

Induction of Lateral Branching of Sweet Cherry and Plum in Fruit Nursery

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Abstract

Modern high quality nursery trees pruning as knipboom grow and crop significantly better during the first years after planting in the orchard. High quality nursery trees perform better in the first years after plantation in the orchard than average or low quality nursery trees. Nurserymen are focused on production of such trees in the shortest time (one year). However, fruit tree species and varieties differ in their abilities to produce these trees. The goal of this work was to increase the quality and branching of one-year-old nursery trees at two plum and two sweet cherry varieties by the treatments of 12 different preparations of plant growth regulators (PGRs). Before lifting, branching and trunk parameters were recorded. Data showed that Ethephone+Putrescine+GA₄₊₇ and Ethephone+Putrescine+6-BAP significantly increased almost all parameters at both plum varieties. Both sweet cherry varieties were sufficiently branching with Globaryl+epibrassinolide treatment, however the lateral shoots of 'Celeste' sweet cherry variety were of low quality. The same was observed after treatment with Progerbalin+4-CPPU+Putrescine. Sweet cherry 'Kordia' branched very well after Globaryl and TIBA+PBZ treatments too, however, the lateral shoots after treatment with the latter preparation were of low quality. 'Kordia' trees after Globaryl treatment were branched in 94% of cases with more than four lateral shoots.

Keywords: apical dominance; feathering; PGRs; *Prunus avium*; *Prunus domestica*

Introduction

The quality of planting material has great effect on an early fruit onset and profitability of high density planting systems. A good root system, strong stem, and well developed crown consisting plentiful lateral shoots with wide crotch angles distributed along the main shoot are characteristic for high quality young trees. Such trees are required by growers for new plantations as they grow better (Preston, 1968) and crop more heavily during first years in the orchard (Zahn, 1996). Nurseries are, thus, improving their cultivation technologies to meet the requested quality of young trees.

Sweet cherries are well known for heterogeneous formation of lateral shoots in nurseries. The capability of young sweet cherry trees to produce lateral shoots depends on the variety. Varieties with weaker apical dominance have better branching characteristics than varieties with strong apical dominance, which form only low amount of lateral shoots (Hrotkó *et al.*, 1999). Compared to sweet cherries, young plum trees branch relatively well in nurseries, however, the heterogeneous formation of lateral shoots between varieties could be also observed. In recent years,

nurseries, especially in case of sweet cherries, are focused on improvement of the management to efficiently induce lateral branching (Sazo and Robinson, 2011).

Apart from a knipboom system, which adds higher cost due to an extra year of cultivation in the nursery, one-year-old nursery tree production system could be used to produce well branched trees as well. Here, pinching and chemical agents are used to induce lateral branching (Looney and Jackson, 2011). To lower the price of the nursery tree, nurserymen prefer to use chemical agents (Looney and Jackson, 2011). From these, the best results have been obtained from application of cytokinins and gibberelins.

Studies proved positive effect of 6-benzylaminopurine (6-BAP, cytokinin) and gibberellic acids₄₊₇ (GA₄₊₇, gibberellins) in different concentrations and doses on branching of sweet cherry (Hrotkó *et al.*, 1999; Elfving and Visser, 2006; Koyuncu and Yidirim, 2008) and plum trees (Magyar and Hrotkó, 2002). GA₄₊₇ and 6-BAP have been commercially available in combination as Perlan, Promalin or Progerbalin LG and also separately as Paturyl (6-BAP), Globaryl (6-BAP) or Gibb plus (GA₄₊₇). Besides nursery production, 6-BAP is used as fruit thinning agent and GA₄₊₇ to reduce the russet development on apples, while their combination is used to increase the size of apple or pear fruits (Looney and Jackson, 2011).

In recent decades cyclanilide, which acts as an auxin transport inhibitor (Pedersen *et al.*, 2006), was tested as a promising branching agent of sweet cherry and apple trees (Elfving and Visser, 2006; Sazo and Robinson, 2011). Among plant growth regulators (PGR's) with inhibition effect, paclobutrazol (PBZ, anti-giberrelin) was used together with Promalin to produce compact and well branched sweet cherry trees (Jacyna *et al.*, 1989).

Therefore, PGR's with different impact on growth could be used for production of well branched nursery trees. Currently, a numerous PGR's with these and other effects have been used or tested in fruit production and could be beneficial in nursery production too. In this study, well known branching agents and up-to-date not tested PGR's are tested in their branching abilities with aim to produce well branched high quality one-year-old nursery trees of sweet cherries and plums. Growth inducing PGR's GA₄₊₇, 6-BAP, 24-epibrassinolide (EBR), 4-CPPU; phytohormone inhibitors 2,3,5-Triiodobenzoic acid (TIBA), naphthylphthalamic acid (NPA), PBZ; stress tolerance promotion agent putrescine HCl (Put) and ethephon as an ethylene production agent were tested.

Materials and Methods

Fruit tree nursery description

The experiment was established in 2017 in a fruit tree nursery (Ökoplant International, Slup) located in the South Moravian Region, Czech Republic. The production area of the nursery is each year located in a new field. Weed within and between the rows was removed and trees were fertilized regularly during the year. If necessary, drum irrigation was used to compensate water deficiency. The average temperature and total rainfall in 2017 were 10.9 °C (min. -16.6 °C, max. 40 °C) and 454.08 mm, respectively.

Plant material

Two plum and two sweet cherry varieties were selected for the experiment. From plum varieties, the semi-vigorous variety 'Toptaste', a representative of well-branching variety and a vigorous variety 'Lipnická', a representative of poorly branching variety were tested. From the sweet cherries, a relatively well-branching semi-vigorous variety 'Kordia' and a poorly branching and poorly growing variety 'Celeste' were tested. Plums were grafted on St. Julien a rootstock and sweet cherries on Gizela 5 rootstocks.

