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Effects of Plant Growth Regulators on Plum (*Prunus domestica* L.) Grown on Two Rootstocks at Harvest and at the Postharvest Period

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Abstract: Plant growth regulators (PGRs), such as cytokinins (6-benzyladenine; BA) and gibberellins (GAs), are widely used in fruit production. This study focused on the plum cultivar "Čačanska rodna" (*P. domestica* L.) grafted on vegetative rootstock "WaVit" and generative rootstock *Prunus cerasifera*, with interstock *Prunus spinosa*. PGR treatments included 50 mg L^{-1} and 100 mg L^{-1} of BA and 200 mg L^{-1} of gibberellin A3 (GA_3) and non-treated control. Pomological characteristics of plum fruits were examined at harvest, while physicochemical properties were analyzed at harvest and after 28 days of cold storage and 4 days of shelf life. GA_3 and BA application changed the fruit morphological traits, improved skin strength, and increased carotenoid, anthocyanin and sucrose content while decreasing the titratable acidity at harvest. The beneficial effects of higher sucrose, anthocyanin and carotenoid levels persisted in all PGR-treated fruits after cold storage and shelf life. GA_3-treated fruits had firmer flesh, stronger skin and higher total soluble solids (TSS) content, while in BA-treated plums, these effects were rootstock-dependent. The physical properties and chemical composition of plum fruit in the postharvest period suggest beneficial effects of the applied PGR treatments. Moreover, these chemical treatments might have prolonged the beneficial impact on fruit storability, nutritional profile and sensory properties. Based on our results, GA_3 preharvest treatment can be included in standard cultivation practices within contemporary production systems of European plums not only to improve fruit quality at harvest but also to improve the storage potential and nutritional value, regardless of the rootstock used.

Keywords: European plum; rootstocks; 6-benzyladenine; GA_3; cold storage; shelf-life

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1. Introduction

Unlike Japanese plums (*Prunus salicina* L.), which are mostly eaten fresh, European plums (*Prunus domestica* L.) are usually consumed in processed form, such as dried or canned whole fruit, jams, jellies, purée or brandy [1]. The recently published review provides an overview of the beneficial health effects attributed to plum consumption, including bone health, antioxidant and anti-inflammatory activity, enhanced cognitive ability, reduced risk of cardiovascular diseases, laxative effects, and anti-allergic and anti-microbial properties [2]. To increase the consumption of fresh plums, preharvest and postharvest measures should be encouraged and supported. Among the preharvest measures which are increasingly drawing the attention of both researchers and producers is the application of plant growth regulators (PGRs). The impact of PGR treatments on

tree growth, productivity, and fruit quality has already been confirmed in different fruit species [3–5].

The mechanisms of action and effects of PGRs are diverse and are associated with different fruit development phases [6]. Gibberellins (GAs) represent the most frequently used and thoroughly studied PGRs. These compounds stimulate cell division and cell expansion [7,8]. Their application results in the enlargement of cells in the mesocarp, increased fruit firmness [9,10], decreased fruit drop, and increased fruit size and yield [6]. Among GA isomers, gibberellic acid, also known as GA_3, was found efficient in increasing fruit size and weight, as well as the weight of dry endocarp in sweet cherry [10,11].

The application of a synthetic cytokinin, 6-benzyladenine (BA), stimulates cell division during the early phase of fruit development and promotes cell enlargement, resulting in higher fruit weight and size [12,13]. In apples, BA application was found to affect both cell size and the number of cell layers in the cortex, altering the final fruit size [12]. BA can increase fruit size in apples [14] and pears [9], but it can also affect the size of stone fruit, such as sweet cherries [3] and apricots [15].

However, less attention has been given to the effects of PGR application on the postharvest quality and properties of stored fruit. In terms of plums, the majority of postharvest research was conducted on Japanese plums [16–33] as compared to European plum cultivars (*P. domestica* L.) [34–40]. Only a few of those studies address the postharvest effects of preharvest PGR application (primarily GA_3) [4,41–45] exclusively on Japanese plum [4,34–38]. Preharvest application of GA_3 increased the fruit weight and size of Japanese plums at harvest, as well as fruit firmness and TSS during storage, manipulation and transport, and limited weight loss [36–38], which made fruits more acceptable after the storage period [35]. Preharvest application of GA_3 or BA [46] in European plums also showed the increased fruit weight and solid soluble content at harvest, while no delayed maturation was observed [47]. The influence of BA application on fruit quality at harvest or postharvest properties of plums grown in Europe, to our best knowledge, has not been investigated so far. GA_3 was commonly applied in stone fruits at lower rates from 10 to 100 ppm [4,10,42–45] and in repeated treatments to achieve fruit quality improvements [44]. BA was applied in stone fruits at concentrations from 50 to 400 ppm. However, it was the most effective in improving fruit quality within concentrations from 50 to 150 ppm [3,5,15].

