Original article

Improving the quality of nursery apple and pear trees with the use of different plant growth regulators

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Summary

Modern fruit tree nurseries emphasize high productivity, production intensification, economic justification, and high final quality of the produced trees. The present study is focused on the effect of selected plant growth regulators (PGRs) on knip-boom apple and pear nursery trees with the aim of increasing quality and intensification and at the same time reducing the costs of production borne by wholesale customers. The experiment was performed over a period of three years to verify the effects of commercial products, such as Gibb plus, Globaryll 100 and Progerbalin LG, and of newly developed mixtures of various PGRs in various concentrations. Among the PGRs, BA, GA₄₊₇, 24-epibrassinolide, 4-CPPU, Naptalam, TIBA, Paclobutrazol, Putrescine and Ethephon were used. The experimental verification was performed on 'Admiral', 'Galaval', and 'Rubinola' apple varieties and on 'Bohemica', 'Conference', and 'Decora' pear varieties. The results showed that among the commercial products, the most effective for feather formation were Globaryll for apple trees and Progerbalin for pear trees at concentrations of 1.0% and 1.5%, respectively. Among the newly developed mixtures, the most effective for feather formation were Progerbalin + 4-CPPU + Putrescine (1.5% + 0.01% + 0.15%) for apple trees and Ethephon + GA₄₊₇ + Putrescine (0.125% + 0.2% + 0.15%) for pear trees. The other measured parameters, such as tree height, feather length and trunk thickness were significantly affected by different PGR. Furthermore, the application of Paclobutrazol in a mixture with TIBA or with Naptalam inhibited tree branching. The effect of the PGRs applications depended significantly on the fruit tree variety.

Keywords

phytohormones, cytokinins, gibberellins, auxin inhibitor, knip-boom, PGRs

Significance of this study

What is already known on this subject?

- The possibility of using and effect of some plant growth regulators or phytohormones for branching of nursery trees.

What are the new findings?

- Knowledge of the effects in selected PGRs and their mixtures, which have not been tested in published studies. Knowledge of interaction in selected varieties with evaluated PGRs and their mixtures.

What is the expected impact on horticulture?

- Based on the obtained results, it would be possible to modify the application formulations used for branching and increasing of quality in nursery trees. Production of trees or application of active mixtures can be cheaper and more efficient.

tative growth of fruit trees and their impact on the management of fruit production was studied by Costes et al. (2006).

In particular, nurseries producing knip-boom-type planting material started to follow this trend. The theory of the improvement of the nursery tree character was discussed in Quinlan and Tobutt (1990). The knip-boom method was developed in the Netherlands in the 1980s to provide planting material for high-density orchards (3,000–4,500 pcs ha⁻¹). Generally, this method can be described as nursery tree production from a 3-year-old root system (rootstock), a 2-year-old trunk of a height of 60–80 cm, and a 1-year-old crown formed by sylleptic shoots that have a wide crotch angle of 60°, with the use phytohormones and leaf pinching. Apart from a knip-boom system, which has higher costs due to an extra year of cultivation in the nursery, a one-year-old nursery tree production system (2-year-old root system and 1-year-old trunk with a crown formed by sylleptic shoots with wide crotch angles) could also be used to produce well-branched trees. Here, again, pinching, and chemical agents are used to induce lateral branching (Looney and Jackson, 2011). Such nursery trees make dense orchards possible and, especially with knip-boom trees, produce fruit in the first year after planting (Nicolai and Nicolai, 1998; Lipecki et al., 2013; Dorić et al., 2016).

An essential feature of cultivating such types of nursery trees is the application of plant growth regulators (PGRs) with long-term impacts on the shape development of a tree (Quinlan and Preston, 1973; Quinlan, 1978a, b). Currently,

Introduction

The increasing intensification of fruit production and the rising costs of establishing a new orchard force farmers to speed up the economic return of their orchards. The key factor is to harvest fruits soon after planting, which requires high-quality planting material. The formation and growth of fruit-bearing shoots, the fertility, and the nature of the vege-

commercial products containing various active compounds are being used. In Central Europe, 6-benzylaminopurine (BA, cytokinin) is available as Paturyl 10 SL (10% BA) or Globaryll 100 (10% BA); gibberellic acids (GA₄₊₇) are available as Gibb plus (10% GA₄₊₇); and a mixture of BA and GA₄₊₇ is available as Promalin (1.8% BA + 1.8% GA₄₊₇) and Progerbalin LG (1.8% BA + 1.8% GA₄₊₇). In recent decades, cyclanilide (CYC), commercially named Tiberon (not available anymore), was tested as a promising branching agent for cherry and apple trees (Elfving and Visser, 2005; Sazo and Robinson, 2011). However, these products do not always sufficiently induce branching, as fruit tree varieties differ in their branching capabilities (Elfving, 1985; Lindhagen, 1998). Therefore, the selection and proper application of other PGRs could solve problems with poorly branching varieties.

