Article

The Effect of the Foliar Application of Biostimulants and Fertilisers on the Growth and Physiological Parameters of Maiden Apple Trees Cultivated with Limited Mineral Fertilisation

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Abstract: The article describes an experiment conducted on maiden apple trees of selected cultivars growing in a nursery. The aim of the study was to investigate how the foliar application of four biostimulants (Aminoplant 0.4%, Biamino Plant 0.2%, Bispeed 0.2% and Fylloton 0.4%) and two fertilisers (Basfoliar 6-12-6 and Basfoliar 12-4-6+S both at a concentration of 0.5%) affected the growth of the trees cultivated with the mineral fertilisation reduced by half. The plants were sprayed with the preparations and fertilisers four times at three-week intervals, between late May and late July. Selected parameters of the plants' physiological processes were also assessed: net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (C) and intracellular CO₂ (I). The foliar application of all the biostimulants and fertilisers significantly improved the growth of the maiden apple trees. The lowest values of the plant growth parameters were noted in the control combination, which was treated with a full dose of mineral fertiliser. The foliar spraying of the apple trees particularly improved the total length and number of side shoots and the fresh weight of maiden apple trees. The apple tree cultivars differed in their growth parameters, and the influence of individual foliar treatments was not conclusive. The foliar application of selected preparations intensified the leaf transpiration coefficient and the internal concentration of carbon dioxide, but it did not increase the net photosynthesis intensity or stomatal conductance.

Keywords: nursery; net photosynthetic rate; transpiration rate; stomatal conductance; intracellular CO₂

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1. Introduction

Poland is the largest producer of maiden apple trees in Europe and the third largest apple producer in the world. Large amounts of mineral fertilisers are used in nursery production to intensify the vegetative growth of fruit trees. On the other hand, plants do not use large amounts of nutrients supplied with mineral fertilisers, because these are leached into deeper soil layers, where they degrade the environment [1–5]. Plants can absorb more nutrients at a faster rate if foliar fertilisation is applied, especially at low temperatures [6,7]. According to some authors [1,8,9], the combination of these two methods of plant nutrition guarantees the best results in the cultivation of apple trees. Bi and Scagel [10] recommended the foliar fertilisation of trees in nurseries. Bearing in mind the recommendation to reduce soil fertilisation, foliar fertilisation effectively meets the nutritional requirements of plants [6]. Biostimulants are also used in sustainable horticulture as environmentally friendly products [11–13], which help to reduce the consumption of mineral fertilisers [14,15]. Plant biostimulants contain some nutrients (marine plant extracts, humic acids, amino acids and other natural products such as saponins and compost teas) that stimulate plant growth, even when administered in small amounts [16]. However, research has shown that the concentration of auxins in most biostimulants is too low to effectively stimulate the growth of the plant root system [17]. It is the development of the

root system that influences the absorption of nutrients. However, in sustainable horticulture, fertilisation is less intensive and nutrients are supplied at low concentrations [18]. Biostimulants not only intensify the growth of roots, but also increase the absorption of nutrients [19]. It is believed that the amino acids and peptides contained in biostimulants may act as signalling molecules in a similar manner to hormones [20].

Experiments have confirmed the positive effects of the AminoplantTM amino acid preparation [21,22]. Some authors [23,24] believe that Aminoplant mainly affects the physicochemical parameters (nitrate content, rutoside and kaemperol-3-o-glucoside content in leaves) of plants and has a lower influence on their growth and yield. Earlier studies have shown that such biostimulants increased the yield, because they stimulated the uptake of nitrogen from the soil, especially during droughts [21,25]. Maini [21] used a similar biostimulant on the laboratory studies and observed that it increased the yield of maize seeds, even when the dose of nitrogen fertiliser was reduced by half. The AtonikTM biostimulant (based on three synthetic nitrophenols, with a similar composition to BispeedTM) improved the growth of plants [26,27]. The experiments also showed that protein hydrolysates as a biostimulant improved the photosynthetic activity, which resulted in a higher yield [28]. Biostimulants containing marine algae and seaweeds stimulated the growth of various plant species, e.g., apples [29,30], grapevines [31], pears [32] and strawberries [33]. Zang et al. [34] found that seaweed extract applied to leaves stimulated the activity of enzymes assimilating nitrogen. Kocira et al. [35] observed that the FyllotonTM preparation (brown algae extract) increased the intensity of photosynthesis in leaves. Many studies showed that the marine algal extract increased the content of chlorophyll in leaves [19,21,31,36,37]. The spraying of maize plants with fulvic acid improved their growth as a result of the increased net photosynthetic rate, transpiration rate and the intercellular concentration of CO₂ [38]. Some other studies [39–41] showed that biostimulants (amino acid and animal-derived) applied to various plant species improved their physiological parameters, e.g., the photosynthetic rate, stomatal conductance and transpiration rate, while increasing their carbon assimilation efficiency.