Preparation and application of PGRs

Three commercially available preparations (GIBB plus – GA₄₊₇ 10 g.l⁻¹, L. Gobbi, IT; Globaryl 100 – 6-BAP 100 g.l⁻¹, GLOBACHEM, B and Progerbalin LG – GA₄₊₇ 18.8 g.l⁻¹ + 6-BAP 18.8 g.l⁻¹, GLOBACHEM B) and 9 other PGRs (EBR, ethephon – Sigma-Aldrich, USA; 4-CPPU, TIBA, NPA, PBZ, GA₄₊₇, putrescine, 6-BAP – Duchefa, N) were applied alone or in mixtures in various concentrations (Table 2). Twelve different preparations were prepared for the test (Table 2). In the spring, when the grafted trees reached height of 60 cm, the upper part of the growing terminal of each tree was sprayed with selected preparation. The 60 cm height was selected according to the practice of the nurseryman, as all side branches up to height of 60 cm were removed during the vegetation. Plum trees were treated in two terms (22nd May and 8th June). The second treatment was applied only on 'Lipnická' variety since the 'Toptaste' variety was sufficiently branched after the first application. Sweet cherry trees were treated three times (8th June, 20th June and 4th July). Each preparation was sprayed with a hand sprayer on a non-windy day and contained 0.5 ml.l⁻¹ of Tween 20 as a surfactant. Each preparation was applied to 60 plants (Table 2). A control variant of 60 plants was sprayed the same way as preparations, only distilled water was used instead of active compound.

Table 1. Trade names, formulas and properties of tested PGRs

Trade name	Formula	Properties
2,3,5-Triiodobenzoic acid (TIBA)	C ₇ H ₃ I ₃ O ₂	Noncompetitive inhibitor of polar auxin transport
4-CPPU	C ₁₂ H ₁₀ ClN ₃ O	Cytokinin plant growth regulator
Naphthylphthalamic acid (NPA)	C ₁₈ H ₁₃ NO ₃	Non competitive transport inhibitor of auxin
Paclobutrazol (PBZ)	C ₁₅ H ₂₀ ClN ₃ O	Plant growing retardant. Antagonist of gibberellin
Epibrassinolide (EBR)	C ₂₈ H ₄₈ O ₆	Promotion of cell expansion and cell elongation; works with auxin to do so. It has an unclear role in cell division and cell wall regeneration
Ethephon (Ethe.)	C ₂ H ₆ ClO ₃ P	Plant growing regulator
Putrescine HCL (Put)	C ₄ H ₁₂ N ₂ *2HCl	Polyamine growth regulator affecting the synthesis of macro-molecules, the activity of macro-molecules, membrane permeability and partial processes of mitosis and meiosis
6-Benzylaminopurine (6-BAP)	C ₁₂ H ₁₁ N ₅	Cytokinins
Gibberelic acid ₄₊₇ (GA ₄₊₇)	C ₁₉ H ₂₄ O ₅ , C ₁₉ H ₂₂ O ₅	Gibberellic acid

Table 2. Description of preparations, concentrations of active compounds and number of treated trees

Preparation	Concentration	Number of treated trees
Globaryl+EBR	1% + 0.001 ppm	60
Globaryl	1%	60
Progerbalin	1.5%	60
GIBB	0.5%	60
GIBB+4-CPPU	0.5% + 0.01%	60
Control	x	60
TIBA+4-CPPU	0.1% + 0.01	60
NPA+4-CPPU	0.001% + 0.01%	60
TIBA+PBZ	0.1% + 0.1%	60
NPA+PBZ	0.001% + 0.1%	60
Etephon+Put+GA ₄₊₇	0.125% + 0.2% 0.15%	60
Etephon+Put+6-BAP	0.125% + 0.2% 0.15%	60
Progerbalin+4-CPPU+Put	1.5% + 0.01% + 0.15%	60

Data collection and evaluation

The evaluation was carried out at the end of the vegetation period (in October) before lifting the nursery trees. Measurements were carried out by calliper and meter. To evaluate the efficacy of PGR preparations, following parameters were measured: tree height (the distance from the ground to the tip of the terminal shoot), trunk diameter above the budding site, trunk diameter under the first branching site, number of lateral shoots (in further text – number of shoots) and the length of the lateral shoots (in further text – shoot length). Obtained data were subjected to statistical analyses in program STATISTICA 12 using a single and multi-factor analysis of variance (ANOVA). Subsequently, Duncan’s test was used to calculate differences (p = 0.05) between the treatments. To define the effective branching, a limit of 80% of branched trees for plum and 70 % for sweet cherry trees was determined.

Results

Data of the ‘Lipnická’ variety trees treated with TIBA+4-CPPU and NPA+4-CPPU were distorted as the trees grew in a field depression where more water retained. These variants of this variety were further not evaluated.

Tree height

The average height of ‘Toptaste’ trees ranged from 171.4

cm (TIBA+4-CPPU) to 246 cm (Ethe.+Put+GA₄₊₇). Significantly higher trees compared to the control were measured after treatment with NPA+PBZ, Ethe.+Put+6-BAP, and Ethe.+Put+GA₄₊₇. At ‘Lipnická’ variety, the average height of the trees ranged from 189 cm (TIBA+PBZ) to 309 cm (TIBA+4-CPPU). Compared to the control, significantly higher trees were obtained in all variants except of TIBA+PBZ and GIBB plus variants (Table 3 and Fig. 1).

The average height of ‘Kordia’ trees ranged from 141.54 cm (TIBA+PBZ) to 181.5 cm (control) (Table 4). Compared to the control, all variants had lower trees, from which significantly lower trees, than of the control, were obtained after treatment with TIBA+PBZ, NPA+4-CPPU, TIBA+4-CPPU, GIBB plus and GIBB +4-CPPU. At ‘Celeste’ variety, average tree height ranged from 78.3 cm (Ethe.+Put+6-BAP) to 158.36 cm (NPA+4-CPPU). Compared to the control, none of the variants had significantly higher trees, however, significantly lower trees were obtained in all variants except of Gibb+4-CPPU, NPA+PBZ, Progerbalin+4-CPPU+Put and NPA+4-CPPU (Table 4 and Fig. 1).