Currently, numerous PGRs with different impacts on growth and other effects have been used or tested in fruit production and could be beneficial in nursery production as well. In this study, well-known branching agents and up-to-date not tested PGRs were studied with the aim of producing well-branched, high-quality knip-boom and one-year-old nursery apple and pear trees.

Materials and methods

The experiment was conducted in 2016–2018 on selected apple and European pear varieties at the Ökoplant International Ltd. commercial nursery in Slup, Znojmo district, Czech Republic. For apples, the well-branching 'Galaval' and poorly branching 'Rubinola' and 'Admiral' varieties were budded (by chip budding) on M9, a dwarfing rootstock. For pears, the well-branching 'Bohemica' and poorly branching 'Decora' and 'Conference' varieties were budded on FOX11, a semi-vigorous rootstock. The 'Conference' pear variety, similarly to the 'Admiral' apple variety, is highly reluctant in feathering in knip-boom production. For the experiment, one-year-old unbranched budded trees tipped at 0.7 m (apple varieties) or 0.5 m (pear varieties) of height were used. The leaf blades were not reduced. Plants were irrigated with supplementary irrigation for best performance.

For the purpose of the experiment, commercially available and commonly used products enhancing branching Gibb plus (Globachem NV, Belgium), Progerbalin LG (Globachem

NV, Belgium), and Globaryll 100 (L. Gobbi, Italy), as well as novel mixtures of selected PGRs that could direct the physiological processes of trees towards the desired goals, were tested (Table 1). The following selected PGRs were used in the experiment: TIBA (2,3,5-triiodobenzoic acid, Duchefa, The Netherlands), an inhibitor of basipetal polar auxin transport in plants that stimulates proleptic axillary shoot growth without affecting fruit set (Thorp and Sedgley, 1993; Hedden and Hoad, 1994); Naptalam (NPA) (N-(1-Naphthyl) phthalamic acid, Duchefa, The Netherlands), a non-competitive inhibitor of auxin transport (Klíma et al., 2016; Teale and Palme, 2018); 4-CPPU (cytokinin, Duchefa, The Netherlands), a plant growth regulator for controlling fruit set and tree growth (Nimbolkar et al., 2016; Hota et al., 2017); Paclobutrazol (antagonist of gibberellin biosynthesis, Duchefa, The Netherlands), a plant growth retardant and triazole fungicide that affects rooting in *in vitro* conditions, transplant establishment and growth of fruit plants, and bud proliferation and plant regeneration in liquid cultures (Ogata, 1986; Kishore et al., 2015); Putrescine HCL (polyamine growth regulator, Duchefa, The Netherlands), which affects synthesis of macro-molecules, activity of macro-molecules, membrane permeability and the partial processes of mitosis and meiosis and is used in fruit growing to affect harvest date and fruit quality (Biasi et al., 1988; Khan and Singh, 2010; Shaban et al., 2017); BA (6-BAP, synthetic cytokinin, Duchefa, The Netherlands), which elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division, an inhibitor of respiratory kinase in plants that increases the post-harvest life of green vegetables (Wertheim and Estabrooks, 1994; Bubán, 2000; Kaplan, 2010; Teixeira da Silva, 2012); GA₄₊₇ (gibberellins, Duchefa, The Netherlands), act by stimulating rapid stem and root growth, induces mitotic division in the leaves and increases the seed germination rate (Hedden, 2003; Kaplan, 2010; Gupta and Chakrabarty, 2013; Atay and Koyuncu, 2013); Ethephon (Sigma-Aldrich, USA), promotes fruit ripening, abscission, flower induction, growth regulation and other responses (Byers, 1993; Murray et al., 1995; Rademacher, 2004); 24-Epibrassinolide (EBL, polyhydroxysteroid, Sigma-Aldrich, USA), is the most bioactive form of the growth-promoting plant steroids, termed brassinosteroids (BRs), regulates a wide range of physiological processes during the plant life cycle, from seed development and cell division to flowering and senescence (Bishop and Koncz, 2002; Gruszka, 2013).

The substances were applied each year on a sunny day in June when the day temperatures were between 25–30°C. The second treatment was applied 10 days later. Each substance supplemented with a surfactant Tween 20 (0.5 mL L⁻¹) was applied to 20 plants with 0.7 m of height in three repetitions (altogether 60 plants) per fruit tree variety. Application was carried out by hand spraying of the rosette and the shoot tip of the terminal (top 50 mm of terminal shoot).

