79.33	*	**	ns
Branch angle/Kut grananja (F)	35.86	**	**	ns
Branch number/Broj grana (E)	4.12	**	**	**

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; ns - non significant

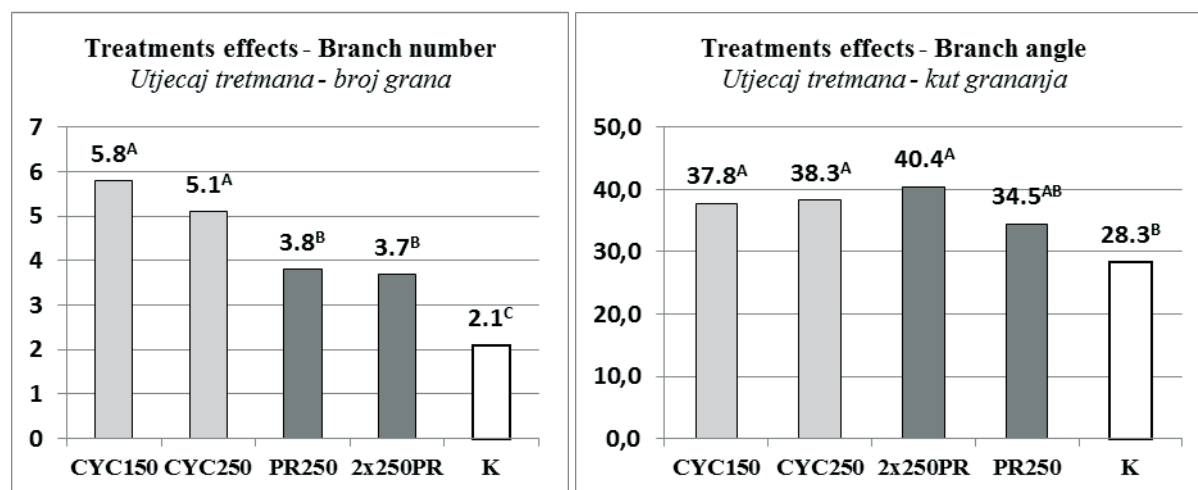


Figures 1 and 2. Cultivar effect on the branch number and branch angle, means with the same letter are not significantly different from each other: <sup>AB</sup> at  $p \leq 0.01$

Grafikoni 1. i 2. Utjecaj sorte na broj grana i kut grananja, vrijednosti iste slovne oznake nisu statistički značajne: <sup>AB</sup> razina  $p \leq 0,01$

Cv. 'Black Star' has averaged a significantly higher number of branches (5.4) than the other two cultivars, while the cv. 'Carmen' had a significantly

sharper angle branches (30.9°) than the cv. 'Grace Star' and 'Black Star' (Figures 1 and 2).



Figures 3 and 4. Treatments effect on the branch number and branch angle, means with the same letter are not significantly different from each other: <sup>AB</sup> at  $p \leq 0.01$

Grafikoni 3. i 4. Utjecaj tretmana na broj grana i kut grananja, vrijednosti iste slovne oznake nisu statistički značajne: <sup>AB</sup> razina  $p \leq 0,01$

Treatment Cyclanilide (150 ppm and 250 ppm) had very significant impact on the number of branches (CYC150 - and CYC250 5.8 - 5.1) (Figure 3). Treatment BA + GA<sub>4</sub> + 7 (PR250 - and 2x250PR 3.8 - 3.7) also very significantly differed compared to untreated plants (K - 2.1). Branching angle influenced by treatment (2x250PR, CYC250 and CYC150) differed significantly in comparison to the control (28.3 °) (Figure 4).

Statistical analysis showed significant differences at observed parameters between the treatments per

individual cultivar. Cv. 'Carmen' resulted in significantly lower overall height in comparison to the control (211.6) in the treatment CYC250 (182.4), PR250 (178.0) and 2x250PR (178.0) between which there were no differences (Table 3). Similar results were obtained by Bank and Stefani (2007) with cyclanilide applications and responses to the final height state in their experiments on woody ornamentals (Fraser Photinia). They claimed that a final height of the stems of plants also decreased with increasing concentrations of cyclanilide (0ppm-