On average, the highest trees were measured at ‘Lipnická’ variety (235.2 cm) and the lowest at variety ‘Celeste’ (129.72 cm). Significant differences in tree heights were calculated between all varieties (Table 5).

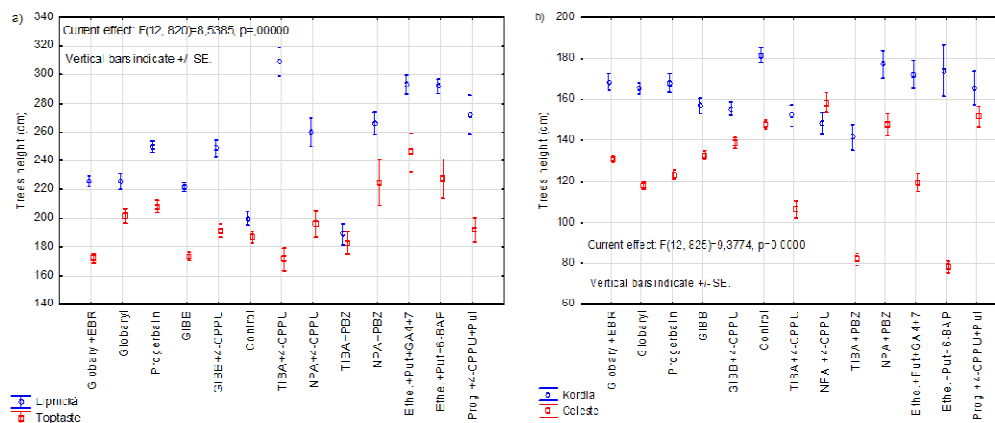


Fig. 1. The tree heights measured at all variants of a) plum varieties ‘Toptaste’ and ‘Lipnická’; b) sweet cherry varieties ‘Celeste’ and ‘Kordia’

Table 3. Average values (\pm standard errors) of parameters measured at plum varieties 'Toptaste' and 'Lipnická'. Grouping was calculated on the significance level of $p=0.5$

Preparation	'Lipnická'					Branching (%)	'Toptaste'					
	Average tree height (cm)	Average trunk diameter above the budding (mm)	Average trunk diameter under branching site (mm)	Average number of shoots	Average length of the shoots (cm)		Average tree height (cm)	Average trunk diameter above the budding (mm)	Average trunk diameter under branching site (mm)	Average number of shoots	Average length of the shoots (cm)	Branching (%)
Globaryl+EBR	225.76 \pm 3.83cd	23.48 \pm 0.36cde	15.09 \pm 0.31bc	6.77 \pm 0.3 bcde	45.85 \pm 1.83ab	96.61	172.03 \pm 3.21a	19.96 \pm 0.36a	16.36 \pm 0.37ab	13.94 \pm 0.44abc	45.73 \pm 1.23ab	100
Globaryl	225.59 \pm 5.5cd	21.89 \pm 0.48abc	15.12 \pm 0.37bc	7.10 \pm 0.5cde	59.34 \pm 2.03cde	84.75	201.49 \pm 4.88bc	23.19 \pm 1.03abc	18.16 \pm 0.41bcd	15.05 \pm 1.03abc	55.03 \pm 1.51cd	100
Progerbalin	249.71 \pm 3.83cd	23.70 \pm 0.32cde	16.96 \pm 0.3cde	6.47 \pm 0.36bcd	69.67 \pm 2.03c	93.22	208.13 \pm 4.44bcd	22.74 \pm 0.37abc	18.50 \pm 0.34cd	12.96 \pm 0.43abc	58.20 \pm 1.45def	100
GIBB plus	221.77 \pm 3.02bc	22.50 \pm 0.29bcd	14.23 \pm 0.25b	4.94 \pm 0.36abcd	38.16 \pm 1.37a	91.53	173.64 \pm 3.15a	19.87 \pm 0.31a	15.18 \pm 0.23a	13.79 \pm 0.39abc	45.77 \pm 1.01ab	100
GIBB+4-CPPU	248.64 \pm 5.52cd	24.28 \pm 0.28def	16.13 \pm 0.31bcde	5.25 \pm 0.29abcd	60.82 \pm 2.97cde	94.92	191.37 \pm 4.74ab	20.80 \pm 0.42ab	16.30 \pm 0.37ab	12.36 \pm 0.55ab	51.85 \pm 1.41bcd	100
Control	199.96 \pm 4.38ab	20.30 \pm 0.44a	12.52 \pm 0.35a	7.74 \pm 0.52c	34.80 \pm 2.14a	91.53	186.69 \pm 3.88ab	22.72 \pm 0.44abc	15.45 \pm 0.42a	12.00 \pm 0.6ab	38.73 \pm 1.59a	100
TIBA+4-CPPU	309.00 \pm 9.93g	26.50 \pm 1h	17.10 \pm 0.68def	7.10 \pm 0.65cde	69.70 \pm 5.5c	100	171.40 \pm 7.7a	20.45 \pm 0.79ab	16.75 \pm 0.88abc	13.60 \pm 1.11abc	45.71 \pm 2.98ab	100
NPA+4-CPPU	260.00 \pm 9.88c	22.40 \pm 0.89bcd	15.33 \pm 0.75bcd	4.00 \pm 0.55a	51.60 \pm 6.65bc	90	196.00 \pm 9.09ab	22.85 \pm 0.52abc	19.60 \pm 0.54de	14.70 \pm 0.49abc	56.65 \pm 1.81de	100
TIBA+PBZ	189.00 \pm 7.21a	20.90 \pm 0.6ab	14.40 \pm 0.32b	4.70 \pm 0.73ab	52.19 \pm 4.4bc	100	183.00 \pm 7.6ab	23.70 \pm 1.19bc	18.90 \pm 0.83de	14.00 \pm 0.76abc	58.55 \pm 3.33def	100
NPA+PBZ	266.00 \pm 7.77c	23.75 \pm 0.82cde	15.75 \pm 0.64bcd	5.20 \pm 0.72abcd	55.43 \pm 6.9bcd	100	224.80 \pm 15.74cde	24.95 \pm 0.9c	20.95 \pm 0.92c	16.40 \pm 1.13c	64.42 \pm 4.34ef	100
Erephon+Put+GA ₄₊₇	293.00 \pm 6.5fg	25.50 \pm 0.47gh	18.80 \pm 0.5g	7.10 \pm 0.8cde	67.07 \pm 3.29de	100	246.00 \pm 13.37c	24.40 \pm 1.02c	19.80 \pm 0.94de	15.50 \pm 1.16bc	65.80 \pm 4.32f	100
Erephon+Put+6-BAP	292.00 \pm 5.12fg	25.85 \pm 0.54gh	17.55 \pm 0.54fg	7.30 \pm 0.89de	66.23 \pm 2.81de	100	227.77 \pm 13.43de	24.16 \pm 1.04c	19.05 \pm 0.66de	16.33 \pm 0.91c	59.30 \pm 2.01def	100
Progerbalin+4-CPPU+Put	272.00 \pm 13.48ef	22.40 \pm 0.77bcd	15.25 \pm 1.21bcd	4.87 \pm 0.78abc	69.19 \pm 6.98c	80	192.00 \pm 8.53ab	20.65 \pm 1.1ab	16.75 \pm 0.71abc	11.70 \pm 0.91a	48.57 \pm 4.56bc	100