The goal of the experiment was to test the effect of selected PGRs on the branching and overall quality of the nursery fruit trees. To assess these substances, the following parameters were evaluated: number of feathers on a tree, total feather length in a tree, tree height, thickness of the trunk under the first branching, and thickness of the trunk at the root neck (above the budding). Three years of results were statistically analysed in Statistica 12 software, using analysis of variance with subsequent Duncan's test of homogeneous subsets ($p=0.05$). The results represent the average values recorded in years 2016–2018 in apple and pear trees.

TABLE 1. The commercial products used, novel substances and concentrations for apple and pear varieties. Each application included an addition surfactant Tween 20 (0.5 mL L⁻¹).

Application variant	Concentration used
GIBB plus	0.5%
Globaryll 100	1.0%
Progerbalin LG	1.5%
Globaryll 100 + EBL	1% + 0.001 ppm
Gibb plus + 4-CPPU	0.5% + 0.01%
TIBA + 4-CPPU	0.1% + 0.01%
Naptalam + 4-CPPU	0.001% + 0.01%
TIBA + Paclobutrazol	0.1% + 0.1%
Naptalam + Paclobutrazol	0.001% + 0.1%
Ethephon + GA ₄₊₇ + Putrescine HCL	0.125% + 0.2% + 0.15%
Ethephon + BA + Putrescine HCL	0.125% + 0.2% + 0.15%
Progerbalin + 4-CPPU + Putrescine HCL	1.5% + 0.01% + 0.15%
Control variant	Untreated

TABLE 2. Overall assessment of the effect of applied PGR variants on observed apple tree characteristics and statistical significance (without varietal influence).

Application variant in apple trees	Average number of feathers (pcs)		Average height of trees (cm)		Average length of feathers (cm)		*Average thickness (mm)		**Average thickness (mm)	
	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup
Globaryll	10.16 ± 0.30 / d		187.41 ± 1.16 / ab		28.51 ± 1.00 / ab		19.78 ± 0.21 / a		18.69 ± 0.17 / bcd	
Progerbalin	8.23 ± 0.30 / c		191.87 ± 1.16 / bcd		29.14 ± 1.00 / b		19.78 ± 0.21 / a		19.00 ± 0.17 / cd	
Gibb plus	5.98 ± 0.30 / b		196.91 ± 1.16 / defg		26.50 ± 1.00 / ab		19.26 ± 0.21 / a		18.52 ± 0.17 / bc	
Globaryll + EBL ***	8.81 ± 0.30 / cd		194.53 ± 1.16 / cdef		29.00 ± 1.00 / ab		19.46 ± 0.21 / a		18.64 ± 0.17 / bcd	
Gibb plus + 4-CPPU	6.24 ± 0.30 / b		197.26 ± 1.16 / defg		25.36 ± 1.00 / ab		19.68 ± 0.21 / a		18.41 ± 0.17 / bc	
TIBA + 4-CPPU	6.57 ± 0.69 / b		192.93 ± 2.67 / bcde		28.52 ± 2.31 / ab		19.82 ± 0.49 / a		18.6 ± 0.39 / bcd	
Naptalam + 4-CPPU	6.04 ± 0.67 / b		200.61 ± 2.60 / fg		25.85 ± 2.24 / ab		19.49 ± 0.47 / a		18.53 ± 0.38 / bc	
TIBA + Paclobutrazol	4.36 ± 0.68 / a		185.30 ± 2.63 / a		23.07 ± 2.27 / a		19.29 ± 0.47 / a		17.40 ± 0.40 / a	
Naptalam + Paclobutrazol	5.59 ± 0.65 / ab		199.12 ± 2.54 / efg		24.82 ± 2.20 / ab		19.51 ± 0.46 / a		17.98 ± 0.37 / ab	
Ethephon + GA ₄₊₇ + Putrescine	6.64 ± 0.67 / b		212.78 ± 2.58 / h		27.36 ± 2.23 / ab		20.49 ± 0.46 / a		19.53 ± 0.39 / d	
Progerbalin + 4-CPPU + Putrescine	9.80 ± 0.68 / cd		193.43 ± 2.65 / bcde		30.21 ± 2.29 / b		19.39 ± 0.48 / a		18.14 ± 0.38 / abc	
Ethephon + BA + Putrescine	8.94 ± 0.68 / cd		188.89 ± 2.63 / abc		28.32 ± 2.27 / ab		19.75 ± 0.47 / a		18.20 ± 0.38 / abc	
Untreated variant	6.63 ± 0.30 / b		202.66 ± 1.16 / g		27.38 ± 1.00 / ab		22.42 ± 0.21 / b		18.27 ± 0.17 / abc	

* Diameter of the trunk above the budding; ** Diameter of the trunk below the first branching; *** EBL: 24-Epibrassinolide.