52 cm to 106 ppm - 42 cm). Robinson and Sazo (2011) confirmed the same effect of cyclanilide (Tiberon) on trees of the apple where cyclanilide was rapidly reduced trunk elongation and significantly reduced the final height of the sapling. Farris et al. (2011) exploring the impact of cyclanilide in the promotion of shoots and flowering ornamental flowers (Coreopsis and Coneflower) stated that the total height of the plant decreased linearly with increasing cyclanilide concentration (up to 6% compared to the control). Other tested

cultivars in our experiment (Grace Star and Black Star) showed no significant difference on overall height (A) which is in agreement with the results of Elfving and Visser (2006a) on cultivars of cherry Rainer, Bing, Lapins and Skeena (mazzard), according to which less effect of cyclanilide to the length of the central trunk was not considered so important. Cyclanilide does not result in long-term reduction or deformation growth of terminal meristem, but only a temporary disruption and interruption of apical dominance.

**Table 3. Influence of cyclanilide (CYC) or BA + GA<sub>4+7</sub> (PR) applications to observed parameters of the sweet cherry cv. 'Carmen' in the nursery (Total height - A, First branch height - B, Last branch height - C, Average branch length - D, Branch number - E, Branch angle - F)**

Tablica 3. Utjecaj ciklanilida (CYC) i BA + GA<sub>4+7</sub> (PR) za promatrane parametre na sorti trešnje Carmen (ukupna visina – A, visina od korijena do prve grane – B, visina od korijena do najviše grane – C, prosječna dužina grana – D, broj grana – E, kut grananja – F)

Treatments/Tretmani	Carmen					
	Cultivar/Sorta					
	A	B	C	D	E	F
	Observations/Parametri					
150 ppm CYC	213.2 <sup>A</sup>	68.0 <sup>A</sup>	81.2 <sup>A</sup>	77.5 <sup>B</sup>	4.8 <sup>A</sup>	34.9 <sup>AB</sup>
250 ppm CYC	182.4 <sup>B</sup>	66.2 <sup>A</sup>	82.8 <sup>A</sup>	73.8 <sup>B</sup>	4.2 <sup>A</sup>	36.6 <sup>A</sup>
2x250 ppm PR	178.0 <sup>B</sup>	61.8 <sup>A</sup>	75.2 <sup>A</sup>	90.4 <sup>AB</sup>	3.0 <sup>B</sup>	34.3 <sup>BA</sup>
250 ppm PR	166.4 <sup>B</sup>	66.2 <sup>A</sup>	74.8 <sup>A</sup>	77.3 <sup>B</sup>	3.0 <sup>B</sup>	26.6 <sup>BC</sup>
Control	211.6 <sup>A</sup>	67.4 <sup>A</sup>	82.0 <sup>A</sup>	105.4 <sup>A</sup>	2.0 <sup>B</sup>	21.9 <sup>C</sup>
<i>F test</i>	5.788	0.208	0.789	3.764	10.517	3.772
<i>P</i>	0.003	0.931	0.546	0.019	0.000	0.019

Means with the same letter are not significantly different from each other: <sup>AB</sup> at  $p \leq 0.05$  - Vrijednosti iste slovne oznake nisu statistički značajne: <sup>AB</sup> razina  $p \leq 0,05$

Testing of differences between mean values significance was not determined for the height of the first branch (B) from the roots at cv. 'Carmen' (Table 3). Cv. 'Grace Star' (Table 4) only in variant PR250 (54.2) resulted in significantly lower induction of the first branch in relation to the control (69.0), while the cv. 'Black Star' (control / 79.2) induced significantly lower branches in the treatments CYC150 (55.8), CYC250 (57.6) and 2x250PR (59.6) without significance between (Table 5). Elfving and Visser (2006) reported in exp. no. 1 (CYC 50, 100 and 200 mg L-1; CYC 100 mg L-1 + PR 500 mg L-1) and exp. no. 3 (CYC 50, 100 mg L-1, PR 250 mg L-1 in combination with each concentration of PR CYC) on cv. Bing / mazzard, and in exp. no. 4 (CYC 100 mg L-1 with or without PR 500 mg L-1) on cv. Skeena / mazzard that cyclanilide each treatment resulted in the induction of a shorter height of the first branches relative to the control which was in agreement with our results. Similar results for the height of the first induction branch on apple seedling (Fuji, Macintosh and Macoun) were reported by Sazo and Robinson (2011), by which the height of the first induced branch was lower in all the cyclanilide

treatments (Tiberon) in relation to the treatment with 6-benzyladenine (Maxcel). None of the cultivars has shown significance at the observed parameter of the height from the root to the top branch (C).