Macroelements, as well as microelements such as B, Zn, Mn and Fe, are regularly applied to counteract their deficiency, especially in fruit trees [42]. It has been proven that fruit plants, both rootstocks [43] and apple trees [44], easily absorb microelements applied to their leaves, which positively affects their growth. Świerczyński et al. [45] and Świerczyński and Stachowiak [46] studied the influence of foliar fertilisation (urea, lime and magnesium saltpetre) on the growth of maiden apple trees in a nursery, but they did not observe a significant improvement in the growth of the plants after these treatments. Biostimulants (humic and fulvic acids or amino acids) intensified the development of the aerial part of almond rootstock, especially in the first year of cultivation in a nursery [47]. The application of biostimulants to year-old almond trees gave similar results [48]. The foliar application of the Humisol biostimulant (humic acids 15%, N 0.5% and K₂O 2%) and other elements (Ca, S, Mg, Zn, Fe, Cu, B and Mn) improved the growth of nut trees in a nursery [49]. Popenoe et al. [50] did not observe any significant improvement in the growth of ornamental plants in a nursery after the application of biostimulants. Differences in the growth of the plants which were treated or not treated with the biostimulants depended on the species.

The aim of the experiment was to find whether the application of foliar biostimulants and fertilisers could reduce the dose of mineral soil fertilisation by half without an adverse effect on the growth of maiden apple trees in the nursery. The research was carried out on the most important fruit tree species in Poland in terms of production and using the biostimulants and fertilisers most commonly found in the market.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiment was conducted on maiden apple trees of four cultivars: ‘Gala Schniga’, ‘Ligol’, ‘Red Boskoop’ and ‘Topaz’, grown on the M.9 rootstock. Between 2016 and 2018,

three series of experiments were conducted in a strip system in a nursery. One series of experiments consisted of two years of cultivation in a nursery. In the first year, apple varieties on the M.9 rootstock were budded. In the second year, during the growth of maiden trees, foliar spraying was applied. Before each new series of experiments, phosphorus-potassium fertilisation was applied in early spring (2015, 2016 and 2017), and nitrogen fertilisation in three divided doses was applied during the growth of the plants in the nursery (2015–2016, 2016–2017 and 2017–2018). A full dose of mineral fertilisation was applied in the first strip, and the dose was reduced by half in the second strip (Table 1).

Table 1. Treatments used in the experiment.

	Doses of Fertilisation	Container of Biostimulants and Fertilisers
Control	N 120 kg·ha ⁻¹ , P ₂ O ₅ 40 kg·ha ⁻¹ , K ₂ O 140 kg·ha ⁻¹	
Aminoplant 0.4%	N 60 kg·ha ⁻¹ , P ₂ O ₅ 20 kg·ha ⁻¹ , K ₂ O 70 kg·ha ⁻¹	18 L-amino acids and bioactive peptides (N 8.5%; organic substance 54%; bioactive peptides 82.7%, amino acids 17.3%)
Biamino Plant 0.2%	N 60 kg·ha ⁻¹ , P ₂ O ₅ 20 kg·ha ⁻¹ , K ₂ O 70 kg·ha ⁻¹	L-amino acids of plant origin (organic nitrogen 7.6%, organic carbon 21.0%, amino acids 42.6%, Fe 1.2%, Mn 0.6%, Zn 0.7%)
BiSpeed 0.2%	N 60 kg·ha ⁻¹ , P ₂ O ₅ 20 kg·ha ⁻¹ , K ₂ O 70 kg·ha ⁻¹	potassium 4-nitrophenolate 0.25–0.30% m/m, potassium 2-nitrophenolate 0.14–0.20% m/m, potassium 5-nitroguaiacolate 0.07–0.10% m/m
Fylloton 0.4%	N 60 kg·ha ⁻¹ , P ₂ O ₅ 20 kg·ha ⁻¹ , K ₂ O 70 kg·ha ⁻¹	brown algal extract (<i>Ascophyllum nodosum</i>) and plant-derived amino acids (organic nitrogen 6%, organic carbon 20.8%, organic substance 35%)
Basfoliar 2.0 6–12-6 0.5%	N 60 kg·ha ⁻¹ , P ₂ O ₅ 20 kg·ha ⁻¹ , K ₂ O 70 kg·ha ⁻¹	N 6%, P ₂ O ₅ 12%, K ₂ O 6%, B 0.01%, Cu 0.01%, Fe 0.02%, Mn 0.01%, Mo 0.005%, Zn 0.05%
Basfoliar 2.0 12–4-6+S 0.5%	N 60 kg·ha ⁻¹ , P ₂ O ₅ 20 kg·ha ⁻¹ , K ₂ O 70 kg·ha ⁻¹	N 12%, P ₂ O ₅ 4%, K ₂ O 6%, sulphur and B 0.01%, Cu 0.01%, Fe 0.02%, Mn 0.01 %, Mo 0.005%, Zn 0.05%