Results

The effect of the application of PGRs on apple trees

The strongest branching effect on apple trees on average was shown by the application of Globaryll + EBL (10.2 feathers tree⁻¹), followed by Progerbalin + 4-CPPU + Putrescine (9.8 feathers tree⁻¹) and Ethephon + BA + Putrescine (8.9 feathers tree⁻¹). Branching was inhibited by application of substances containing Paclobutrazol, i.e., TIBA + Paclobutrazol and Naptalam + Paclobutrazol, which had by 2.27 and 1.04 feathers tree⁻¹, respectively, less than the control, thus showing a decrease by 34.24% and 15.69%, respectively, when compared to the control. Significant differences were calculated between individual variants (Table 2). In both poorly branching varieties, the strongest branching effect was shown by the application of Globaryll (5.1 feathers tree⁻¹ in 'Admiral', 11.7 feathers tree⁻¹ in 'Rubinola'). In the 'Galaval' variety, the most effective treatment was the application of Progerbalin + 4-CPPU + Putrescine (14.0 feathers tree⁻¹) (Figure 1A). In the 'Admiral' variety, insufficient branching was achieved with all substances.

Surprisingly, the average height of the apple trees was influenced the most by the application of Ethephon + GA₄₊₇ + Putrescine (average height of 212.78 cm). Other variants achieved only lower values than the control. Values very close to the control were measured in varieties treated with Naptalam + 4-CPPU and Naptalam + Paclobutrazol (shorter than the control by 2.0 cm and 3.4 cm, respectively). The difference between the variants with the highest and the lowest trees was 27.66 cm (13.0%). Among the varieties, the strongest effect on height was clearly demonstrated by Ethephon + GA₄₊₇ + Putrescine, with which the average tree heights after treatment were 196.3 cm, 231 cm, and 217 cm in the 'Admiral', 'Rubinola', and 'Galaval' varieties, respectively (Figure 2A). Therefore, the application of Ethephon + GA₄₊₇ + Putrescine in the given concentration positively stimulates the height of nursery apple trees.

The strongest effect on the average length of feathers in apple trees was shown by the application of Progerbalin + 4-CPPU + Putrescine (average length 30.21 cm) and Progerbalin (average length 29.14 cm). Only TIBA + Paclobutrazol variant was significantly less effective. The length of feathers was significantly inhibited by treatments containing Paclobutrazol, i.e., TIBA + Paclobutrazol and Naptalam + Paclobutrazol, which had by 7.14 cm and 5.39 cm, respectively, shorter feathers than those of the best-performing variant, thus showing a decrease by 23.63% and 17.84%, respectively, compared to the best-performing variant. The strongest effect on the length of feathers of the 'Galaval' variety was, again, shown by Ethephon + GA₄₊₇ + Putrescine (average length 46.2 cm). In the 'Rubinola' variety, the most effective application was the treatment with Progerbalin + 4-CPPU + Putrescine (average length 23.9 cm); in the 'Admiral' variety, the most effective treatment was TIBA + Paclobutrazol (average length 24.0 cm) (Figure 3A). The values of the trunk thickness above budding in apples on average were significantly lower in all treated variants than in the control (22.4 mm). The difference in trunk thickness between the control and the least effective variant, the commercial product Gibb plus, was 3.2 mm, whereas between the most effective variant (Ethephon + GA₄₊₇ + Putrescine) and the control, the difference was 1.9 mm. Among the

FIGURE 1. Statistical analysis of the data obtained through evaluation of the effect of applications on branching (number of feathers) in apple trees (A); pear trees (B). The analysis confirms that the differences between the applied variants are statistically highly significant.

FIGURE 2. Statistical analysis of the data obtained through evaluation of the effect of applications on tree height in the apple trees (A); pear trees (B). The analysis confirms that the differences between the applied variants are statistically highly significant.

FIGURE 3. Statistical analysis of the data obtained through evaluation of the effect of applications on length of feathers in apple trees (A); pear trees (B). The analysis confirms that the differences between the applied variants are statistically highly significant.

varieties, the strongest effect on trunk thickness was shown by different substances; for the 'Rubinola' variety it was the treatment with Ethephon+GA₄₊₇+Putrescine (23.5 mm), and for the 'Admiral' variety it was the Gibb plus+4-CPPU (19.5 mm). For the 'Galaval' variety, the differences between

the variants were significant in most cases; however, all treatments resulted in lower values than the control. From a practical point of view, the application of PGRs did not have a major effect on trunk thickness above budding in apple trees (Table 2).