Note: Different letters between preparations denote significant differences (Duncan test, $p < 0.05$)

Table 4. Average values (\pm standard errors) of parameters measured at sweet cherry varieties 'Celeste' and 'Kordia'. Grouping was calculated on the significance level of $p=0.5$

Preparation	'Celeste'					Branching (%)	'Kordia'					
	Average tree height (cm)	Average trunk diameter above the budding (mm)	Average trunk diameter under branching site (mm)	Average number of shoots	Average length of the shoots (cm)		Average tree height (cm)	Average trunk diameter above the budding (mm)	Average trunk diameter under branching site (mm)	Average number of shoots	Average length of the shoots (cm)	Branching (%)
Globaryl+EBR	130.93 \pm 1.59def	26.37 \pm 0.35f	13.65 \pm 0.36abc	2.48 \pm 0.22b	30.31 \pm 1.78ab	74.14	168.45 \pm 4.00bcd	19.05 \pm 0.62ab	17.26 \pm 0.65 ab	4.22 \pm 0.31d	59.42 \pm 1.93abc	86.44
Globaryl	117.89 \pm 1.92bc	21.37 \pm 0.37bc	13.63 \pm 0.39abc	1.72 \pm 0.19ab	36.90 \pm 2.07abc	47.46	165.27 \pm 2.99bcd	25.15 \pm 1.00d	17.48 \pm 0.41 ab	4.7 \pm 0.37d	53.17 \pm 1.36ab	98.30
Progerbalin	123.42 \pm 2.23cde	22.47 \pm 0.34cd	13.07 \pm 0.61ab	1.28 \pm 0.16ab	38.45 \pm 3.63abc	23.73	166.27 \pm 4.89bcd	22.69 \pm 0.62abcd	16.76 \pm 0.54ab	2.25 \pm 0.27abc	66.26 \pm 4.15c	47.45
GIBB plus	132.77 \pm 2.4cf	23.78 \pm 0.41de	15.14 \pm 0.76bcd	1.00 \pm 0a	36.57 \pm 3.79abc	12.28	156.88 \pm 3.77abc	23.06 \pm 1.05bcd	15.51 \pm 0.45 ab	2.28 \pm 0.22abc	55.21 \pm 1.99abc	54.23
GIBB+4-CPPU	137.00 \pm 3.17fg	26.74 \pm 0.47f	16.14 \pm 0.53cd	1.95 \pm 0.20ab	44.9 \pm 2.48bc	36.84	155.33 \pm 3.24abc	18.27 \pm 0.56a	16.86 \pm 0.71ab	4.00 \pm 0.57bcd	59.26 \pm 2.36abc	62.71
Control	147.64 \pm 2.05gh	26.57 \pm 0.41f	16.08 \pm 0.53cd	1.32 \pm 0.11ab	49.03 \pm 2.63c	43.86	181.50 \pm 3.57d	19.49 \pm 0.55abc	17.57 \pm 0.60 ab	3.85 \pm 0.44bcd	64.51 \pm 2.23bc	67.79
TIBA+4-CPPU	106.40 \pm 4.05b	22.90 \pm 1.05cd	x	x	x	0	153.70 \pm 4.99abc	22.90 \pm 1.17bcd	16.71 \pm 1.44 ab	1.66 \pm 0.33ab	53.40 \pm 2.42abc	66.67
NPA+4-CPPU	158.36 \pm 4.65h	25.27 \pm 0.92ef	15.20 \pm 1.15bcd	2.00 \pm 0.44ab	46.86 \pm 7.61c	45.45	148.27 \pm 5.25ab	21.27 \pm 1.49abcd	15.88 \pm 1.26 ab	3.50 \pm 0.53bcd	50.53 \pm 3.24a	81.82
TIBA+PBZ	82.20 \pm 3.07a	20.10 \pm 0.01ab	x	x	x	0	141.54 \pm 6.17a	22.81 \pm 0.58bcd	14.91 \pm 0.56a	2.45 \pm 0.36abcd	50.89 \pm 4.23a	100
NPA+PBZ	147.72 \pm 5.34gh	22.18 \pm 0.65bcd	14.50 \pm 0.5abcd	1.50 \pm 0.5ab	30.25 \pm 4.75ab	25	162.80 \pm 17.95abcd	25.70 \pm 0.80d	18.55 \pm 0.91b	4.12 \pm 0.81cd	57.46 \pm 2.00abc	80
Erephon+Put+GA ₄₊₇	119.50 \pm 4.49cd	22.90 \pm 0.95cd	12.25 \pm 0.85a	1.50 \pm 0.28ab	25.50 \pm 5.20a	40	174.83 \pm 6.93cd	18.75 \pm 1.10ab	17.00 \pm 1.87ab	1.00 \pm 0a	61.77 \pm 1.92abc	45.45
Erephon+Put+6-BAP	78.30 \pm 2.84a	18.20 \pm 0.84a	x	x	x	0	174.00 \pm 12.54cd	25.09 \pm 0.69d	16.91 \pm 0.87ab	3.33 \pm 0.68bcd	56.39 \pm 3.95abc	81.82
Progerbalin+4-CPPU+Put	151.05 \pm 5.08h	26.05 \pm 0.95f	16.85 \pm 0.76d	2.00 \pm 0.30ab	50.54 \pm 9.20c	70	165.18 \pm 7.33bcd	23.54 \pm 0.98cd	17.00 \pm 1.36ab	2.20 \pm 0.48abc	62.16 \pm 7.54abc	50

