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The Effect of Foliar Spray Treatments with Various Biostimulants and Fertilisers on the Growth of M.9 Rootstock Stoolings

Sławomir Świerczyński ^{1,*} and Maciej Bosiacki ²

¹ Department of Ornamental Plants, Dendrology and Pomology, Poznan University of Life Sciences
Dąbrowskiego 159, 60-594 Poznan, Poland

² Department of Plant Physiology, Poznan University of Life Sciences, Wołyńska 35, 60-637 Poznan, Poland;
maciej.bosiacki@up.poznan.pl

* Correspondence: slawomir.swierczynski@up.poznan.pl; Tel.: +48-61848-7954

Abstract: An experiment was conducted to compare the effect of applying half a dose of a mineral fertiliser combined with a foliar treatment with four biostimulants and two fertilisers in relation to a full dose of a mineral fertiliser. The M.9 rootstock stoolings were sprayed four times during their growth. Each year of the experiment, the height, the diameter, the fresh weight of leaves and the leaf surface area of all stoolings were measured. The efficiency of rootstocks from one mother plant was assessed. Some parameters of physiological processes as well as the content of micro- and macronutrients in the leaves were also assessed. The research results showed that the reduced dose of the mineral fertiliser with foliar treatment did not significantly decrease the growth parameters of the M.9 rootstock stoolings. Some foliar treatments, especially Bioamino Plant and Bispeed, resulted in higher fresh weight and larger leaf area of the stoolings. The treatment with the Bioamino Plant biostimulant and two foliar fertilisers resulted in parameters of the physiological processes of stoolings that were the same as or better than those in the control plants. After the foliar application of two fertilisers, the content of macronutrients in the leaves of the stoolings was usually the same as in the control. The treatment with the biostimulants resulted in a lower content of most macronutrients in the plants (N, K and Ca). The only exception was the higher magnesium content than in the control after the treatment with all biostimulants and the same phosphorus content after the treatment with most of the biostimulants. The Aminoplant and Bispeed biostimulants increased the accumulation of iron in the leaves, whereas the Basfoliar 6-12-6 fertiliser resulted in higher zinc and copper content than in the control plants.

Keywords: M.9 rootstock; propagation by stooling; mother field; leaves; nutrition; intensity of physiological processes



Citation: Świerczyński, S.; Bosiacki, M. The Effect of Foliar Spray Treatments with Various Biostimulants and Fertilisers on the Growth of M.9 Rootstock Stoolings. *Agronomy* **2022**, *12*, 689. <https://doi.org/10.3390/agronomy12030689>

Academic Editors: Elisa Gamalero, Simone Cantamessa, Patrizia Cesaro and Giorgia Novello

Received: 3 February 2022

Accepted: 10 March 2022

Published: 12 March 2022

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1. Introduction

In the EU, Poland is an important producer of young apple trees. It is necessary to use good-quality M.9 rootstocks to produce maiden apple trees on such a large scale. This involves high intensification of production, based mainly on the increased application of fertilisers to the soil and intensive irrigation of plants propagated in a mother field. This leaches minerals to lower levels of the soil profile, which increases the contamination of the soil environment [1]. It also depends on the type of soil and the cultivation practices used. However, according to some researchers [2], even half of the nitrogen dose is not used by plants. In such a situation, the limited use of mineral fertilisers without negative consequences for the proper growth of plants can be replaced by foliar application of biostimulants [3]. Some authors [4] treat biostimulators as an environmentally friendly and effective form of plant fertilisation which supplements fertilisation with synthetic products. However, it should be mentioned that the lack of minerals in the soil or their unbalanced

ratio may have a negative impact on the proper metabolism of plants [5]. The plants can take up the nutrients faster and more efficiently if they are applied by the foliar route [6].

There are attempts to partly replace chemicals with biostimulants in plant production. So far, it has been observed that the Aminoplant amino acid preparation positively influences the growth and yield of various vegetable species [7–9]. However, according to some experiments [8,9], Aminoplant mainly affects the physical and chemical parameters of plants but has lesser influence on their yield. A study involving laboratory investigations showed that a biostimulant based on amino acids and peptides influences the nitrogen uptake from soil, especially during drought [10]. The foliar treatment of cotton and tomato plants with the Asahi and Atonik preparations, whose composition is similar to that of the Bispeed biostimulant, stimulated their development [11]. Some authors [12] used the Asahi biostimulant and observed that it improves the growth parameters of maiden apple trees when a full dose of mineral fertiliser is applied. Studies conducted on apple trees [13,14], grapevine [15], pear trees [16] and strawberry plants [17] have shown that the foliar application of preparations based on marine algae and seaweed stimulates the production of these plants. Kocira et al. [18] also observed that Fylloton stimulates the vegetative development of plants and affects the efficiency of the photosynthetic apparatus and the leaf chlorophyll content. Some studies have found that sweet basil plants after the application of the Asahi biostimulator [19] and cotton by nitrophenolate spraying [20] have an increased level of photosynthesis.