TABLE 3. Overall assessment of the effect of applied PGR variants on observed pear tree characteristics and statistical significance (without varietal influence).

Application variant in pear trees	Average number of feathers (pcs)		Average height of trees (cm)		Average length of feathers (cm)		*Average thickness (mm)		**Average thickness (mm)	
	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup	Standard error	Homogeneous subgroup
Globaryll	14.33 ± 0.46 / bc		178.59 ± 1.88 / abcde		30.00 ± 0.67 / abcde		19.17 ± 0.24 / ab		17.42 ± 0.29 / a	
Progerbalin	17.65 ± 0.43 / de		188.88 ± 1.76 / e		28.78 ± 0.63 / e		18.97 ± 0.23 / ab		17.42 ± 0.27 / a	
Gibb plus	14.47 ± 0.56 / bc		168.12 ± 2.27 / a		24.78 ± 0.81 / a		17.89 ± 0.30 / a		17.79 ± 0.35 / a	
Globaryll + EBL***	16.82 ± 0.46 / cd		180.38 ± 1.89 / bcde		29.40 ± 0.67 / bcde		19.34 ± 0.25 / ab		16.84 ± 0.29 / a	
Gibb plus + 4-CPPU	16.77 ± 0.46 / cd		183.10 ± 1.88 / cde		27.93 ± 0.67 / cde		19.12 ± 0.24 / ab		17.58 ± 0.29 / a	
TIBA + 4-CPPU	13.43 ± 1.06 / b		177.57 ± 4.37 / abcde		34.70 ± 1.54 / abcde		18.34 ± 0.56 / ab		16.96 ± 0.67 / a	
Naptalam + 4-CPPU	13.69 ± 1.07 / b		184.66 ± 4.37 / cde		30.85 ± 1.56 / cde		19.17 ± 0.57 / ab		17.67 ± 0.67 / a	
TIBA + Paclobutrazol	9.78 ± 1.04 / a		178.77 ± 4.24 / abcde		45.34 ± 1.51 / abcde		21.01 ± 0.55 / c		17.65 ± 0.65 / a	
Naptalam + Paclobutrazol	10.58 ± 1.07 / a		179.11 ± 4.37 / abcde		31.29 ± 1.56 / abcde		18.40 ± 0.58 / ab		17.11 ± 0.68 / a	
Ethephon + GA ₄₊₇ + Putrescine	19.64 ± 0.96 / e		187.25 ± 3.91 / de		30.51 ± 1.40 / de		19.59 ± 0.50 / b		16.86 ± 0.68 / a	
Progerbalin + 4 - CPPU + Putrescine	15.72 ± 1.07 / bcd		176.37 ± 4.43 / abcd		30.70 ± 1.56 / abcd		19.67 ± 0.57 / b		17.25 ± 0.71 / a	
Ethephon + BA + Putrescine	13.09 ± 1.14 / b		170.95 ± 4.66 / ab		30.14 ± 1.67 / ab		18.72 ± 0.61 / ab		17.26 ± 0.71 / a	
Untreated variant	15.06 ± 0.46 / bcd		173.64 ± 1.88 / abc		26.76 ± 0.67 / abc		18.69 ± 0.24 / ab		16.81 ± 0.29 / a	

* Diameter of the trunk above the budding; ** Diameter of the trunk below the first branching; *** EBL- 24-Epibrassinolide.

In apple trees, on average, the difference in trunk thickness below the first branching between the most (Ethephon + GA₄₊₇ + Putrescine) and the least (TIBA + Paclobutrazol) effective application was 2.1 mm. The difference between the most effective application and the control was only 1.3 mm. The effect of treatments of individual apple varieties on trunk thickness below branching was the strongest in 'Galaval' and 'Rubinola' trees treated with Ethephon + GA₄₊₇ + Putrescine (20.0 mm in 'Galaval', 18.8 mm in 'Rubinola') and in 'Admiral' trees treated with Globaryll (20.3 mm) (data not shown). Despite the significant differences between the variants, the differences in the values were minimal. Similar to the trunk thickness above budding, the PGR applications did not have a major effect on trunk thickness below the first branching in apple trees (Table 2).