Treatments CYC150 (77.5), CYC250 (73.8), PR250 (77.3) at the cv. 'Carmen' resulted in significantly shorter average length of a branch (D) compared to the control (105.4) (Table 3). Elfving and Visser (2006) in exp. no. 2 (Rainer/mazzard) reported identical effect of cyclanilide (control 99 cm CYC100 ppm, 44 cm; CYC50 ppm, 62 cm) and BA + GA<sub>4+7</sub> (70 cm) to the average branch length at cherry sapling. In cv. 'Grace Star' treatment CYC150 (67.6) resulted only in a significant reduction in average branch length (D) relative to the control (90.9) and all the other treatments (Table 4). Bank and Stefani (2007) presented their results on woody ornamentals (Girard Rose - azalea and Eleanor Taber - Indian hawthorn) on successive reduction of the average length of branches with increasing concentrations of cyclanilide (0 ppm=7.2 cm to 212 ppm=5.0 cm). Significance for this parameter D (Table 5) was not recorded at Black Star.

**Table 4. Influence of cyclanilide (CYC) or BA + GA<sub>4+7</sub> (PR) applications to observed parameters of the sweet cherry cv. 'Grace Star' / Gisela 6 in the nursery (Total height - A, First branch height - B, Last branch height - C, Average branch length - D, Branch number - E, Branch angle - F)**

Tablica 4. Utjecaj ciklanilida (CYC) i BA + GA<sub>4+7</sub> (PR) za promatrane parametre na sorti trešnje Grace Star (ukupna visina – A, visina od korijena do prve grane – B, visina od korijena do najviše grane - C, prosječna dužina grane – D, broj grana – E, kut grananja – F)

Treatments/Tretmani	Grace Star					
	Cultivar/Sorta					
	A	B	C	D	E	F
	Observations/Parametri					
150 ppm CYC	166.6 <sup>A</sup>	56.8 <sup>BA</sup>	71.2 <sup>A</sup>	67.6 <sup>B</sup>	4.2 <sup>A</sup>	38.0 <sup>A</sup>
250 ppm CYC	193.0 <sup>A</sup>	59.0 <sup>BA</sup>	73.6 <sup>A</sup>	77.2 <sup>BA</sup>	4.2 <sup>A</sup>	38.1 <sup>A</sup>
2x250 ppm PR	172.4 <sup>A</sup>	59.6 <sup>BA</sup>	74.6 <sup>A</sup>	80.9 <sup>BA</sup>	3.2 <sup>A</sup>	36.6 <sup>BA</sup>
250 ppm PR	171.2 <sup>A</sup>	54.2 <sup>B</sup>	66.2 <sup>A</sup>	79.0 <sup>BA</sup>	4.4 <sup>A</sup>	41.2 <sup>A</sup>
Control	156.8 <sup>A</sup>	69.0 <sup>A</sup>	74.0 <sup>A</sup>	90.9 <sup>A</sup>	1.8 <sup>B</sup>	28.1 <sup>B</sup>
<i>F test</i>	0.511	1.796	0.599	1.139	6.457	2.478
<i>p</i>	0.728	0.169	0.667	0.369	0.002	0.077

Means with the same letter are not significantly different from each other: <sup>AB</sup> at  $p \leq 0.05$  - Vrijednosti iste slovne oznake nisu statistički značajne: <sup>AB</sup> razina  $p \leq 0,05$

Treatments applied at the cv. 'Grace Star' and 'Black Star' resulted in a significantly greater number of the induced branch (E) relative to the control (Tables 4 and 5). There are significant differences in the number of branches from the treatment in the cv. 'Black Star'. Treatment CYC150 (8.4) resulted in a significantly greater number of branches relative to the control (2.6) and all treatments BA + GA<sub>4+7</sub>, except the treatment CYC250 (7.8) without significant difference between (CYC150 > CYC250 > PR250 > 2x250PR). Robinson and Sazo (2013) reported significant increases in branching with Cyclanilide treatment of the apple trees where some cultivars developed more than 20 lateral branches. The positive effects of cyclanilide (25-100 ppm) to the number of new shoots at ornamental flowers (Coreopsis and Coneflower) were reported Farris et al. (2011). A significantly higher number of branches (E) compared to control (2.0) and other treatments for the cv. 'Carmen' (Table 3) was obtained with both variants of cyclanilide (CYC150 / CYC250 and 4.8 / 4.2) with no significance between. Identical results on cherry seedling were stated by Elfving and Visser (2006) according to which the branch induction proportionately related to an increase in the concentration of cyclanilide (Tiberon). Positive effect of cyclanilide was cited in relation to BA + GA<sub>4+7</sub> for the number of branches in the cultivar Lapins known for more difficult inducing lateral branches (Wustenberghs and Keulemans, 1999). Similar results of the two applications BA + GA<sub>4+7</sub> (2xPR) were reported by Elfving and Visser (2006a) on the Rainier cherries (experiment no. 1) where this combination did not result in improved induction of branching compared to one application of the same preparation