In recent years, it has been recognised that the foliar application of biochemical organic substances containing a set of micro- and macronutrients can be a modern form of agricultural practice that is environmentally friendly and increases the possibility of sustainable plant production [21]. So far, the influence of the foliar treatment of maiden apple trees growing in a nursery with urea and calcium nitrate fertilisers has been investigated [22]. However, foliar treatments with these fertilisers did not significantly improve plant growth. Other researchers [23] compared the influence of various biostimulants and organic fertilisers applied to the soil with that of a full dose of mineral fertiliser. They observed that the treatment improved the growth of the M.26 rootstock in a nursery. Foliar fertilisation of apple maiden trees with multi-component fertilisers was also performed [24]. Improvement of the majority of maiden growth parameters after the application of these fertilisers was found. So far, there has been no research on the effect of these treatments combined with a reduced dose of a mineral fertiliser on a mother plantation of M.9 rootstock.

The aim of our experiment was to verify whether the foliar application of biostimulants and fertilisers combined with a reduced dose of mineral fertiliser by half would not adversely affect the growth of M.9 rootstock stoolings, their nutrition and the intensity of physiological processes.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Between 2016 and 2018, research on the effect of the foliar spraying of M.9 rootstock stoolings was conducted in a mother plantation established in spring 2014. The experiment was carried out on two parallel plots in the mother field. On one of them, full mineral fertilisation ($120 \text{ N} \cdot \text{h}^{-1}$, $40 \text{ P}_2\text{O}_5 \cdot \text{h}^{-1}$ and $140 \text{ K}_2\text{O} \cdot \text{h}^{-1}$) was carried out and the stoolings were not treated with foliar treatment during their growth. In the second plot, the dose of mineral fertilisation was reduced by half ($60 \text{ N} \cdot \text{h}^{-1}$, $20 \text{ P}_2\text{O}_5 \cdot \text{h}^{-1}$ and $70 \text{ K}_2\text{O} \cdot \text{h}^{-1}$) and foliar treatment with four biostimulators and two fertilisers was performed four times during the growth of stoolings. The mother rootstocks in the mother plantation were grown at a distance of $180 \times 30 \text{ cm}$ from each other. Before starting the exploitation of the mother field, a laboratory analysis of the content of macronutrients in the soil was performed. The soil was characterised by the following soluble macronutrient content: 93 phosphorus ($\text{mg} \cdot \text{dm}^{-3}$), 125 potassium ($\text{mg} \cdot \text{dm}^{-3}$), 435 calcium ($\text{mg} \cdot \text{dm}^{-3}$) and 78 magnesium ($\text{mg} \cdot \text{dm}^{-3}$). The sum of rainfall in individual years was 500 mm in 2016, 338 mm in 2017 and 228 mm in the last year of study.

2.2. Biostimulants and Fertiliser Experiment

Four biostimulants and two foliar fertilisers were applied at the followed concentrations (Aminoplant 0.4%, Biamino Plant 0.2%, Bispeed 0.2%, Fylloton 0.4%, Basfoliar 6-12-6 - 0.5% and Basfoliar 4-6-12 + S - 0.5%). Aminoplant contained 18 L-amino acids and bioactive peptides (N 8.5%, organic substance 54%, bioactive peptides 82.7% and amino acids 17.3%). The composition of Biamino was dominated by L-amino acids of plant origin (organic nitrogen 7.6%, organic carbon 21.0%, amino acids 42.6%, Fe 1.2%, Mn 0.6% and Zn 0.7%). Bispeed was composed of potassium 4-nitrophenolate 0.25–0.30% m/m, potassium 2-nitrophenolate 0.14–0.20% m/m and potassium 5-nitroguaiacolate 0.07–0.10% m/m. Fylloton contained brown algae extract (*Ascophyllum nodosum*) and plant-derived amino acids (organic nitrogen 6%, organic carbon 20.8% and organic substance 35%). Basfoliar 6-12-6 was a multi-component liquid foliar fertiliser composed of N 6%, P₂O₅ 12%, K₂O 6%, B 0.01%, Cu 0.01%, Fe 0.02%, Mn 0.01%, Mo 0.005% and Zn 0.05%. Basfoliar 12-4-6 + S was composed of N 12%, P₂O₅ 4%, K₂O 6%, sulphur and all microelements. Each of the seven experimental treatments (four biostimulants, two fertilisers and one control combination) was represented in one plot by three mother plants in triplicate. Between 15 May and 15 July, the plants were sprayed with the biostimulants and fertilisers four times at 3-week intervals. The control plants were treated with distilled water only. A 0.2% Slippa adjuvant was added to the solution with which the shoots were treated. To thoroughly moisturise all the leaves on the plant, an appropriate amount of a biostimulator solution or foliar fertiliser was used.