Before the nursery was established, the soil had been analysed chemically. It contained the following amounts of soluble forms of macronutrients: 107 phosphorus (mg·dm⁻³), 145 potassium (mg·dm⁻³), 520 calcium (mg·dm⁻³) and 96 magnesium (mg·dm⁻³) (mean value for 2015–2017). The total rainfall in individual years of the study was: 500 mm (2016), 338 mm (2017) and 228 mm (2018).

2.2. Biostimulants and Fertiliser Experiment

In the second year of the nursery production, which was the first year of growth of maiden apple trees growth, seven combinations were randomly distributed in the strips of maiden trees of each cultivar. Following that, six of them were subjected to foliar treatment with biostimulants and fertilisers with half doses of mineral fertilisers, and one of them was the control combination with full doses of mineral fertilisers with distilled water spraying. Each of the 28 experimental combinations (6 with foliar spraying and one control, 4 apple varieties) was represented in one plot by 10 maiden apple trees, in triplicate. The trees were treated with four preparations at concentrations recommended by the manufacturers (Aminoplant 0.4%, Biamino Plant 0.2%, BiSpeed 0.2% and Fylloton 0.4%) and two foliar fertilisers: Basfoliar 6-12-6 and Basfoliar 12-4-6+S, both at a concentration of 0.5%. The plants were sprayed with the preparations and fertilisers by added Slippa adjuvant (0.7%) four times at three-week intervals, between late May and late July. In each plot where 10 trees were used, 0.5 L of the prepared biostimulant or fertiliser solution was used during spraying.

2.3. Plant Measurements and Experimental Design

All maiden trees in the plot were measured and observed in triplicate for each combination. The following parameters of the maiden trees were measured: height—from the ground surface of soil to the top (cm), trunk diameter—20 cm above the budding site (mm), the length and number of side shoots. The side shoots longer than 5 cm were taken into account. After being removed from the nursery, the maiden trees without soil were weighed to determine their fresh weight (g).

In 2017, on the maiden apple trees growing in the field, directly before, during and immediately after the last foliar spraying, the following parameters were measured with a CI-340 Handheld Photosynthesis device (CID BIOSCIENCE Inc., Camas, WA, USA): net photosynthetic rate (P_n) ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), transpiration rate (E) ($\mu\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), stomatal conductance (C) ($\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), intracellular CO_2 (I) ($\text{mol CO}_2 \cdot \text{mol}^{-1}$). The research was conducted at a constant intensity of photosynthetically active radiation (PAR) ($1000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) supplied to the plants and at a constant concentration of carbon dioxide (CO_2) ($390 \mu\text{mol CO}_2 \cdot \text{mol}^{-1}$ of air). One leaf from the middle part of the long shoot was randomly selected for the measurements, which were made in four replications, four maiden apple trees for each combination.

2.4. Data Analysis

The results of the study were analysed with the STATISTICA 13.1 (Statsoft Polska, Kraków, Poland). Pearson's linear correlation coefficient between the studied growth features and the separately assessed physiological parameters was calculated, and its statistical significance was verified. Next, homogeneous groups for individual traits and treatments were calculated with Tukey's HSD test. Multivariate analyses of variance (MANOVA) based on Wilk's test were conducted. The statistical significance level was $p = 0.05$.