Note: Different letters between preparations denote significant differences (Duncan test, $p < 0.05$).

Table 5. Overall average values (\pm standard errors) of parameters measured at sweet cherry and plum varieties. Grouping was calculated on the significance level of $p=0.5$

Variety	Tree height (cm)	Trunk diameter above the budding (mm)	Trunk diameter under branching site (mm)	Number of shoots	Length of the shoots (cm)
Plum					
'Toptaste'	191.61 \pm 1.74c	21.79 \pm 0.2b	17.01 \pm 0.15b	13.41 \pm 0.17d	50.48 \pm 0.62b
'Lipnická'	235.20 \pm 2.03d	22.88 \pm 0.15c	15.23 \pm 0.13c	6.27 \pm 0.15c	53.14 \pm 1.01b
Sweet Cherry					
'Kordia'	164.90 \pm 1.44b	20.36 \pm 0.32a	18.20 \pm 0.25d	3.56 \pm 0.14b	58.28 \pm 0.82c
'Celeste'	129.72 \pm 1.11a	24.22 \pm 0.18d	14.53 \pm 0.21a	1.84 \pm 0.09a	38.60 \pm 1.17a

Note: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$).

Trunk diameter above the budding site

The trunk diameter above the budding site of 'Toptaste' variety ranged between 19.87 mm (GIBB) to 24.95 mm (NPA+PBZ). No significant differences were observed between the treatments and the control. At 'Lipnická' variety, the trunk diameter above the budding site ranged from 20.3 mm (control) to 26.5 mm (TIBA+4-CPPU). Compared to the control, significantly higher values were measured at all variants except for the TIBA+PBZ, and Globaryl. At both plum varieties the highest values of this parameter were measured in trees treated with

Ethe.+Put+GA₄₊₇ and Ethe.+Put+6-BAP (Table 3).

The trunk diameter above the budding site of 'Kordia' variety ranged between 18.27 mm (Gibb+4-CPPU) to 25.7 mm (NPA+PBZ). Compared to the control, significantly higher values were measured at variants treated with NPA+PBZ, Ethe.+Put+6-BAP and Globaryl. At 'Celeste' variety, the diameter of the trunk above the budding site ranged from 18.2 mm (Ethe.+Put.+6-BAP) to 26.74 mm (Gibb+4-CPPU), however no variants with significantly thicker trunks above the budding site were observed compared to the control (Table 4).

On average, the highest values of trunk diameter above budding site were measured at ‘Celeste’ variety (24.22 mm) and the lowest at ‘Kordia’ variety (20.36 mm). Significant differences were calculated between all varieties (Table 5).

Trunk diameter below the first branching site

The trunk diameter below the first branching site of ‘Toptaste’ variety ranged from 15.18 mm (GIBB plus) to 20.95 mm (NPA+PBZ). Compared to the control, significantly higher values were measured at variants treated with Globaryl, Progerbalin, TIBA+PBZ, NPA+4-CPPU, NPA+PBZ, Ethe.+Put+GA₄₊₇ and Ethe.+Put+6-BAP. At ‘Lipnická’ variety, the trunk diameter below the first branching site ranged from 12.52 mm (control) to 18.8 mm (Ethe.+Put+GA₄₊₇). All treated variants had significantly higher values of trunk diameters below the branching site as the control (Table 3).

The trunk diameter below the first branching site of ‘Kordia’ variety ranged from 14.91 mm (TIBA+PBZ) to 18.55 mm (NPA+PBZ). Significant differences were calculated only between variants treated with NPA+PBZ and TIBA+PBZ. At ‘Celeste’ variety the trunk diameter below the first branching site ranged between 12.25 mm (Ethe.+Put+GA₄₊₇) to 16.85 mm (Progerbalin+4-CPPU+Put). None of the variants had significantly thicker trunks below the first branching site compared to the control (Table 4).

On average, the lowest values of trunk diameter below the first branching site were measured at ‘Celeste’ variety (14.53 mm) and the highest at ‘Kordia’ variety (18.2 mm). Significant differences were calculated between all varieties (Table 5).

Branching

All trees in all variants of ‘Toptaste’ variety were branched, whereas at ‘Lipnická’ variety at least 80 % of the trees were branching in each variant. The lowest branching at ‘Lipnická’ variety was observed at treatment with Progerbalin+4-CPPU+Put (80 % of branched trees) see in Table 3.

At ‘Kordia’ variety Globaryl+EBR, Globaryl, NPA+4-CPPU, TIBA+PBZ, NPA+PBZ and Ethe.+Put+6-BAP variants and at ‘Celeste’ variety only Globaryl+EBR and Progerbalin+4-CPPU+Put variants had more than 70 % of trees branched. On the other hand, at ‘Celeste’ variety no branching was observed at trees treated with TIBA+4-CPPU, TIBA+PBZ and Ethe.+Put+6-BAP (Table 4).