2.3. Morphological Stoolings Growth and Gas Exchange Measurements

All stoolings (1-year-old shoots) were cut off from the three mother plants in the plot, and the height of the stoolings (cm) and the diameter of the base of their aerial part (mm) were measured. When the stoolings were taken away, the number of rooted stoolings from one mother plant (efficiency of mother plants) and the number of roots per one stooling were estimated. To determine the total assimilation area (cm²), the leaves of all stoolings collected from one mother plant were scanned and then processed with the SKWER software. Before the leaves fell, all of them had been plucked and the fresh matter of the leaves (g) per mother plant was weighed.

During the growth of stoolings of M.9 rootstock in the mother field in 2017, the following parameters were measured three times, directly after the treatments: net photosynthetic rate Pn (μmol CO₂ · m⁻² · s⁻¹), transpiration rate E (μmol H₂O · m⁻² · s⁻¹), stomatal conductance C (mol H₂O · m⁻² · s⁻¹) and intracellular CO₂ I (mol CO₂ · mol⁻¹). They were performed using a manual device for photosynthesis CI-340 aa (CID Bio-Science Inc., USA), and it was done at a set intensity of active photosynthetic radiation (PAR) (1000 μmol · m⁻² · s⁻¹) and a constant level of carbon dioxide (390 μmol CO₂ · mol⁻¹ of air). Four leaves from the middle part of long shoots of stoolings from four different mother plants of each combination were randomly selected for the measurements.

2.3.1. Chemical Analyses of Soil Samples

Soil samples were collected for chemical analyses in late July. Collected samples were chemically analysed by the universal method. Extraction of macronutrients (N-NH₄, N-NO₃, P, K, Ca, Mg and S-SO₄), Cl and Na was carried out in 0.03 M CH₃COOH with a quantitative 1:10 proportion of substrate-to-extraction solution. After extraction, the following were determined: N-NH₄ and N-NO₃ by microdistillation according to Bremner in Starck's modification; P colorimetrically with ammonium vanadomolybdate; K, Ca and Na photometrically; Mg by atomic absorption spectrometry (ASA); S-SO₄ nephelometrically with BaCl₂; and Cl nephelometrically with AgNO₃. Micronutrients (Fe, Mn, Zn and Cu) were extracted from the soil with Lindsay's Solution containing in 1 dm³ the following: 5 g of ethylenediaminetetraacetic acid (EDTA), 9 cm³ of 25% NH₄ solution, 4 g of citric acid and 2 g of Ca (CH₃COO)₂ · 2H₂O. Micronutrients were determined by the 150 ASA method. Salinity was identified conductometrically as an electrolytic conductivity (EC in

mS·cm⁻¹) (substrate:water = 1:2), and pH was determined by the potentiometric method (substrate:water = 1:2).

2.3.2. Chemical Analyses of Leaf Samples

In early August in 2017, the research samples of leaves were collected from the middle part of the long shoots of randomly selected stoolings for analyses of the content for macro- and micronutrients. The total nitrogen content was measured by the Kjeldahl method on a Parnas–Wagner apparatus. The phosphorus content was measured by the colorimetric method with ammonium molybdate. The potassium and calcium content was measured by flame photometry, whereas the magnesium content was measured by atomic absorption. The content of macronutrients was expressed as percentage. The content of the total forms of micronutrients, such as iron, zinc, manganese and copper (ppm), was measured by means of atomic absorption spectrometry (ASA) after wet mineralisation of 2.5 g samples in a mixture of nitric acid and perchloric acid at a volume ratio of 3:1.

2.4. Data Analysis

Data analysis was processed with the STATISTICA 13.1 software (Statsoft Polska, Kraków, Poland). One-way analysis of variance with Tukey's test was applied separately for individual years and for the characteristics of the growth of stoolings. The results of the physiological processes and the leaf content of macro- and micronutrients were subjected to the same analysis of variance based on the measurement result from 1 year. The differences were considered significant at $\alpha = 0.05$.

3. Results

3.1. The Growth Parameters of Stoolings

The height of the M.9 rootstock stoolings exhibited foliar-treatment-dependent differences only in the first year of observation (2016). The Aminoplant biostimulant gave a significantly better result whereas the Fylloton biostimulant gave an inferior result than that of the control (Table 1).

Table 1. The height of M.9 rootstock stoolings depending on the tested treatments (cm).