The effect of the application of PGRs on pear trees

The strongest average branching effect on pear trees was shown by the application of Ethephon + GA₄₊₇ + Putrescine (19.4 feathers tree⁻¹), followed by the commercial product Progerbalin (17.7 feathers tree⁻¹) and Globaryll + EBL (16.8 feathers tree⁻¹). Both mixtures containing Paclobutrazol inhibited branching and resulted in a decrease of 5.3 feathers tree⁻¹ (35.1%) compared to the control. Significant differences were calculated between individual variants (Table 3). Among the varieties, the most effective branching treatment for 'Bohemica' and 'Decora' varieties was Ethephon + GA₄₊₇ + Putrescine (16.9 feathers tree⁻¹ and 28.0 feathers tree⁻¹, respectively). For the 'Conference' variety, the most effective branching application was Ethephon + BA + Putrescine (9.7 feathers tree⁻¹) (Figure 1B). The average height of the pear trees was influenced the most by the application of the commercial product Progerbalin (average height 188.9 cm) and Ethephon + GA₄₊₇ + Putrescine (average height 187.3 cm). Apart from Gibb plus and Ethephon + BA + Putrescine, all other treatments increased the height of pear nursery trees. Similar to apple trees, the difference of the heights of pear trees between the most and the least effective variants was substantial (20.8 cm, 11.0%). The effects of substances on the height of the trees varied substantially among individual varieties (Figure 2B). For the 'Bohemica' variety, the most effective application was Naptalam + 4-CPPU (average height 203.3 cm), for 'Decora' the most effective application was the commercially available Progerbalin (average height 211.2 cm), and for 'Conference' the most effective application was Ethephon + GA₄₊₇ + Putrescine (average height 182.1 cm).

The highest impact on the average length of feathers of the pear trees had both substances containing TIBA, namely, TIBA + Paclobutrazol (average feather length 45.0 cm) and TIBA + 4-CPPU (average feather length 34.7 cm), whereas the lowest effect had the treatment with Gibb plus, where the trees had shorter feathers, however not significantly, than the control. The difference between the least and the most effective treatment was 20.6 cm (45.5%). The strongest effect on the length of feathers in all varieties had the treatment with TIBA + Paclobutrazol (Figure 3B): average feather length: 46.5 cm, 35.4 cm, and 60.7 cm in 'Bohemica', 'Decora', and 'Conference', respectively.

Statistically significant differences were calculated between average values of trunk thickness above bud-

ding between variants in pear trees; however, as with apple trees, the differences were not substantial. The strongest effect was shown by TIBA + Paclobutrazol (21.0 mm), whereas the weakest effect was shown, as in apple trees, by the application of the commercially available Gibb plus. The difference between the trunk thicknesses above budding in the most and the least effective treatment was 3.1 mm, and compared to the control, the difference was 2.3 mm. Despite the significant differences between the varieties, the value differences were not important from a practical point of view. For each variant, the differences between varieties did not exceed 1.0 mm. The most effective substance appeared to be TIBA + Paclobutrazol, as it stimulated trunk thickness in all three varieties evenly. The effect of PGRs on trunk thickening above budding was slightly more evident in pear trees than in apple trees.

Similar results were obtained for measurements of trunk thickness below the first branching. Here, the differences between variants in pear trees were not significant on average. The difference between the most effective variant (surprisingly, Gibb plus) and the control was a mere 0.9 mm. Among varieties, the impact of the substances on trunk thickness below the first branching varied greatly. The strongest influence of treatment was shown for the 'Decora' variety, which had values by 3.1 mm and 4.5 mm higher than those of the 'Bohemica' and 'Conference' varieties, respectively. The strongest effect on the 'Decora' variety had the treatment with Gibb plus + 4-CPPU (21.1 mm), the most effective for 'Bohemica' was the treatment with Naptalam + 4-CPPU (17.6 mm), and the most effective for 'Conference' was the treatment with Ethephon + GA₄₊₇ + Putrescine (16.8 mm) (data not shown). Similar to the trunk thickness above budding, PGRs applications affected trunk thickening below the first branching more significantly in pear trees than in apple trees.

Discussion

The main objective of this experiment was to verify the effect of selected PGRs on the branching of apple and pear trees (both knip-boom systems) in a nursery with a focus on varieties that do not branch with commercial products. Currently, the market offers a relatively wide selection of such products, however, only a fraction of these products are allowed for use in fruit tree production in the Czech Republic. Among these, many products are too expensive for typically small Czech nurseries.