Number and length of the shoots

At ‘Toptaste’ variety, the average number of shoots ranged from 11.7 (Progerbalin+4-CPPU+Put) to 16.4 (NPA+PBZ.) (Table 3). Significantly higher number of shoots compared to the control were recorded only at NPA+PBZ and Ethe.+Put+6-BAP variants (Fig. 2). The average shoot length at ‘Toptaste’ variety ranged from 38.73 cm (control) to 65.8 cm (Ethe.+Put+GA₄₊₇). Compared to the control, significantly longer shoots were measured at all variants except for TIBA+4-CPPU, Globaryl+ERB and GIBB plus (Fig. 3). The average number of shoots at ‘Lipnická’ variety ranged from 4.00 (NPA+4-CPPU) to 7.74 (control). None of the variants had higher number of shoots than the control, moreover, NPA+4-CPPU, TIBA+PBZ, Progerbalin+4-CPPU+Put, GIBB plus, NPA+PBZ and GIBB+4-CPPU variants had significantly lower number of shoots than the control. The average shoot length of ‘Lipnická’ variety ranged from 34.80 cm (control) to 69.70 cm (TIBA + 4-CPPU). Compared to the control, significantly longer shoots were measured at all variants except for those treated with GIBB plus and Globaryl+EBR.

The average number of shoots at ‘Kordia’ variety ranged from 1.00 (Ethe.+Put+GA₄₊₇) to 4.70 (Globaryl) (Table 4). When compared to the control, significant difference was calculated only at Etephon+Put+GA₄₊₇, showing however significantly lower number of shoots than the control (Fig. 2). The average shoot length at ‘Kordia’ variety ranged between 50.53 cm (NPA+4-CPPU) and 66.26 cm (Progerbalin) (Table 4). No significantly higher differences were observed between the treatments and the control.

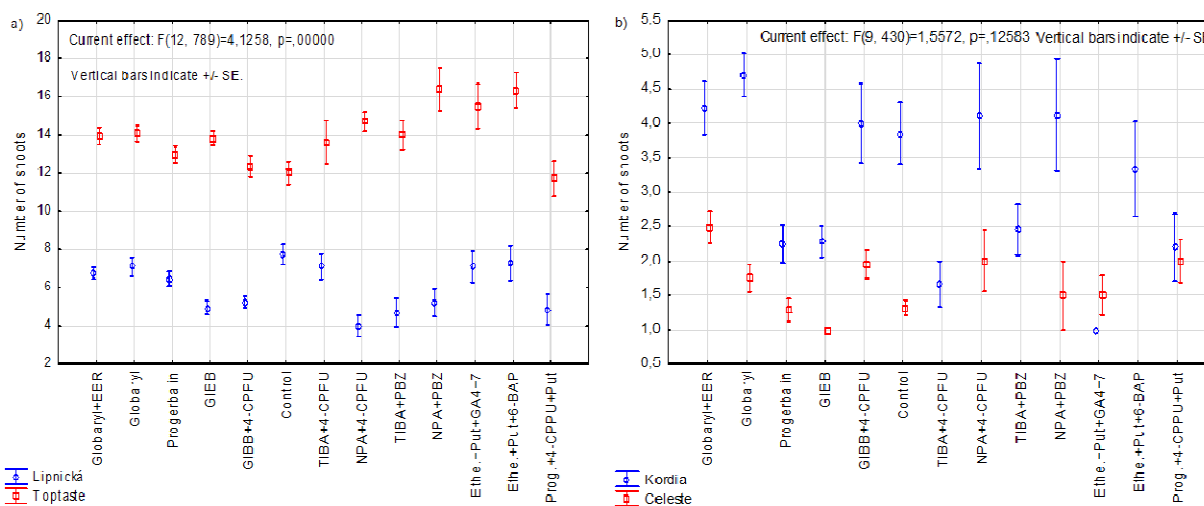


Fig. 2. The number of shoots counted at all variants of a) plum varieties ‘Toptaste’ and ‘Lipnická’; b) sweet cherry varieties ‘Celeste’ and ‘Kordia’

On contrary, NPA+4-CPPU a TIBA+PBZ had significantly lower shoot length than the control (Fig. 3). At 'Celeste' variety no branching was observed after treatment with TIBA+4-CPPU, TIBA+PBZ, and Ethe.+put+6-BAP and therefore were not evaluated at number of shoots and shoot length parameters. At the rest variants, the average number of shoots ranged from 1.00 (GIBB plus) to 2.48 (Globaryl+EBR). Significant difference was found only between the variants treated with Globaryl+EBR and GIBB plus. The average shoot length at 'Celeste' variety ranged between 25.5 cm (Ethe.+Put+GA₄₊₇) and 50.54 cm (Progerbalin+4-CPPU+Put). None of the treated variants

had significantly longer shoots than the control, however, significantly shorter shoots were measured at Globaryl+EBR, NPA+PBZ, and Ethe.+Put+GA₄₊₇ variants.

On average, the highest number of shoots had the plum variety 'Toptaste' (13,41) and the lowest sweet cherry variety 'Celeste' (1,84). Significant differences were calculated between all varieties. On average, the longest shoots were measured at variety 'Kordia' (58.28 cm) and the shortest at 'Celeste' variety (38.6 cm). Significant differences were calculated between all varieties except for between 'Toptaste' and 'Lipnická' varieties (Table 5).