Year	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Average
2016	59.0 ^{bc}	61.6 ^d	58.6 ^b	60.8 ^{cd}	54.7 ^a	61.0 ^{cd}	57.8 ^b	59.1
2017	51.6 ^a	55.3 ^a	59.6 ^a	58.5 ^a	56.8 ^a	62.8 ^a	53.5 ^a	56.9
2018	87.3 ^a	85.7 ^a	83.9 ^a	95.1 ^a	84.8 ^a	85.7 ^a	83.3 ^a	86.5
Average treatment	66.0	67.5	67.4	71.5	65.4	69.8	64.9	

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each year according to Tukey's test.

In all 3 years of the experiment, the individual foliar treatments had no effect on the diameter and the number of the stoolings from one mother plant (Tables 2 and 3). Only in the third year of the study did the foliar application of the Basfoliar 12-4-6 + S fertiliser result in a significantly greater number of roots than in the control (Table 4).

Table 2. The diameter of M.9 rootstock stoolings depending on the tested treatments (mm).

Year	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Average
2016	6.6 ^a	6.5 ^a	6.6 ^b	6.4 ^a	6.4 ^a	6.9 ^a	6.2 ^a	6.5
2017	6.4 ^a	6.2 ^a	6.6 ^a	6.3 ^a	6.4 ^a	7.0 ^a	6.6 ^a	6.5
2018	8.7 ^a	8.3 ^a	8.0 ^a	9.0 ^a	7.6 ^a	7.6 ^a	7.8 ^a	8.1
Average treatment	7.2	7.0	7.0	7.2	6.8	7.2	6.8	

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each year according to Tukey's test.

Table 3. The number of M.9 rootstock stoolings from one mother plant depending on the tested treatments.

Year	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Average
2016	13.7 ^a	15.7 ^a	18.7 ^b	18.0 ^a	16.0 ^a	14.7 ^a	14.7	15.9
2017	17.3 ^a	20.3 ^a	20.0 ^a	18.3 ^a	21.0 ^a	17.0 ^a	14.3 ^a	18.3
2018	10.3 ^a	11.3 ^a	17.7 ^a	18.3 ^a	12.0 ^a	12.7 ^a	15.3 ^a	14.0
Average treatment	13.8	15.8	18.8	18.2	16.3	14.8	14.8	

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each year according to Tukey's test.

Table 4. The root number of M.9 rootstock stoolings depending on the tested treatments.

Year	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Average
2016	14.3 ^a	16.3 ^a	12.6 ^a	14.0 ^a	14.9 ^a	14.1 ^a	14.0 ^a	14.3
2017	6.7 ^a	6.0 ^a	5.2 ^a	5.5 ^a	5.5 ^a	6.3 ^a	6.7 ^a	6.0
2018	11.7 ^a	9.4 ^a	15.7 ^{ab}	17.7 ^{ab}	17.7 ^{ab}	16.4 ^{ab}	20.4 ^b	14.5
Average treatment	10.9	10.6	11.2	12.4	10.2	12.2	13.7	

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each year according to Tukey's test.

The foliar treatments significantly differentiated the results of measurements of the fresh weight and the total leaf area. In the first year, the application of the Bispeed biostimulant resulted in significantly better values of these parameters than in the control plants (Tables 5 and 6). In the second year of experiment, the treatment of the stoolings with the Aminoplant, Bispeed and Biamino Plant biostimulants and the Basfoliar 6-12-6 fertiliser resulted in higher values of the parameters under analysis. In the last year of the research, the treatment with the Bispeed and Biamino Plant biostimulants resulted in larger fresh weight and total leaf area than in the control plants.

Table 5. The fresh weight of M.9 rootstock stooling leaves depending on the tested treatments (g).

Year	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Average
2016	96.0 ^{ab}	142.0 ^{ab}	143.5 ^{ab}	185.0 ^b	114.5 ^{ab}	169.0 ^{ab}	88.8 ^a	134.1
2017	118.5 ^a	266.5 ^c	216.5 ^{bc}	216.0 ^{bc}	165.0 ^{ab}	251.5 ^c	136.0 ^{ab}	195.7
2018	171.0 ^a	236.0 ^{ab}	333.5 ^{bc}	363.0 ^c	154.5 ^a	230.0 ^{ab}	274.0 ^{a-c}	251.7
Average for treatment	128.5	214.8	231.2	254.7	144.7	216.8	166.3	

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each year according to Tukey's test.

Table 6. The total leaf area of M.9 rootstock stoolings depending on the tested treatments (dm²).