(Promalin®) being in full compliance with our results. Jacyna and Linden (2008) confirmed the positive effects of BA + GA<sub>4+7</sub> in the induction of branching at Regina and Schneiders cherry but in contradictory with results by Elfving and Visser (2007), where good results of BA + GA<sub>4+7</sub> were induced only in places with deliberately damaged bark.

The angle of branching (F) at cv. 'Carmen' (Table 3) in the variants CYC150 (34.9), CYC250 (36.6) and 2x250PR (34.3) resulted in a significantly greater deviation compared to the control (21.9). Sazo and Robinson (2011) in the experiments on apples (Fuji, McIntosh and Macoun) presented similar results of cyclanilide impact (Tiberon) to increase the angle of branching at more than 60° (Fuji), while the 6-benzyladenine (MaxCel) resulted in the angle 45° or smaller with increasing concentrations of BA (Empire and Fuji). Significantly higher branching angle (F) relative to the control (28.1) at the cv. 'Grace Star' (Table 4) was induced by treatment CYC150 (38.0), CYC250 (38.1) and PR250 (41.2) without major differences between. This is in disagreement with experiments by Elfving and Visser (2006) who have not noticed significance between the treatment at this parameter on cherry seedling (Rainer, Bing, Lapins and Skeena). The treatment 2x250PR (50.4) at the cv. 'Black Star' (Table 5) resulted only in a significantly greater angle of branching compared to the other treatments and the control (35.0). Elfving reported in exp. no. 4 on apple (Scarletspur Delicious) that the angle of branching is acceptable in all treatments, and that treatment with the BA + GA<sub>4+7</sub> (250 ppm) resulted in a small, not significant increase in the angle relative to the cyclanilide (50 to 100 ppm).

**Table 5. Influence of cyclanilide (CYC) or BA + GA<sub>4+7</sub> (PR) applications to observed parameters of the sweet cherry cv. 'Black Star' + MIX\*\* treatments in the nursery (Total height - A, First branch height - B, Last branch height - C, Average branch length - D, Branch number - E, Branch angle - F)**

Tablica 5. Utjecaj ciklanilida (CYC) i BA + GA<sub>4+7</sub> (PR) za promatrane parametre na sorti trešnje Black Star + MIX\*\* tretman (ukupna visina – A, visina od korijena do prve grane – B, visina od korijena do najviše grane - C, prosječna dužina grane – D, broj grana – E, kut grananja – F)

Treatments/Tretmani	Black Star + MIX**					
	Cultivar/Sorta					
	A	B	C	D	E	F
	Observations/Parametri					
150 ppm CYC	227.8 <sup>A</sup>	55.8 <sup>C</sup>	95.2 <sup>BA</sup>	71.2 <sup>A</sup>	8.4 <sup>A</sup>	40.5 <sup>AB</sup>
250 ppm CYC	204.6 <sup>A</sup>	57.6 <sup>C</sup>	76.6 <sup>B</sup>	71.6 <sup>A</sup>	7.0 <sup>BA</sup>	40.2 <sup>AB</sup>
250 ppm PR	196.2 <sup>A</sup>	75.8 <sup>BA</sup>	103.2 <sup>A</sup>	73.0 <sup>A</sup>	4.0 <sup>DC</sup>	35.6 <sup>B</sup>
2x250 ppm PR	205.0 <sup>A</sup>	59.6 <sup>BC</sup>	86.4 <sup>BA</sup>	74.7 <sup>A</sup>	5.0 <sup>BC</sup>	50.4 <sup>A</sup>
Mix**	202.0 <sup>A</sup>	57.6 <sup>C</sup>	84.0 <sup>BA</sup>	84.6 <sup>A</sup>	7.8 <sup>A</sup>	52.0 <sup>A</sup>
Control	191.6 <sup>A</sup>	79.2 <sup>A</sup>	97.6 <sup>BA</sup>	79.4 <sup>A</sup>	2.6 <sup>D</sup>	35.0 <sup>B</sup>
<i>F test</i>	0.860	2.973	1.329	0.297	11.043	2.573
<i>p</i>	0.522	0.032	0.286	0.910	0.000	0.053