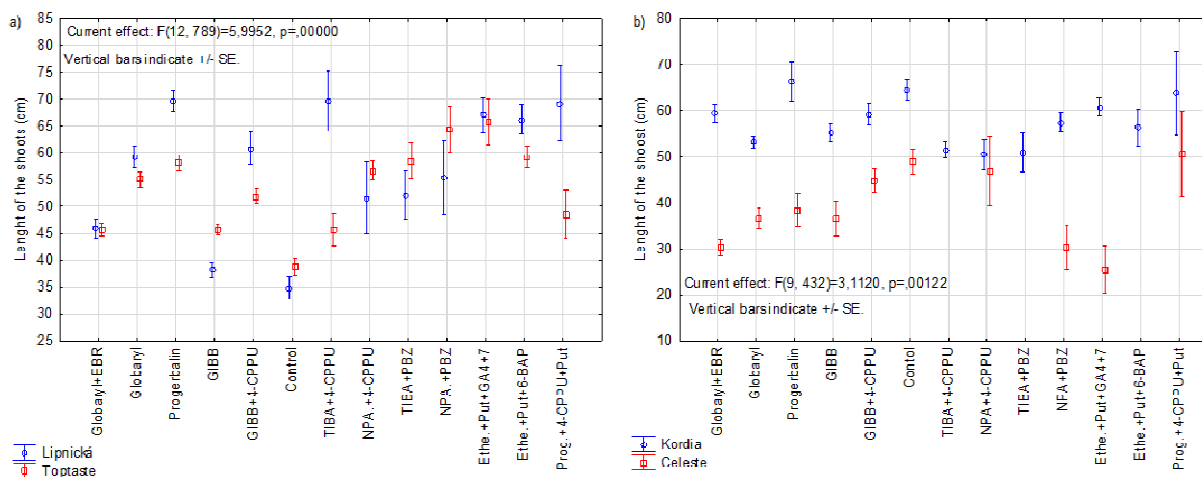


Fig. 3. The shoot lengths measured at all variants of a) plum varieties 'Toptaste' and 'Lipnická'; b) sweet cherry varieties 'Celeste' and 'Kordia'

Discussion

Managing the production of one-year-old trees is important for all fruit tree nurseries. Most of the plum varieties produce well-branched one-year-old trees even without application of PGRs. On the other hand, branching of some sweet cherry varieties is problematic and has been under research. Several publications (Hrotkó *et al.*, 1999; Magyar and Hrotkó, 2005; Koyuncu and Yildirim, 2008) have studied branching promotion of sweet cherry trees, however, with low efficiency. Previous studies have shown positive effects of cytokinins (6-BAP) and gibberellins (GA₄₊₇) on branching of one-year-old trees (Elfving and Visser, 2007, 2009; Elfving *et al.*, 2011). Except for these, several other PGRs were tested in this study to produce well-branched one-year-old nursery trees.

In the Czech Republic, according to the Decree 332/2006 of Act 219/2003, the nursery tree must have at least 80 and 100 cm of height on a dwarfing and semi-vigorous to vigorous rootstocks, respectively. All tested plum trees in this study were grafted on a semi-vigorous St. Julien A rootstock and reached at least 150 cm of height. Sweet cherry trees were grown on a dwarfing rootstock Gisela 5 and except the variant Ethe.+Put+6-BAP (78.3 cm) all trees reached the legal tree height of 80 cm (Table 4).

Tree height of sweet cherry varieties was negatively affected by all preparations, whereas at plum varieties this

effect was observed only at 'Toptaste' treated with Globaryl+EBR and GIBB plus. An active compound of Globaryl and Progerbalin is 6-BAP, which decreases the tree height by up to 10% (Hrotkó *et al.*, 2000; Doric *et al.*, 2016). The stronger effect of height decrease at sweet cherry varieties could be promoted by a significantly weaker growth (Table 5) and a higher number of applications of tested preparations compared to the plum varieties.

At both plum varieties, the highest nursery trees were obtained after treatment by Ethe.+Put+6-BAP, Ethe.+Put+GA₄₊₇. Ethephon, an ethylene-releasing substance, is used to suppress the growth of terminal shoots due to an anti-gibberellin effect of ethylene (Lieberman, 1979). Putrescine, however, suppresses the effect of ethylene (Khan and Singh, 2010; Khan *et al.*, 2008) and promotes growth in conjunctions with other phytohormones, for example with indole-3-butyric acid (Özkaya and Celik, 1994). In our case, the ethephon was applied in 10 times lower concentration than the effective concentration in Byers (1993) and its effect was probably revoked by putrescine, which led to the promotion of apical dominance. Another possible explanation could be the vigorous growth of both plum varieties which suppressed the effect of ethephon and at the same time promoted the growth through the synergism of putrescine with 6-BAP and GA₄₊₇. The same could be the reason at sweet cherry variety 'Kordia', which showed similar results with these

preparations. At 'Celeste' variety, Ethe.+Put+6-BAP, and Ethe.+Put+GA₄₊₇ preparations negatively affected the tree heights, where trees treated with Ethe.+Put.+6-BAP preparation did not meet the standards for the nursery tree height (80 cm according to the Decree 332/2006 of Act 219/2003). All varieties had lower tree heights after treatment with preparation containing 6-BAP (Ethe.+Put+6-BAP) compared to that containing GA₄₊₇ (Ethe.+Put+GA₄₊₇).

Growth of 'Celeste' variety was negatively affected also by TIBA+4-CPPU and TIBA+PBZ. Application of TIBA on stem and leaf reduces or completely interrupts apical dominance (Snyder, 1949; Panigrahi and Audus, 1966). A combination of poorly growing variety and TIBA, an auxin inhibitor, could negatively affect the height of treated 'Celeste' trees. The negative effect of TIBA was observed also on 'Toptaste' variety few days after the first application of TIBA+4-CPPU and TIBA+PBZ, however, due to vigorous growth, the negative effect was suppressed and at the evaluation period, no effect of TIBA was observed. At 'Lipnická' variety no significant difference was calculated between the control and the TIBA+PBZ variant too, however, trees treated with TIBA+PBZ had the smallest trees among all variants. Growth suppression of vigorous varieties is beneficial, however, it should be kept in mind that paclobutrazol is deposited in the plants for a longer time and could lead to unexpected growth reduction in the future (Jacyna *et al.*, 1989).