Year	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Average
2016	33.9 ^a	51.2 ^{ab}	51.8 ^{ab}	66.7 ^b	41.3 ^{ab}	59.6 ^{ab}	31.3 ^a	48.0
2017	41.8 ^a	96.1 ^c	78.1 ^{bc}	77.9 ^{bc}	59.5 ^{ab}	88.8 ^{bc}	48.0 ^a	70.0
2018	57.4 ^a	87.2 ^{ab}	126.3 ^b	131.0 ^b	56.0 ^a	83.2 ^{ab}	98.9 ^{ab}	91.4
Average treatment	44.4	78.2	85.4	91.9	52.3	77.2	6.8	

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each year according to Tukey's test.

3.2. The Physiological Parameters of Stooling Leaves

All the parameters referring to the physiological processes occurring in the leaves exhibited significant variability depending on the applied foliar treatment (Table 7). The treatment with the Biamino Plant biostimulant and the Basfoliar 12-4-6 + S fertiliser resulted in a significantly better net leaf photosynthesis level (Pn) than in the control. The foliar application of Basfoliar 12-4-6 + S also resulted in a better leaf transpiration coefficient (E) than in the control. The treatment with the Aminoplant and Biamino Plant biostimulants as well as the Basfoliar 12-4-6 + S fertiliser resulted in the highest stomatal conductivity (C), as compared with the control plants. The Biamino Plant and Aminoplant biostimulants significantly improved the internal concentration of carbon dioxide (I_CO2) as compared with the control combination (Table 7).

Table 7. Results of physiological parameters of M.9 rootstock stoolings depending on the tested treatments.

Parameter	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Standard Deviation
Pn	11.7 ^c	10.8 ^b	15.1 ^e	9.5 ^a	9.6 ^a	12.2 ^c	13.0 ^d	1.9
E	3.4 ^c	2.7 ^b	3.2 ^c	2.7 ^b	1.5 ^a	3.4 ^c	4.0 ^d	0.7
C	136.2 ^c	380.0 ^e	380.0 ^e	78.9 ^b	60.0 ^a	137.2 ^c	170.9 ^d	127.0
I_CO2	261.6 ^{cd}	458.2 ^e	477.1 ^f	192.6 ^b	109.6 ^a	257.9 ^c	271.4 ^d	127.0

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each parameter according to Tukey's test. The results presented in the table are the average results of three repetitions of measurements.

3.3. The Concentration of Macro- and Microelements in Stooling Leaves

Only the foliar application of the Basfoliar 12-4-6 + S fertiliser guaranteed leaf nitrogen content similar to that of the control combination. The other treatments resulted in lower leaf nitrogen content (Table 8). The treatment with the Fylloton biostimulant reduced the phosphorus level. After the other foliar treatments, the phosphorus level was the same as in the control. The foliar application of the Basfoliar 6-12-6 fertiliser increased the leaf potassium content, whereas the other treatments reduced it to a lower level than in the control plants. The foliar application of the same fertiliser also increased the leaf calcium content. The other treatments resulted in leaf calcium content similar to or lower than that of the control. All four biostimulants increased the magnesium content, which was higher than in the control plants (Table 8).

The Aminoplant and Bispeed biostimulants significantly increased the iron content in comparison with the control plants. The Bioamino Plant treatment resulted in a similar iron content to the control combination (Table 9).

Table 8. Content of macronutrients in leaves of M.9 rootstock stoolings depending on the tested treatments.

Nutrient (%)	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Standard Deviation
N	2.10 ^c	1.71 ^b	1.55 ^a	1.72 ^b	1.51 ^a	1.75 ^b	2.07 ^c	0.22
P	0.11 ^b	0.12 ^b	0.11 ^b	0.11 ^b	0.08 ^a	0.13 ^b	0.13 ^b	0.02
K	1.66 ^d	1.40 ^{bc}	1.35 ^b	1.23 ^a	1.15 ^a	1.81 ^e	1.50 ^c	0.22
Ca	0.70 ^c	0.68 ^{bc}	0.42 ^a	0.64 ^b	0.45 ^a	1.19 ^d	0.70 ^c	0.24
Mg	0.34 ^{ab}	0.53 ^e	0.43 ^c	0.48 ^d	0.47 ^d	0.32 ^a	0.36 ^b	0.08

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each macronutrient according to Tukey's test.

Table 9. Content of micronutrients in leaves of M.9 rootstock stoolings depending on the tested treatments.