Means with the same letter are not significantly different from each other: <sup>AB</sup> at  $p \leq 0.05$  - Vrijednosti iste slovne oznake nisu statistički značajne: <sup>AB</sup> razina  $p \leq 0,05$

There is no significance with additional treatments on the cv. 'Black Star' (MIX - a combination of cyclanilide 100 ppm and BA + GA<sub>4+7</sub> 250 ppm) for the observed parameters: total height (A), the height of the last branch (C) and the average length of a branch (D) (Table 5). The same results were reported by Elving and Visser (2005) on the apple sapling (Scarletspur Delicious) without influencing the differences in the final height of sapling and length of the induced branch. The combination of cyclanilide and BA + GA<sub>4+7</sub> (MIX) resulted in significantly shorter height from the root to the first branch (B / 57.6) and more spread branching angle (F / 52.0) compared to one control (B / 79.2 and F / 35.0) and treatment PR250 (B / F and 75.8 / 35.6), which is in disagreement with the results of Elfvig and Visser (2005) showing no effect on the height to the first branch. This treatment (MIX) resulted in a significant induction of a number of branches (7.8) compared to the control (2.6), treatment PR250 (4.0) and 2x250PR (5.0). From the results obtained it is evident that the combination of cyclanilide and BA + GA<sub>4+7</sub> (MIX / 7.8) has a similar effect on the number of branches of (C) as well as the application of cyclanilide alone (CYC150 / 8.4 and / or CYC250 / 7.0). There were no significant differences between the impact of cyclanilide (CYC150 and / or CYC250) in comparison to the mix in all the other parameters observed being in full compliance with the allegations stated by Elfvig and Visser (2005 and 2006) by which the combination of cyclanilide with BA + GA<sub>4+7</sub> did not lead to reducing or synergistic effect on the activity of buds in their experiment (apple and cherry).

Cyclanilide treatments in concentrations of 150 ppm and 250 ppm in all the tested cultivars of cherries have resulted in severe burns of leaves within 24

hours as a result of phytotoxicity preparations. This phytotoxicity was confirmed in studies on apple cultivars Wiltons<sup>®</sup> and Camspur<sup>®</sup> (unpublished research, Stanisavljević et al.). The symptoms can be seen both as a sudden change of color (red-purple spots) with the presence of large marginal necrosis of leaf. Identical phytotoxicity stated and Elfvig (2005a, 2006a). Most of the damaged leaves were permanently lost, resulting in short-term reduction of the assimilation potential of sapling. Regeneration of less damaged leaves was not complete. This phenomenon due to the aggressive effect of cyclanilide has resulted in increased revegetation of new leaves. Phytotoxicity was short-lived and its duration correlated with the appearance of lateral shoots after which the growth of the top portion under the influence of apical dominance was again expressed. We can say that vigor and final height of sapling were not disturbed by this phenomenon.

## CONCLUSION

Great genetic variability and potential of certain cultivars in the formation of lateral shoots impose the use of growth regulators as a necessary measure in a continuous automation of nursery production. Skeletons uniformity of two and three-year sapling constitutes an indispensable base in establishing early high productivity intensive plantations. Efficiency of BA + GA<sub>4+7</sub> is confirmed through repetitiveness of the results in increasing the number of lateral shoots. Pinching as a measure, remains indispensable independent or supplementary measure to control apical dominance.

Cyclanilide in this study resulted in a significantly greater number of branches compared to untreated



plants and BA + GA<sub>4+7</sub> treatments. The results confirm the allegations stated by Elfving and Visser (2006a) on the proper distribution and uniformed length of lateral branches. Identified phytotoxicity due cyclanilide applications above 150 ppm had no effect on the observed parameters ie. the final look (habitus) of sapling. The combination of cyclanilide and BA + GA<sub>4+7</sub> did not result in synergistic increase in the number of branches in relation to the cyclanilide treatment. In general, it can be concluded that the rapid increase in the number of branches regarding the whole plant was disincensive to the length of the branches, or the vigor of lateral shoots was less pronounced looking at their diameter and angle openness. We can not conclude for sure that it was due to chemistry of products, or trees loaded with shoots number in the rapid growth phase through a disturbed source-sink ratio. The cultivars of cherries in the trial due to the genetic variability differently responded to the applied treatment. The consistency of the results in our study indicates the potential use of cyclanilide as standard measures in nursery production of high quality planting material.