The growth parameters of the maiden trees were subjected to three-way analyses of variance. The experimental factors were: the apple tree cultivars, years of the experiment and foliar spray combinations. The results of measurements of the physiological parameters were analysed separately by means of two-way analyses of variance, where the apple tree cultivars and foliar treatments were the factors.

3. Results

3.1. Biometric Parameters

The measurements of the mean heights of the four apple tree cultivars showed that the shortest maiden trees were found in the control combination, whereas the trees in all the other combinations sprayed with the foliar preparations were significantly taller (Table 2). Over all the treatments, the cultivars 'Gala Schniga' and 'Red Boskoop' were the tallest and cultivar 'Topaz' was the shortest (Table 2).

The maiden trees of all the cultivars in the control combination were characterised by the smallest trunk diameter. The value of this parameter was significantly higher in all the other combinations subjected to the foliar treatments (Table 2). Over all treatments, cultivar 'Red Boskoop' had the largest trunk diameter and cultivar 'Ligol' had the smallest (Table 2).

The mean total length of the side shoots of the maiden trees of all the cultivars was significantly lower in the control combination. All foliar treatments yielded greater total lengths of the side shoots (Table 2). The trees of the 'Gala Schniga' cultivar had the largest total length of the side shoots. The smallest total length of side shoots was noted in the 'Topaz' and 'Red Boskoop' cultivars (Table 2).

The maiden trees of all the cultivars in the control combination were characterised by the smallest mean number of side shoots. All of the four biostimulants applied to the trees resulted in a greater number of side shoots (Table 2). The smallest number of side shoots was observed in the maiden trees of the 'Red Boskoop' cultivar; the largest number—in the 'Gala Schniga' cultivar (Table 2).

The maiden trees treated with the Aminoplant, Biamino Plant and Bispeed biostimulants were characterised by the largest fresh weight. The lowest fresh weight was noted in the control plants (Table 2). The fresh weight of the ‘Red Boskoop’ trees was significantly lower than the fresh weight of the trees of the other cultivars (Table 2).

Table 2. Growth parameters of four cultivars of maiden apple trees subjected to four biostimulants and two fertiliser treatments (mean from years 2016–2018).

		Control	Amino-Plant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6–12–6	Basfoliar 12–4–6+S	Mean for Cultivar
Height (cm)	Gala Schniga	136.0 hi	152.6 lm	146.6 j-m	149.5 k-m	149.4 k-m	147.9 k-m	144.4 i-l	146.6 c
	Ligol	106.3 a	118.6 c-f	122.9 e-g	125.8 fg	130.8 gh	116.3 b-e	123.2 e-g	120.6 b
	R. Boskoop	138.6 h-j	149.0 k-m	147.5 k-m	143.1 i-k	138.4 h-j	150.7 k-m	154.8 m	146.0 c
	Topaz	107.9 ab	121.7 c-f	124.8 fg	122.5 d-f	117.5 c-f	113.7 a-c	114.4 a-d	117.5 a
	Mean for treatment	122.2 a	135.5 c	135.5 bc	135.3 bc	134.0 bc	132.1 b	134.2 bc	
Diameter (mm)	Gala Schniga	11.4 a-c	12.6 f-l	12.4 d-k	12.9 j-l	12.2 c-k	12.4 d-l	12.1 b-j	12.3 b
	Ligol	10.7 a	11.1 ab	11.8 b-h	11.2 a-c	12.6 f-l	11.5 a-d	11.7 b-f	11.5 a
	R. Boskoop	11.9 b-i	12.8 i-l	12.8 h-l	12.7 g-l	12.2 c-k	13.3 l	12.8 i-l	12.6 c
	Topaz	11.6 a-e	12.5 e-l	12.5 e-l	13.1 k-l	12.5 e-l	12.6 e-l	11.8 b-g	12.4 b
	Mean for treatments	11.4 a	12.3 bc	12.4 bc	12.5 c	12.4 bc	12.5 bc	12.1 b	
Sum of long shoots	Gala Schniga	75.6 f-i	156.3 n	133.5 mn	159.4 n	124.5 lm	114.9 k-m	108.9 j-m	124.7 c
	Ligol	36.7 a-d	51.4 b-f	82.7 g-j	57.5 c-g	97.3 i-l	39.9 a-d	90.8 h-k	65.2 b
	R. Boskoop	33.4 a-d	30.0 a-c	27.4 ab	42.3 a-e	40.9 a-d	39.8 a-d	53.0 b-g	38.1 a
	Topaz	19.7 a	40.7 a-d	72.1 e-i	62.1 d-h	45.8 a-f	38.7 a-d	41.7 a-d	45.9 a
	Mean for treatment	41.4 a	69.6 bc	78.9 c	80.3 c	77.1 c	58.2 b	73.6 c	
Number of lateral shoots	Gala Schniga	3.6 i-k	5.7 m	4.9 lm	5.2 lm	5.1 lm	4.3 kl	3.8 jk	4.7 c
	Ligol	1.2 ab	2.3 c-f	3.3 g-j	2.4 d-g	3.4 h-k	1.6 a-e	2.6 e-h	2.4 b
	R. Boskoop	1.1 ab	0.7 a	0.9 a	1.2 ab	1.4 a-d	1.3 a-c	1.4 a-d	1.2 a
	Topaz	1.2 ab	2.7 f-i	2.6 e-h	2.5 e-h	2.5 e-h	2.1 b-f	2.3 d-g	2.3 b
	Mean for treatment	1.8 a	2.7 cd	2.9 cd	2.8 cd	3.1 d	2.3 b	2.5 bc	
Fresh weight of plants (g)	Gala Schniga	401.1 b-e	550.0 mn	517.2 k-n	505.6 i-n	476.1 f-k	476.7 g-k	472.2 f-k	485.6 b
	Ligol	388.9 b-d	451.1 e-j	538.9 l-n	524.4 k-n	494.4 h-m	486.7 g-l	504.4 h-m	484.1 b
	R. Boskoop	321.7 a	448.9 d-i	402.2 b-e	426.7 c-g	353.9 ab	429.4 c-g	416.1 c-f	399.8 a
	Topaz	375.0 a-c	510.0 j-n	542.8 l-n	565.6 n	482.8 g-l	473.9 f-k	444.4 d-h	484.9 b
	Mean for treatment	371.7 a	490.0 cd	500.3 d	505.6 d	451.8 b	466.7 bc	459.3 b	