Nutrient (ppm)	Control	Aminoplant	Biamino Plant	Bispeed	Fylloton	Basfoliar 6-12-6	Basfoliar 12-4-6 + S	Standard Deviation
Fe	361.9 ^d	379.6 ^e	364.3 ^d	407.0 ^f	357.8 ^c	350.9 ^b	334.8 ^a	21.8
Mn	149.5 ^e	143.0 ^d	141.4 ^{cd}	148.3 ^e	138.7 ^c	98.0 ^b	69.7 ^a	29.2
Zn	26.4 ^d	19.2 ^b	21.4 ^c	20.9 ^c	13.9 ^a	30.6 ^e	29.9 ^e	5.1
Cu	8.5 ^f	7.8 ^c	7.5 ^b	8.1 ^d	6.5 ^a	9.3 ^g	8.3 ^e	0.8

Data followed by the same letters do not differ significantly at $p = 0.05$ separately for each micronutrient according to Tukey's test.

There were higher manganese levels in the control combination and after the treatment with the Bispeed biostimulant. The other foliar treatments resulted in the lowest level of this element (Table 3). Only the foliar application of the two Basfoliar fertilisers increased the zinc level and the Basfoliar 6-12-6 fertiliser the copper levels, which were higher than in the control plants (Table 3).

4. Discussion

4.1. Growth Parameters of Stoolings

The experiment showed that foliar spray treatments with biostimulants and fertilisers combined with a limited dose of mineral fertiliser do not deteriorate the growth parameters of the M.9 rootstock stoolings, which did not show significant differences with control plants in all variables except for fresh weight and total leaf area of stoolings. Some of these treatments even increased the fresh weight and the leaf area of the stoolings. A different experiment [25] confirmed that thanks to the application of biostimulants into soil, a lower dose of mineral fertilisers may be sufficient for the normal growth of apple and sour cherry rootstocks in a nursery. The experiment also showed that the biostimulants considerably improved the growth of the M.26 rootstock under different soil and climatic conditions, as compared with mineral fertilisers [25]. The analysis of the results of our experiment did not show such improvement in the growth of the M.9 rootstock stoolings. However, it is noteworthy that in the experiment used for comparison, higher doses of the biostimulants were applied into the soil. The same authors in later experiment studies [26] did not observe any improvement in the growth of maiden sour cherry trees after the application of biostimulants into the soil in comparison with a full dose of mineral fertiliser. The research authors of other studies have also observed that various types of biostimulants do not stimulate the growth of apple trees [27,28], grapevines [29] and strawberries [30,31]. It is difficult to unequivocally assess how the treatments applied during the cultivation of crops influence their vegetative development, because the results depend on various factors, e.g., the age of the plant, the cultivar [32,33] and the type of treatment applied [34].

According to Van Trump et al. [35], some biostimulants, such as humic substances, have a positive effect on plants grown in a nursery. In the experiment under consideration,

the stoolings treated with the Aminoplant biostimulant were higher than control plants only in 1 of 3 years. Other authors have also not observed any significant effect of this biostimulant on the growth of plants [7,36]. However, a biostimulant with a similar composition as Aminoplant (BioFeed Amin) improved the growth of maiden sour cherry trees [26] as well as the growth of apple trees in an orchard [27,37]. According to Walch-Lui et al. [38], the foliar application of plant amino acids results in the formation of root branches. In our experiment, we did not have a greater number of M.9 rootstock stooling roots.

The treatment of the plants with the Biamino Plant biostimulant resulted in the same growth of the M.9 rootstock in the mother plantation as in the control. This treatment even resulted in greater fresh weight and leaf area during 2 of the 3 years of the experiment. According to observations made by the authors of various studies [39–41], biostimulants of a similar composition improve the growth of strawberry plants. However, the authors of another experiment [31] observed that the foliar treatment of mother strawberry plants with the Aminoflor biostimulant (similar composition to Biamino Plant) does not have any positive effect on their growth. Moreover, the treatment significantly decreased the fresh weight of the plants.

During 3 years of our experiment, the treatment of the plants with the Bispeed biostimulant led in similar growth results to those in the control, whereas the fresh weight and the total area of the leaves of the M.9 rootstock stoolings improved. The treatment of maiden apple trees with the Asahi biostimulant, which had a composition similar to that of Bispeed, significantly improved the growth parameters of maiden apple trees [12]. The same biostimulant combined with the Tytanit fertiliser increased the number of strawberry runners from one mother plant [42]. A Bispeed preparation also had a positive effect on the rooting of shoot cuttings of two species of gymnospermous trees and their fresh weight [43]. The application of the Bispeed biostimulant in our experiment did not improve the rooting of the M.9 rootstock stoolings.

The Fylloton biostimulant did not significantly influence the growth parameters of the stoolings in the mother plantation. The authors [44,45] of other studies who used the BioFeed Quality biostimulant (seaweed extract with humic and fulvic acids) noted the opposite dependence. They found that the product stimulates the growth of maiden apple trees and the development of their root system. Other studies [46,47] have confirmed the positive effect of this preparation on the dynamics of the growth of apple tree roots in an orchard. Basak [13] also observed that these biostimulants have a positive influence on the growth of apple trees in an orchard, especially on a larger leaf surface area. None of these observations, except for the larger leaf surface area of the stoolings, was confirmed in our experiment. However, it is noteworthy that in our experiment, the biostimulants were used with a reduced dose of mineral fertilisers, whereas a full dose of mineral fertiliser was applied in the above-mentioned studies.