## REFERENCES

1. Abbas, M.F. (1978): Association between branching in maiden apple trees and levels of endogenous auxins. *Acta Hort.*, 80: 59–62.
2. Baldini, E., Sansavini, S., Zocca, A. (1973): Induction of feathery by growth regulators on maiden trees of apple and pear. *J. Hort. Sci.* 48:327–337.
3. Banko, T.J., and Stefani, M.A. (2007): Cyclanilide Promotes Lateral Branching in Nursery Production of Woody Landscape Species. *J. Environ. Hort.*, 25(4): 215–220.
4. Cline, M.G. (1997): Concepts and terminology of apical dominance. *Amer. J. Bot.*, 4(9): 1064-1069. doi: 10.2307/2446149
5. Cody, C.A., Larsen, F.E., Fritts, Jr. R. (1985): Stimulation of lateral branch development in tree fruit nursery stock with GA<sub>4+7</sub> + BA. *HortScience*, 20: 758–759.
6. Čmelik, Z., Tojnko, S. (2005.): Pospješivanje razvoja prijevremenih izbojaka na sadnicama jabuke u rasadniku. *Pomologia Croatica*, 11(3-4): 155.-166.
7. Elfving, D.C. (1984): Factors affecting apple tree response to chemical branchinduction treatments. *J. Ame. Soc. Hort. Sci.*, 109: 476-481.
8. Elfving, D.C., Visser, D.B. (2005): Cyclanilide induces lateral branching in apple trees. *HortScience*, 40:119–122.
9. Elfving, D.C. (2005a): Induction of branches (feathers) in sweet cherry trees in the nursery and orchard (2005). Final project report WTFRC, Project #CH-02-202, Organization Project #13C-3655-3298, WSU Tree Fruit Research and Extension Center, Wenatchee, WA
10. Elfving, D.C., Visser, D.B. (2006): Timing Cyclanilide and Cytokinin Applications in the Nursery to Obtain Desired Lateral Branch Height in Apple and Sweet Cherry Trees. *HortScience*, 41(5): 1238–1242.
11. Elfving, D.C., Visser, D.B. (2006a): Cyclanilide Induces Lateral Branching in Sweet Cherry Trees. *HortScience*, 41(1):149–153.
12. Elfving, D.C., Visser, D.B. (2007): Improving the efficacy of cytokinin applications for stimulation of lateral branch development in young sweet cherry trees in the orchard. *HortScience*, 42: 251-256.
13. Elfving, D.C. (2010): Plant bioregulators in the deciduous fruit tree nursery. XI International Symposium on Plant Bioregulators in Fruit Production 2010.
14. Farris, M.E., Keever, G.J., Kessler, J.R., Olive, J.W. (2011): Cyclanilide Promotes Shoot Production and Flowering of Coreopsis and Coneflower during Nursery Productio. *J. Environ. Hort.*, 29(3): 108–114.
15. Hoying, S.A., Robinson, T.L., Andersen, R.L. (2001): Improving Sweet Cherry Branching. *New York Fruit Quarterly*, 9(1): 13-16.
16. Hrotkó, K., Magyar, L., Öri, B. (1999): Improved feathering on one-year-old 'Germersdorfi FL 45' sweet cherry trees in the nursery. *Gartenbauwiss*, 64: 75–78.
17. Jacyna, T., Lipa, T. (2008): Induction of lateral shoots in unpruned leaders of young sweet cherry trees. *Journal of Fruit and Ornamental Plant Research*, 16: 65-73.
18. Martin, G.C. (1987): Apical dominance. *Hort. Sci.*, 22(5): 824-833.
19. Miller, P. (1983): The use of Promalin for manipulation of growth and cropping of young sweet cherry trees. *J. Hort. Sci.*, 58: 497–503.
20. Pederson, M.K., Burton, J.D., Coble, H.D., Collins, J.R., Fritz, D. (1997): Efficacy of Finish and its mechanism of action. *Proc. Beltwide Cotton Conf.*, 2: 1363–1365.
21. Plich, H., Basak, A. (1978): Further trials on induction of feathering in young apple and cherry nursery trees. *Fruit Sci. Rpt.*, 5: 23–33.
22. Preston, A.P. (1968): Pruning and rootstock as factors in the production of primary branches on apple trees. *J. Hort. Sci.*, 43: 17–22.
23. Quinlan, J.D., Preston, A.P. (1973): Chemical induction of branching in nursery trees. *Acta Hort.*, 34: 123–127.
24. Quinlan, J.D., Preston, A.P. (1978): The use of branching agents to replace hand pruning of young trees of Bramley's Seedling apple. *J. Hort. Sci.*, 53: 39–43.
25. Robinson, T.L., Sazo, M.M. (2013): Effect of Promaline, Benzyladenine and Cyclanilide on lateral branching of apple trees in the nursery. *ISHS Acta Horticulturae 1042: XII International Symposium on Plant Bioregulators in Fruit Production.*
26. Sazo, M.M., Robinson, T.L. (2011): The Use of Plant Growth Regulators for Branching of Nursery Trees in NY State. *New York Fruit Quarterly* 19(2): 5-9.
27. Sazo, M.M. (2013): Feathering Techniques Are Critical For a Modern Apple. *Lake Ontario Fruit Program - Lake Ontario Fruit - Profitability in Fruit Production Nursery in NY or Elsewhere in the US.*
28. Warner, G. (2012): Branching agent discovered by chance. *Good Fruit Grower Magazine*, 1 May 2012, pp 10-11.