Data followed by the same letters do not differ significantly at $p = 0.05$ for each parameter according to Tukey’s test. The influence of the years was not statistically significant.

There were significant correlations between all the growth parameters of the maiden apple trees. The only exception was the nonsignificant relationship between the height of the maiden trees and their fresh weight (Table 3).

Table 3. The correlations between the studied parameters of growth (averages for the three years 2016–2018).

Parameters	Height	Diameter	Slss	Number of Shoots	Weight of Plants
Height	1.00				
Diameter	0.44 *	1.00			
Slss	0.34 *	0.42 *	1.00		
Number of shoots	0.27 *	0.33 *	0.94 *	1.00	
Weight of plants	0.07	0.31 *	0.48 *	0.50 *	1.00

The level of significance $p = 0.05$, Pearson’s linear correlation coefficient. Slss—Sum of lengths of side shoots. * Significant relationship between parameters.

To sum up the results of all growth parameters, the lowest values were noted in the control combination (Table 2).

3.2. Physiological Parameters

The highest significant value of the net photosynthetic rate (Pn) in all the cultivars was noted after the treatment with the Basfoliar 6-12-6 fertiliser (Table 4). The smallest net photosynthetic rate was observed after the treatment with the Fylloton biostimulant,

followed by Bispeed—these values were lower than in the control plants. The Pn value of the ‘Red Boskoop’ cultivar was significantly lower than those in the other cultivars (Table 4).

The treatment of the maiden trees with all the preparations resulted in a significantly higher transpiration rate than in the control plants (Table 4). The highest transpiration rate was observed after the treatments with Basfoliar 6-12-6 and Basfoliar 12-4-6+S. The ‘Topaz’ maiden trees were characterised by the highest transpiration rate (E) of all the cultivars (Table 4).

The lowest stomatal conductance (C) was noted in the maiden trees treated with the Bispeed biostimulant; the highest—after the treatment with Basfoliar 6-12-6 and Fylloton (Table 4). These results did not differ from the control combination. The value of this parameter in the ‘Topaz’ trees was significantly higher than those in the other cultivars (Table 4).

The intracellular CO₂ values were not significantly different among all of the treatments (Table 4). The highest value of intracellular CO₂ was noted in the leaves of the ‘Topaz’ trees; the lowest—in the leaves of the ‘Red Boskoop’ trees (Table 4).