Interesting effects on branching were observed after treatment of both fruit species with commercial products. For apple trees, the most effective commercial product was Globaryll (containing BA), which is allowed for use in nurseries, followed by Progerbalin (containing GA₄₊₇ + BA), which is allowed for other purposes in Czech fruit growing. In pear trees, the most effective substance was Progerbalin (containing GA₄₊₇ + BA), followed by Globaryll (containing BA) in a mixture with 24-Epibrassinolide (EBL). Various products of a similar composition have been tested in other studies. Basak et al. (1993) tested Promalin (GA₄₊₇ + BA) and Paturyl 10 WSC (BA) in a similar study on apple trees. Their results showed higher effectiveness of Promalin than of Paturyl, yet the difference between varieties in feather formation ranged from 3.8 (pcs) in the control to 5.0 (pcs) with Paturyl and 8.3 (pcs) with Promalin application, showing high varietal dependence of the substance effect. Similar results were also obtained by Hrotko et al. (2000), whose results showed that the treatment of the 'Idared' variety with six consecutive BA applications stimulated feather development more than

only two applications. Bektaş and Ersoy (2010) experimented with Perlan (GA₄₊₇ + BA) applied to three apple varieties and compared the effectiveness of PGR application and PGR application combined with pinching. Similar to our conclusion, the effectiveness of the application depended on the variety; the best results were observed in the 'Gala Galaxy' variety, with the exception of the feather length, which did not show a significant difference between treatments. In our study, application of Progerbalin significantly stimulated the trunk thickness below branching and the apical growth in pear trees where differences from the control were as high as 15.0 cm. Jacyna et al. (1994) applied Promalin (BA + GA₄₊₇) and Dikegulac-sodium (Atrimmec) to ornamental Asian pear nursery trees to promote branching. The application of Promalin was effective in all the observed parameters, but Dikegulac-sodium had an inhibiting effect on tree height, and the crotch angle of the feathers was too narrow. The effect of different concentrations of Progerbalin and Globaryll on the branching of nursery trees was also studied by Lañar et al. (2015a). The results indicate that Globaryll at a concentration of 20 mL L⁻¹ and Progerbalin at a concentration of 50 mL L⁻¹ had the strongest effects on feather formation, and, although the differences were not significant, the more effective application was that of Globaryll. The largest effect on the height of the nursery trees was observed after the application of Progerbalin at a concentration of 10 mL L⁻¹. Our results showed that the application of Globaryll on apple trees stimulated their branching but inhibited apical growth, as did the application of TIBA + Paclobutrazol. According to Lañar et al. (2015b), the effectiveness of Progerbalin is significantly affected by the type of surfactant used. In general, as stated by Robinson and Sazo (2014) and Doric et al. (2015), BA, applied in combination with GA₄₊₇, has a stronger positive effect on the total feather number, feather length, branching zone and tree height than when applied separately. Compared to BA + GA₄₊₇, BA has a stronger effect on the growth rate of the shoot, resulting in a greater feather angle and a lower tree height, which can be eliminated by the addition of GA₄₊₇. Same effects of joint and separate application of BA and GA₄₊₇ concerning the feather formation was observed in our study too. The effect of joint application on feather formation could be then enhanced with the application of auxin inhibitors of the Naptalam/TIBA type or growth retardants such as Ethephon/Paclobutrazol (Teale and Palme, 2018).

In most cases, significant results were obtained from analyses of the results of the newly developed PGR mixtures in this study. An interesting result was the improvement of most of the parameters after the application of Globaryll in a mixture with 24-Epibrassinolide (EBL). According to the results, inhibition of branching (feather formation) was observed after the application of Paclobutrazol in a mixture with TIBA or with Naptalam. This corresponds with the natural effect of all three substances belonging to the growth inhibitors (see the methodology). Interestingly, the feather length after the application of TIBA + Paclobutrazol was stimulated in pear trees, yet inhibition was observed in apple trees. The application of Paclobutrazol to reduce growth and promote tree productivity has been demonstrated in many studies (e.g., Antognozzi and Romani, 1989; El Hodairi and Canham, 1990; Elfving et al., 1990; Deyton et al., 2014), but the assessment of its effects on nursery trees is completely novel. According to our results, the reactions of pear trees and apple trees to PGR applications are often very different.

Among the newly developed substances, Ethephon + GA₄₊₇ + Putrescine had the highest effect on feather formation

in pear trees and increased height the most in apple trees. A positive effect of Ethephon on the formation of lateral branches in fruit-bearing apple trees was described by Ebert et al. (1987), and its growth reduction effect on young trees in new orchards has been described in many studies (e.g., Edgerton, 1983; Guak et al., 2001, 2004). However, direct use of Ethephon in nurseries is rare. In the work of Palmer et al. (2011), the application of BA as well as GA₃ had a stronger effect on the branching of the 'Doyenné du Comice' pear variety than the application of GA₄₊₇. However, the gradual application of 1× BA followed by 3× GA₄₊₇ had the greatest effect on branching and feather length. In our study in apple trees, Progerbalin + 4-CPPU + Putrescine had the second-best results, which was only by 7.5% less effective than the best newly developed PGR mixture. This may indicate that increased application of cytokinins with 4-CPPU stimulated feather formation as well as in the third-most effective application, Ethephon + BA + Putrescine. According to our results, substances with the polyamine growth regulator Putrescine, which was tested up to now by Costa (1986), Schoonjans et al. (1989), and Baninasab (2017) to improve fruit onset and fruit quality in apple and stone fruit trees, generally increased both feather formation and height. This is the first time when Putrescine was tested for promotion of feather formation and to increase the quality of nursery fruit trees and with these promising results it should be tested more in further experiments.