The application of two foliar fertilisers resulted in a similar growth of the stoolings to that of the control combination. Świerczyński et al. [48] conducted an experiment that showed that only the foliar treatment of maiden sweet cherry trees with the Maxi Grow Excel fertiliser significantly improved their growth results. It is noteworthy that when the dose of mineral fertiliser is reduced, additional foliar fertilisation only compensates for the possible lack of soil nutrients but it does not improve the growth of plants.

4.2. Physiological Parameters of Stoolings

The foliar application of two Basfoliar fertilisers resulted in physiological processes of the same intensity as or higher intensity than in the control combination. However, the biostimulants usually reduced the intensity of physiological processes occurring in the plants. These observations do not correspond with the results of researchers [18,49] who obtained a higher level of net photosynthesis while the transpiration and stomatal conductance of plant leaves exposed to biostimulants slowed down. Own results confirmed that, only the Aminoplant and Bioamino Plant biostimulants increased two of the parameters of the life processes of stoolings (C and I_CO₂). The Bioamino Plant biostimu-

lant additionally improved the net leaf photosynthesis level (Pn). The use of the Bispeed and Fylloton biostimulants did not improve any of the parameters of life processes. It was found that after the use of biostimulants, young citrus trees were characterised by increased photosynthesis by 32% [50]. In the experiments of these authors, after the use of sea algae extract, higher values of stomatal conductivity and net photosynthesis were obtained in comparison to the control plants. Other researchers [51] in 1 of the 2 years of observation after using the *Ascophyllum nodosum* extract found an increased net CO₂ assimilation by cherry trees. The considered results have not been confirmed by other researchers [52,53], who applied biostimulants based on seaweed extracts and observed intensified physiological processes in celeriac and rape plants. It is worth emphasising that not all biostimulants increased the intensity of photosynthesis, which may be related to the stomatal conductance reduced by up to 40% in some treatments. However, the foliar application of two Basfoliar fertilisers gave similar or higher values of most of the considered parameters of the physiological processes of plants in relation to the control. Two applied zinc-containing foliar fertilisers, as a rule, increased the intensity of plant photosynthesis. Zinc-metalloenzymes are essential for the activity of an important enzyme in the carboxylation process [54]. It has been found that the deficiency of this element in the plant slows down the plant's photosynthetic activity [55]. In the conducted experiment, the increased level of photosynthesis measured in plants treated with Basfoliar fertiliser could be due to the higher zinc content in the leaves. According to some authors [56–58], when trees are treated with biostimulants, they grow larger than control trees due to increased photosynthesis. This is due to the fact that plants have a larger leaf surface, which captures more solar radiation compared to plants not treated with biostimulants. The conducted experiment did not fully confirm the relationship between the better growth of the M.9 rootstock and a higher level of photosynthesis intensity. In addition, more leaf surface did not always increase photosynthesis and, as a result, plant growth.

4.3. Content of Macro- and Micronutrients in Stooling Leaves

The treatment of the plants in the mother plantation with foliar fertilisers usually resulted in a higher content of macronutrients in the leaves than the treatment with the biostimulants, which resulted in a lower content of most macronutrients than in the control plants. The only exception was magnesium, whose content was higher after the treatment with the biostimulants. The treatment of the stoolings with the Basfoliar 12-4-6 + S fertiliser resulted in the same leaf nitrogen content as in the control combination. The biostimulant treatments resulted in a lower content of nitrogen. It did not confirm the thesis presented by Maini et al. [10], who observed that the Aminoplant biostimulant increases the nitrogen uptake. The research by Von Bennewitz et al. [59] showed that biostimulants increase the phosphorus uptake. However, this effect was not observed in our experiment. Definitely the lowest phosphorus content was recorded for Fylloton. This resulted in the lowest parameters of physiological processes measured in plants. Phosphorus deficiency has a significant influence on leaf photosynthesis in plants [60] and could result in a smaller size of stomatal opening [61]. Furthermore, Khan et al. [62] mentioned that phosphorus plays an important role in photosynthesis, energy transfer, signal transduction and respiration in the plant. The obtained results were also not fully consistent with the findings made by Shehata et al. [52], who observed that an amino-acid-based biostimulant does not significantly affect the potassium content but its level increases after the application of a seaweed extract. Another experiment [63] showed a significant increase in the leaf potassium content after the treatment of maiden apple and cherry trees with various biostimulants. However, this effect was not confirmed in the conducted experiment. Most of the biostimulants used in the aforementioned experiment [63] did not decrease the magnesium level, but the calcium level was lower than in the control combination. There were similar dependencies in the performed experiment.