Table 4. Physiological parameters of maiden apple trees depending on treatment and cultivar.

		Control	Amino-Plant	Biamino Plant	Bispeed	Fylloton	Basf. 6-12-6	Basf. 12-4-6+S	Mean for Cultivar
Pn	Gala S.	24.6 e-j *	22.4 ab	22.9 b-d	23.8 c-g	25.0 h-j	25.7 j	24.2 e-i	24.1 b
	Ligol	25.5 j	25.4 ij	24.7 f-j	22.8 bc	22.2 ab	25.6 j	24.1 e-h	24.3 b
	R.	21.5 a	23.9 c-g	23.9 d-h	23.9 d-g	23.7 c-f	24.4 e-i	23.6 c-e	23.6 a
	Boskoop	24.8 f-j	24.8 g-j	23.8 c-f	24.1 e-h	22.3 ab	25.0 h-j	24.6 e-j	24.2 b
	Topaz	24.1 c	24.1 c	23.8 bc	23.6 b	23.3 a	25.2 d	24.1 c	
Mean for treatment									
E	Gala S.	1.7 b *	1.4 a	1.8 bc	2.1 f-h	2.3 hi	2.4 i	2.3 g-i	2.0 a
	Ligol	1.8 bc	2.1 fg	2.0 d-f	1.9 cd	1.9 c-e	2.0 d-f	1.8 bc	2.0 a
	R.	1.8 bc	1.8 bc	1.8 bc	2.1 e-g	2.0 d-f	2.2 f-h	2.2 f-h	2.0 a
	Boskoop	2.9 j	3.2 k	3.3 kl	3.5 lm	3.4 lm	3.4 lm	3.5 m	3.3 b
	Topaz	2.1 a	2.2 b	2.3 c	2.4 d	2.4 de	2.6 f	2.5 ef	
Mean for treatment									
C	Gala S.	125.9 b-f *	102.5 ab	123.7 b-e	139.5 c-j	147.1 d-j	133.5 c-h	142.5 c-j	130.7 a
	Ligol	144.2 c-j	140.3 c-j	124.9 b-e	125.7 b-e	131.2 c-g	135.2 c-h	119.7 a-c	131.6 a
	R.	132.5 c-h	133.8 c-h	132.3 c-h	97.5 a	138.7 c-i	163.0 j	122.1 b-d	131.4 a
	Boskoop	147.4 e-j	154.8 h-j	148.9 f-j	132.9 c-h	161.1 ij	150.8 g-j	153.7 h-j	149.9 b
	Topaz	137.5 bc	132.8 b	132.4 b	123.9 a	144.5 c	145.6 c	134.5 b	
Mean for treatment									
I	Gala S.	211.0 b-d *	181.7 a	225.7 d-f	258.1 j-m	251.2 h-k	222.7 d-f	250.5 g-k	228.7 b
	Ligol	228.5 d-f	235.6 e-h	224.7 d-f	238.8 f-i	258.0 j-l	230.0 d-g	226.5 d-f	234.6 c
	R.	187.0 a	196.7 ab	193.5 ab	228.2 d-f	217.8 c-e	242.9 f-j	199.2 a-c	209.3 a
	Boskoop	256.4 i-l	271.7 l-n	281.8 n	270.1 k-n	277.5 mn	251.9 h-k	271.5 l-n	268.7 d
	Topaz	220.7 a	221.4 a	231.4 b	248.8 c	251.1 c	236.9 b	236.9 b	
Mean for treatment									

* Data followed by the same letters do not differ significantly at $p = 0.05$ for each parameter, according to Tukey's test. Pn—net photosynthesis rate ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), E—transpiration rate ($\mu\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), C—stomatal conductance ($\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), I—intracellular CO₂ ($\text{mol CO}_2 \cdot \text{mol}^{-1}$).

There was a significant negative correlation between the net photosynthetic rate (Pn) and transpiration rate (E). On the other hand, there were significant positive correlations between the transpiration rate (E) and stomatal conductance (C), transpiration rate (E) and intracellular CO₂ (I), as well as stomatal conductance (C) and intracellular CO₂ (I) (Table 5).

Table 5. The correlations between the studied physiological parameter of maiden apple trees in the year 2017.