Serbia, the USA, China and Romania are among the leading plum producers [48]. Plum breeding programs in Serbia have resulted in the creation of several plum cultivars with excellent production properties, which are widely used in production. Nowadays, old plum cultivars widely spread in production, like "Čačanska rodna", created in the early 1960s, are adapted to contemporary production systems through grafting on new rootstocks, which alter their properties towards reduced vigor, smaller tree size and increased yield. It has already been shown that utilization of different rootstocks results in differences in fruit size, quality [49,50] and sensory traits [51,52] of plum cultivars, including "Čačanska rodna".

We hypothesized that PGR application alters not only plum fruit properties at harvest but also its postharvest behavior and quality after storage and that this influence is rootstock-dependent. Accordingly, the fruit quality traits, and their changes during cold storage and shelf life, were examined on European plum cv. "Čačanska rodna" grafted on two rootstocks, treated with GA_3 and two doses of BA, in the early stage of fruit development.

2. Materials and Methods

2.1. Plum Production and Preharvest Treatments

Plum fruits were cultivated at the Experimental Orchard of the Faculty of Agriculture, Novi Sad, Serbia (45°19′ N and 19°50′ E, 86 m a.s.l.). The plum cultivar "Čačanska rodna" (*P. domestica* L.) grafted on vegetative rootstock "WaVit," and generative rootstock *Prunus cerasifera* with interstock *Prunus spinosa* (P/P) was used for the experiment. The trial was set up in a completely randomized design. Plums were subjected to a single PGR treatment and applied when the fruit diameter reached 10 mm (April 27). The treat-

ments included 50 and 100 mg L^{-1} of 6-benzyladenine (BA50 and BA100, respectively), 200 mg L^{-1} of gibberellin A3 (GA_3) and non-treated control, prepared from Gerba 4 LG (4% active ingredient of BA) and Gibrelin (1.8% active ingredient of GA_3), respectively, both purchased from "L-Gobbi", Italy. Treatments were applied with a backpack sprayer (Stihl SR-420) until run-off. The total volume of each PGR solution was 2.4 L per treatment. Fruits were harvested as commercially ripe with appropriate total soluble solids (TSS, above 15%) and fruit firmness (105 to 140 N) values typical for "Čačanska rodna".

For postharvest analysis, approximately 5 kg of plum fruits were distributed in wooden crates of 50 × 30 × 8 cm dimensions and were placed in a cooling chamber (at 1 ± 1 °C; 80 ± 10% RH). After 28 days, fruits were removed from cold storage and subjected to shelf-life conditions (24 ± 2 °C) for 4 days. Pomological properties were analyzed only at harvest, while fruit physicochemical properties were analyzed at harvest, after cold storage and after shelf life.

2.2. Pomological Properties, Respiration Rate and Ethylene Production

Fruit weight (g) was measured on 30 fruits per treatment using a technical balance (Kern 572-35, Kern & Sohn, GmbH, Balingen, Germany). Fruit size (mm) was determined by measuring three linear dimensions (length, width, and thickness) of each fruit with a digital caliper gauge (0.01 mm) (Mitutoyo, CD-6"CX, Tokyo, Japan). The fruit shape index was calculated by applying the following formula: length × length/width × thickness [14].

Respiration rate and ethylene production were determined on approx. 300 g of fruit (equivalent to 10 plums), placed in a 700 mL container and hermetically sealed with multilayer foil at 24 ± 2 °C. Ethylene production and respiration rate were measured on harvested fresh fruit and stored fruit once a day for seven consecutive days.

Ethylene detection was carried out from 2 mL of gas sampled by a plastic syringe and injected into a 10 mL headspace vial sealed with silicone septa. The detection was performed by gas chromatography (GC7890, Agilent, Santa Clara, CA, USA), equipped with an FID detector (Agilent, Santa Clara, CA, USA) and an autosampler (COMBIPAL, CTCAnalytics AG, Zwingen, Switzerland), according to the protocol described by Mandić et al. [53]. A DB-WAX column was used for separation. The temperature gradient varied from 60 °C to 150 °C, and the flow rate was set to 30 mL min^{-1}, with nitrogen (N_2) as the carrier gas. The injection was in split mode (10:1). The ethylene content was calculated from the calibration developed using different injections of 4% ethylene in N_2. The ethylene production (nL g^{-1} h^{-1}) was calculated from ethylene concentration, taking into account the weight of fruit in the dish, its volume, the volume of the dish and the exact time that elapsed from the moment the dish was sealed until the sampling was performed.