According to Westwood [64], amino acids have a chelating effect and they increase the permeability of the cell membrane, thus facilitating the absorption and transport of

micronutrients inside the plant. In the experiment under consideration, this dependence was manifested only by the higher iron content after the treatment of the plants with the Bispeed and Aminoplant biostimulants. Maini [10] observed that the foliar application of Aminoplant increases the uptake of micronutrients and suggested that it could be used in combination with FeSO_4 to combat the symptoms of iron deficiency. The obtained results confirm this statement. Soppelsa et al. [65] observed an increase in the Fe and Zn concentrations after the application of several different biostimulants. In the experience under consideration, two biostimulants increased the concentration of iron only. The higher levels of zinc and copper after the foliar treatment of the maiden trees with Basfoliar 6-12-6 may be caused by the fact that this fertiliser contained these micronutrients.

There were diversified results of other studies in terms of the influence of biostimulants on the nutrition of plants. Some authors [66] observed that the treatment of soybean seeds with a seaweed extract as well as the treatment of pear leaves with biofertilisers and magnesium sulphate [67] results in a higher content of N, P, K and Mg. Similarly, the foliar application of biostimulants alone and in combination with potassium and zinc increased the levels of N, P, K, Ca, Mg and Zn in mandarin leaves [68]. In addition, the treatment of tomato seedlings with a biostimulant containing amino acids, peptides and nutrients increased the levels of K, Ca, Mg, Fe, Cu and Zn in the leaves, thus affecting the nutrition of the plants [69]. Protein hydrolysates increased the K and Mg content in spinach leaves and proved to be more effective than the seaweed extract [70]. However, Chitu et al. [71] did not observe any positive effect of various biostimulants on the content of macronutrients in apple leaves, whereas Soppelsa et al. [65] did not observe such an effect in strawberry leaves. In our experiment, the full dose of the mineral fertiliser resulted in better nutrition of the plants compared with their treatment with biostimulants. The only exceptions were the magnesium and iron levels.

5. Conclusions

The foliar application of biostimulants and fertilisers combined with a half lower dose of mineral fertiliser did not deteriorate the growth of the M.9 rootstock stoolings. Some treatments even increased the weight and surface area of the leaves. In comparison with the control combination, the application of the Bioamino Plant biostimulant and two fertilisers resulted in similar or better parameters of the physiological processes occurring in the leaves. Aminoplant also improved two parameters (C and I_{CO_2}). Compared with the use of the biostimulants, the foliar application of the two fertilisers resulted in a higher content of macronutrients in the leaves. Only the magnesium and iron levels were higher after the application of some biostimulants. The treatment of the plants with a full dose of mineral fertiliser usually resulted in a higher content of micronutrients than foliar treatments combined with half the dose of mineral fertiliser. Generally, after foliar application of biostimulants, similar levels of macro- and micronutrients were found in leaves. This could be due to the fact that the plants showed no symptoms of deficiency of these nutrients. In addition, all foliar treatments compensated for the reduced mineral fertilisation without increasing the efficiency of mother plants compared to the control.

The experiment showed that part of the dose of the mineral fertiliser could be replaced by foliar biostimulants and fertilisers in the propagation by stooling of M.9 rootstock. The foliar fertilisation fully compensated for the reduced dose of mineral fertiliser, as it did not deteriorate the growth of the plants. The biostimulants Bispeed and Bioamino Plant turned out to be the most suitable for additional foliar treatments due to plant growth parameters. However, when deciding on the use of these treatments, you should consider both the benefits, i.e., limiting the use of mineral fertiliser, and the additional costs of implementing these treatments. Considering the current price of mineral fertilisers, around EUR 700 per ton, this gives a saving of around EUR 470 when using half the dose (150 kg; full dose 300 kg). This fully compensates for the purchase price of a biostimulant or foliar fertiliser for four treatments. The disadvantage of foliar treatments results only from the cost of their implementation, and this is due to ecological reasons. The experiment needs to be

continued to compare the effects of biostimulants and foliar fertilisers applied together with full and reduced doses of mineral fertiliser.

Author Contributions: S.Ś. contributed to the study conception and design. Material preparation, data collection and analysis were performed by S.Ś. and M.B. The first draft of the manuscript was written by S.Ś., and authors commented on the previous version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The publication was co-financed within the framework of the Polish Ministry of Science and Higher Education's program: 'Regional Initiative Excellence' in the year 2019–2022 (No. 005/RID/2018/19), financing amount 1,200,000 PLN.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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