Parameters	Pn	E	C	I
Pn	1.00			
E	−0.17 *	1.00		
C	0.05	0.65 *	1.00	
I	−0.05	0.71 *	0.67 *	1.00

The level of significance $p = 0.05$, Pearson's linear correlation coefficient. For an explanation, see Table 4.
 * Significant relationship between parameters.

4. Discussion

4.1. Growth

The experiment showed that both the biostimulants and fertilisers applied to the leaves of the maiden apple trees improved all growth parameters of the maiden apple trees. According to Grzyb et al. [51], thanks to biostimulants, reduced doses of mineral fertilisers may be sufficient for good growth of maiden apple trees in a nursery. A further study conducted by Grzyb et al. [52] on maiden apple trees showed that biostimulants (seaweed extract) applied to soil resulted in similar values of growth parameters to full mineral fertilisation. Other authors did not always observe the positive effect of different biostimulants on the growth of fruit trees in orchards [53,54] and nurseries [55].

In our experiment, the Aminoplant preparation significantly increased the growth parameters of the maiden apple trees, as compared with the control combination. Grabowska et al. [22] applied this biostimulant in a carrot plantation and observed a similar effect. On the other hand, Kunicki et al. [23] applied this biostimulant to spinach, but it had no significant effect on the growth of the plants. Perhaps the area of uptake of the biostimulator by the leaves of individual plant species influenced different results. Grzyb et al. [52] found BioFeed Amin (plant amino acid extract) to be the most effective in improving the growth of maiden apple trees. According to studies conducted in orchards, the same preparation stimulated the growth of apple trees [56,57].

In our experiment, the foliar treatment of the maiden apple trees with the Biamino Plant preparation significantly improved their growth parameters, as compared with the control combination. Other authors applied preparations based on amino acids and peptides in a strawberry plantation and observed a similar beneficial effect [58,59]. On the other hand, Lisiecka et al. [60] conducted a study on the foliar application of Aminoflor (an organic complex of amino acids and peptides) to strawberry mother plants and found that the preparation did not stimulate their growth. However, the results of the impact of biostimulants on the same plant species depend on many factors, including weather conditions, soil quality, the level of basic fertilisation and even the cultivar.

In our experiment, the treatment of the maiden trees with the Bispeed preparation resulted in the highest values of the following parameters: diameter, total length of side shoots and fresh weight. However, these results were not significantly different with respect to some other foliar treatments. Similarly, Klimek et al. [61] applied the Asahi preparation (sodium para-nitrophenolate 0.3%, sodium ortho-nitrophenolate 0.2% and sodium 5-nitroguaiacolate 0.1%) to maiden apple trees and observed its significant effect on their height, number and length of side shoots. Wysocki et al. [62] used this biostimulant in a strawberry plantation but observed no improvement in the yield.

Similar to other biostimulants, the Fylloton preparation had positive effects on the growth parameters of the maiden apple trees in the nursery, especially on the number of side shoots. However, this result was not significantly different with respect to three other biostimulant treatments. Grzyb et al. [52,63] also observed that another preparation based on marine algae improved the growth of maiden apple and cherry trees in a nursery. Basak [30] observed that the foliar application of preparations made from seaweed positively affected the growth and yield of apple trees. Norrie et al. [64] observed a similar effect by using marine plant extracts in a study on grapevines.

Our experiment showed good results of the foliar application of Basfoliar 6-12-6 and Basfoliar 12-4-6+S fertilisers. Mosa et al. [57] used the Florovit Natura fertiliser in combination with beneficial bacteria in an orchard and observed the highest increase in the apple tree trunk diameter.

Only the 'Red Boskoop' maiden trees did not differ significantly in the number and total length of their side shoots after the treatment with the biostimulants. There was a significant improvement in these growth parameters in the other three cultivars. Basak [29] also found that Kelpak® (contains natural auxins and cytokinins extracted from *Ecklonia maxima*) and Goëmar BM 86® (contains amino acids, vitamins, phytohormones and oligosaccharides extracted from *Ascophyllum nodosum*, and the mineral nutrients N, Mg and B') stimulated the elongation of shoots and sometimes induced the better branching of trees. There were different results of the studies conducted by Grzyb et al. [65,66], who observed that only some biostimulant containing extracts from several seaweed species reinforced with humic and fulvic acids induced the growth of side shoots of apple trees to a greater extent than soil fertilisation. In our experiment, the growth parameters of the maiden trees of the four cultivars improved in a preparation-dependent manner. This may have been caused by the fact that each of the cultivars was characterised by different genetic conditions for growth. Other authors [52,66] also observed that the maiden apple trees of two cultivars in a nursery reacted differently to the same fertilisers and biopreparations. Basak [29] also noted that the same biostimulants applied to apple trees of different cultivars resulted in their different ability to develop side shoots.