The height of fruit trees was promoted by the application of synthetic cytokinin 4-CPPU, especially in combination with Naptalam, an auxin transport inhibitor. TIBA, which has a similar effect as Naptalam, combined with 4-CPPU did not demonstrate the same effect as 4-CPPU+Naptalam. Similar to branching, the application of TIBA + Paclobutrazol decreased the height of apple trees. In general, the most effective application for both apple and pear trees was the application of Ethephon + GA₄₊₇ + Putrescine. An experiment on the branching of apple trees in a nursery performed by Robinson and Sazo (2014) showed that separate application of cyclanilide (product Tiberon) and BA inhibited tree height; however, a combination of BA + GA₄₊₇ did not have the same inhibiting effect. When evaluating the results in the experiment, consideration of the growth vigour of different varieties cannot be excluded. Blanco (1990) confirmed the inhibitory effect on shoot growth and trunk thickening, with a simultaneous negative reduction in flower bud differentiation from Ethephon and Paclobutrazol in peach trees. However, in this case, the effect of Ethephon was not significant.

The feather length of both apple and pear trees was influenced by different substances. In pear trees, the dominant PGRs were auxin transport inhibitors, which significantly supported the lateral growth of feathers at the expense of tree height. In apple trees, Progerbalin + 4-CPPU + Putrescine as well as Progerbalin and Globaryll + EBL had the strongest effect on feather length. 24-Epi brassinolide is an active by-product of the biosynthesis of brassinolide, which can stimulate various metabolic processes in plants, such as photosynthesis (Sairam, 1994) and biosynthesis of nucleic acids and proteins (Bajguz, 2000). Brassinosteroids act against many abiotic stressors (Bajguz and Hayat, 2009; Shahzad et al., 2018).

Trunk thickness at both measured heights (above budding and below branching) was affected by the substances in different ways. In apple trees, the lower trunk thickness was influenced the most by substances containing both cytokinins and gibberellins, Ethephon + GA₄₊₇ + Putrescine, and

by the commercial products Progerbalin and Globaryll. On the other hand, the lower trunk thickness was decreased by the substances containing Paclobutrazol in apple trees. In an experiment carried out by Doric et al. (2015), the application of BA + GA₄₊₇ had different effects on trunk diameter; in the 'Gala' variety, BA had minor adverse effects on trunk diameter, whereas it had a positive effect on the 'Jonagold' variety. In pear trees, both diameter parameters increased with the application of TIBA + Paclobutrazol. The effects of other substances on the two studied trunk thicknesses varied, and the differences were not significant.

Conclusions

Influence of tested PGRs on apple trees

- Application of the commercial product Globaryll 100 significantly stimulated branching but inhibited apical growth of the terminal shoot.
- Application of Progerbalin + 4-CPPU + Putrescine stimulated the elongation growth of feathers.
- Application of Ethephon + GA₄₊₇ + Putrescine stimulated apical growth of the terminal shoot and trunk thickness below branching.
- Application of TIBA + Paclobutrazol significantly inhibited branching in both apple and pear trees.
- Application of Putrescine had a positive effect on branching, tree height and on feather length and trunk thickness.

Influence of tested PGRs on pear trees

- Application of the commercial product Progerbalin stimulated apical growth of the terminal shoot and branching.
- Application of Gibb plus stimulated trunk thickness below branching (height of feather formation) but inhibited apical growth of the terminal shoot.
- Application of Ethephon + GA₄₊₇ + Putrescine significantly stimulated branching (feather formation).
- Application of TIBA + Paclobutrazol affected feather length and trunk thickness above budding.

General influence of tested PGRs

- Application of Ethephon + GA₄₊₇ + Putrescine had a positive effect on the number of feathers, height of the tree and the diameter of the trunk.
- Of the tested apple varieties, the least affected by PGR substances was a resistant triploid apple variety 'Admiral' ('Mira' × 'Bohemia' crossbreeding), whereas 'Galaval' (a clone of the 'Gala' variety) turned out to be very plastic with good reactions to the applied PGRs.
- Of the tested pear varieties, the least affected by PGR substances was 'Conference'; the reactions of 'Bohemica' and 'Decora' varieties to PGR substances were more or less similar.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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