29. Wertheim, S.J. (1978): Manual and chemical induction of side-shoot formation in apple trees in the nursery. *Scientia Hort.*, 9: 337–345.  
doi: 10.1016/0304-4238(78)90043-2
30. Wustenberghs, H., Keulemans, J. (1999): Le cerisier a fruits doux: Amélioration de la qualité des plants. *Le Fruit Belge*, 67: 106–110.

## INDUKCIJA LATERALNIH IZBOJA TREŠNJE NA PODLOZI GISELA 6

### SAŽETAK

*Broj lateralnih grana, odnosno ukupna razvijenost sadnica, danas predstavlja ključni parametar za postizanje potrebnih standarda kvalitete namijenjenim suvremenim intenzivnim sustavima uzgoja. Razgranate sadnice imaju formiranu strukturu krošnje, čime je definiran i uzgojni model budućega nasada. Pored uniformiranosti nasada, postiže se bolja i ranija produktivnost. Istraživanje u cilju standardiziranja tehnike indukcije lateralnoga grananja provedeno je 2013. godine u rasadniku voćnih sadnica (Koprivna, Istočna Hrvatska) na 3 sorte trešnje cijepljenih na podlozi Gisela 6. Tretmani su uključivali aplikaciju različitih kombinacija bioregulatora 6-benzyladenina i giberelina ( $BA + GA_{4+7}$ ) te ciklanilida na sortama Carmen, Grace Star i Black Star. Svi su primijenjeni tretmani rezultirali značajnim povećanjem broja novih grana u odnosu na kontrolu. Sorta Black Star inducirala je razvoj najvećega broja grana. Tretman  $BA + GA_{4+7}$  povećao je broj lateralnih izboja u odnosu na kontrolu kod svih ispitivanih sorti. Najznačajnija indukcija broja grana postignuta je pri tretmanu s ciklanilidom, koji je poboljšao pravilniju distribuciju i rezultirao uniformnijom dužinom induciranih izbojaka. Kombinacija tretmana ciklanilid i  $BA + GA_{4+7}$  nije rezultirala sinergičnim povećanjem broja grana u odnosu na tretman s ciklanilidom. Primijenjeni su tretmani, također, značajno utjecali na dužinu grana i kut grananja. Rezultati ovog istraživanja ukazuju na potrebu daljnjih ispitivanja ciklanilida, kao potencijalnoga regulatora rasta u rasadničarskoj proizvodnji. Tretman  $BA + GA_{4+7}$  rezultirao je povećanjem broja lateralnih izboja u odnosu na kontrolu kod svih ispitivanih sorti.*

**Ključne riječi:** ciklanilid, lateralne grane, trešnja

(Received on 20 April 2015; accepted on 15 May 2015 - *Primljeno 20. travnja 2015.; prihvaćeno 15. svibnja 2015.*)