The plants used in our experiment reacted to the type of fertilisers in a cultivar-specific manner. Apart from that, some authors [67,68] believe that biostimulants better adapt plants to changing cultivation conditions. In addition, our experiment showed that limited mineral fertilisation did not decrease the growth parameters of the maiden apple trees, although there were lower values of some physiological parameters under analysis.

4.2. Physiological Parameters

The values of the net photosynthetic rate and the stomatal conductance in the leaves of the maiden apple trees in the control combination were higher or equal to the values of these parameters compared to the other treatments. Our observation is not in line with the opinion of other authors [11,69] who found an increased net photosynthetic rate and reduced transpiration rate and stomatal conductance in plants treated with biostimulants. Conesa et al. [70] proved that the treatment of maiden citrus trees grown in containers with biostimulants increased their photosynthetic capacity by 32%. However, there was an additional stress factor in this experiment in the form of a limited amount of substrate in the container, which could have resulted in better results of using biostimulants. In this experiment, the values of the net photosynthetic rate and stomatal conductance in the trees treated with sea algae extracts were higher than those in the untreated ones. In another experiment [71], in one of the two years of observation, the cherry trees treated with an *Ascophyllum nodosum* extract exhibited greater net CO₂ assimilation than the control plants. Kocira et al. [35] proved that the Fylloton biostimulant positively affected the function of the photosynthetic apparatus of Moldavian dragonheads. Fylloton improved most of the physiological parameters analysed in this experiment. There were similar effects observed in other studies [19,30], in which plants were treated with biostimulants based on seaweed extracts or seaweed extracts with amino acids [72]. The differences in the effect of the Fylloton preparation in our experiment in relation to the results of other authors may result from the fact that in the analysed experiment, half the dose of mineral fertilisation was used. The treatment of the plants with Basfoliar 6-12-6 resulted in higher values of most of the physiological parameters tested in our experiment compared to the rest of the treatments. Mosa et al. [57] observed that the treatment of plants with the Florovit Natura fertiliser (93 N%, 1.3 P₂O₅%, 3 K₂O%, 0.008 B%, 0.004 Cu%, 0.018 Fe%, 0.006 Mn%, 0.006 Zn% and humic substances) improved their photosynthesis index. A high photosynthetic rate was found to increase the yield of crops [73]. According to Proietti et al. [74], in general, the

values of the leaf transpiration rate (E), stomatal conductance (C), and intracellular CO₂ (I CO₂) are associated with that of the net photosynthetic rate (Pn). However, the values of the correlation coefficients in our experiment did not confirm the relations between these parameters. According to some authors [75–77], trees grow larger as a result of increased photosynthesis. This happens because they develop a larger leaf area and capture more light. In consequence, their source activity increases and they grow larger. Our experiment did not fully confirm the dependence between the higher intensity of photosynthesis and stomatal conductance and the better growth of maiden apple trees. The lowest values of the leaf transpiration coefficient and internal carbon dioxide concentration were noted only in the control combination, which resulted in the poorest growth of the maiden apple trees. According to Zhao et al. [78] and Bown et al. [79], if there is a reduced supply of nitrogen for plants, and almost always if there is a lower supply of phosphorus and potassium, the chlorophyll concentration decreases and so does the rate of photosynthesis. In our experiment, this effect was observed for the parameters of some physiological processes occurring in the maiden apple trees cultivated with reduced mineral fertilisation. However, this reduction in fertilisation should be monitored on an ongoing basis through soil and plant material analyses because the initial content of nutrients in the soil may be depleted.

5. Conclusions

The results of the experiment led to the conclusions that the foliar spraying of maiden apple trees with selected biostimulants and fertilisers, while mineral fertilisation was reduced by half, usually significantly improved the plants' growth parameters in comparison with mineral fertilisation at a full dose. The biostimulants and fertilisers applied in the experiment had diversified effects on the growth of the maiden apple trees of individual cultivars. For the future, it is worth examining the effect of foliar biostimulants under more stressful conditions, such as no soil fertilisation and no irrigation in periods of drought. An analysis of the costs of using this type of plant care treatment should also be performed.

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