The trunk diameter above budding site and below first branching site generally does not affect the quality of the nursery tree. On average, 'Lipnická' variety had significantly thicker trunks above the budding site than the 'Toptaste' variety. On contrary, significantly thicker trunks under the first branching site had the 'Toptaste' variety, which was however, branching by 13.22 cm higher than 'Lipnická' variety (63.56 cm) (data not shown).

Among all varieties, the highest trunk diameter above budding site was measured at poorly growing sweet cherry variety 'Celeste' (24.22 mm). At the same time, 'Celeste' variety had the lowest trunk diameter below the first branching site (14.53 mm) among all varieties. This result was probably caused by the weak growth of this variety. At sweet cherry varieties, the trunk diameters above the budding site varied considerably between the treatments, however, very similar results were obtained at both varieties at trunk diameters below the first branching site.

The thickest trunks were expected at paclobutrazol-containing preparations (PBZ+TIBA and NPA+PBZ) as paclobutrazol significantly increases trunk diameter (Werner, 1993). However, increased trunk diameters were observed only in few cases in this study. Possible cause of this result could be the concentration of the paclobutrazol in our preparations, being 1000 ppm, which in the work of Werner (1993) showed weaker effect on trunk diameter than at the concentration of 750 ppm.

According to the Decree 332/2006 of Act 219/2003, all branched nursery trees must have at least three shoots with a minimum length of 30 cm. The number and also the quality of the shoots are important parameters of nursery trees (Zahn, 1996). Both plum varieties met these requirements. 'Toptaste' variety gave very good results in number and

length of the shoots after treatment with Ethe.+Put+6-BAP, Ethe.+Put+GA₄₊₇ and NPA+PBZ and very similar results were obtained at 'Lipnická' variety too. Higher number of shoots were counted after treatment by preparation containing 6-BAP (Ethe.+Put+6-BAP), which agrees with positive effect of 6-BAP on branching described in several studies (Hrotkó *et al.*, 1999; Magyar and Hrotkó, 2005; Koyuncu and Yildirim, 2008). The longest shoots were measured at trees treated with preparation containing GA₄₊₇ (Ethe.+Putre+GA₄₊₇), which agrees with the results of several authors (Schmidt *et al.*, 2009; Atay and Koyuncu, 2016) where GA₄₊₇ prolonged the length of shoots in different apple varieties by 12–138 %. In this study, the shoot length of the 'Toptaste' variety increased by 70 % and at 'Lipnická' variety by 92.7 % after treatment containing GA₄₊₇ (Ethe.+Put+GA₄₊₇) compared to control. Good results of Ethe.+Putre+6-BAP, Ethe.+Putre+GA₄₊₇ preparations could be enhanced by ethephon, which significantly promotes branching (Lieberman, 1979).

At both plum varieties, all variants had longer shoots than the control. Increase in the shoot length is beneficial since plums often produce fruits at the ends of short shoots. After fruit drop, the end of the shoot shrivels and a truncated shoot is formed, which results in its degradation.

Best results of branching of sweet cherry variety 'Kordia' were observed after treatment with preparations containing 6-BAP (Globaryl - 98.3%, Globaryl+EBR - 86.44% and Ethe.+Putre.+6-BAP - 81.82%) and with preparations NPA+4-CPPU (81.82%), TIBA+PBZ (100%) and NPA+PBZ (80%). Positive branching effect of preparations containing 6-BAP was expected, however, branching of 'Kordia' variety could be also promoted by suppression of apical dominance with auxin inhibitors (TIBA and NPA) in association with paclobutrazol, which has shown branching promotion in the work of Werner (1993).

Efficient branching ($\geq 70\%$ of branched trees) of the poorly growing 'Celeste' variety was achieved only at treatment with Globaryl+EBR (74.14 %) and Progerbalin+4-CPPU+Put (70%). Although the efficiency of branching in these two variants was sufficient, low number of shoots and their position on the trunk resulted in their poor quality. Strong apical dominance and the dwarfing rootstock could be the reasons of unsuccessful branching of the 'Celeste' variety. For efficient branching, the optimal ratio between the concentration of auxins and cytokinins (reduction of auxin and increase of cytokinin concentrations) (Klee and Lanahan, 1995) is necessary in the shoot tissues. Thus, careful selection, higher number of applications or adjusting the concentrations of PGRs will be necessary in order to achieve effective branching at 'Celeste' variety.

Interesting effect was observed in variants treated with PBZ+NPA, where paclobutrazole caused abnormal thickening and internode shortening of the terminal shoot in the application site. Similar effect of paclobutrazole was described by Werner (1993) too. Another interesting effect was observed at 'Toptaste' variety treated with 6-BAP in variants Globaryl and Globaryl+EBR, where second-order sylleptic shoots reaching 10 cm of length were formed. However, the asset of this effect is questionable and could be revoked by lower concentrations of 6-BAP.

Conclusions

In conclusion, no significant results were observed at preparations containing NPA and 4-CPPU. An exception at NPA was NPA+PBZ preparation, which showed promising results in plum varieties. Here, PBZ could have greater effect on the trees than NPA or a synergistic effect between NPA and PBZ occurred. However, the long-term effect of PBZ on plants is questionable. Overall at plums, the treatments with Ethephon+Put+GA₄₊₇ and Ethephon+Put+6-BAP significantly increased almost all measured parameters. Both sweet cherry varieties were sufficiently branching after treatments with Globaryl+EBR, however the lateral shoots of 'Celeste' sweet cherry variety were of low quality. The same effect was observed after treatment with Progerbalin+4CPPU+Put as well. Sweet cherry variety 'Kordia' branched very well after treatment with Globaryl and TIBA+PBZ too, however TIBA+PBZ, the lateral shoots after treatment with the latter preparation were of low quality. 'Kordia' trees after Globaryl treatment were branched in 94% of cases with more than four lateral shoots.

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Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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