Respiration rate was determined by measuring carbon dioxide (CO_2) concentration directly in the atmosphere surrounding fruits by puncturing multiple sealed foils with a sampling needle from an OXYBABY 6.0 instrument (WIT-Gasetechnik GmbH & Co KG T, Witten, Germany). CO_2 production (μL g^{-1} h^{-1}) was calculated from the difference in CO_2 concentration before sealing the dish and after 4 h by taking into account the weight of fruit in the dish, its volume, the volume of the dish and the exact time period from the moment the dish was sealed until the sampling was performed.

2.3. Physicochemical Properties

Fruit skin and flesh color were measured in CIELAB color space using a CR-400 Chroma Meter (Konica-Minolta, Osaka, Japan). After removing the wax layer, the skin color was measured on 20 randomly selected specimens, with two measurements on the opposite sides of each fruit, in the equatorial region. After removing the stone, the flesh color was measured on 20 randomly selected specimens on both halves of each fruit.

Texture analysis was performed using TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK). The skin and flesh textural properties were analyzed on 20 randomly chosen plum fruits. Skin strength and elasticity were measured by conducting the penetration test, whereby a 2 mm diameter stainless steel flat cylinder probe (P/2) was

inserted into each specimen at a constant penetration speed of 3 mm s^{-1} to the pre-set penetration distance of 10 mm. The load cell mass was 30 kg, while the trigger force was set to 5 g. The skin elasticity was measured for each fruit at two opposite points in the equatorial region. Skin elasticity is the distance (mm) to which the skin inflects prior to probe penetration into the fruit, while skin strength is the force (N) needed for puncturing the fruit skin. The fruit flesh firmness was measured using a penetration test with a stainless-steel rounded cylinder probe of 8 mm diameter. Prior to the measurements, the skin was removed from the equatorial region with a sharp peeler (15–20 mm diameter). Penetration was performed to the pre-set distance of 4 mm at a 10 mm s^{-1} penetration speed with a 25 g trigger force. Fruit firmness was recorded as the force (N) needed for penetration.

Total soluble solids (TSS; %) and pH were measured directly from the previously homogenized sample at 20 °C using a refractometer (ATR-ST plus, Schmidt + Haensch, Berlin, Germany) and pH meter (AMT12, Amtast, Phoenix, AZ, USA), respectively. The titratable acidity (TA; %) was measured from 3 g of sample diluted in 30 mL of deionized water. After homogenization, the sample was centrifuged at 13,776× *g* for 5 min (Centrifuge 5804 R, Eppendorf, Germany). Titration was carried out on 10 mL of supernatant with 0.1 M NaOH.

The anthocyanin content was determined according to Lee et al. [54] with minor modifications. Briefly, anthocyanins were extracted from approx. 2 g of homogenized sample in 20 mL 0.1% HCl in methanol. The sample extract (1 mL) was transferred to 9 mL of buffer (pH 1.0 or pH 4.5). After stabilization for 2 h at 4 °C, absorbance was measured at 515 and 700 nm using a spectrophotometer (Cintra 303, GBC Scientific Equipment, Braeside, VIC, Australia). A blank was prepared by dissolving 1 mL of 0.1% HCl in methanol in 9 mL of buffers. The total anthocyanin content was calculated using measured absorbance, molar extinction coefficient and molar mass of cyanidin-3-glucoside and was expressed as cyanidin-3-glucoside (mg kg^{-1} of fresh weight).

Carotenoids were extracted from 0.5 g of plant material with 20 mL of acetone. The carotenoid content was determined from the supernatant obtained after centrifugation for 5 min at 13,776× *g* (Centrifuge 5804 R, Eppendorf, Germany) by measuring the absorbance at 470, 653 and 666 nm using a spectrophotometer (Cintra 303, GBC Scientific Equipment, Braeside, VIC, Australia). The carotenoid content (mg kg^{-1} of fresh weight) was calculated using the molar extinction coefficient [55].

The composition of sugars and organic acids was determined using liquid chromatography according to the method described by Milenković et al. [56].

2.4. Statistical Analysis

The univariate analysis consisted of determining the significance of the applied treatments using ANOVA followed by Duncan's multiple range test. The Tukey's HSD (honestly significant difference) test was adopted for ethylene production and respiration rate, while multivariate explanatory analysis was performed using principal component analysis (PCA). The data were analyzed using Statistica (TIBCO Software Inc., Palo Alto, CA, USA, 2018, version 13) commercial software.

3. Results

Obtained results were presented in the form of a comparison of the average values of determined plum properties accompanied by an ANOVA-based analysis of the significance of effects and in the form of multivariate PCA.

3.1. Univariate Results

All plums treated with PGRs were characterized by higher weight than the controls, but the increase was statistically significant only in BA50-treated fruits grown on "WaVit" rootstock (Figure 1A). PGR treatments affected fruit width and thickness, while fruit length remained unchanged (Figure 1C). Differences in the fruit shape index (due to fruits being

more round) were noted only in fruits grown on "WaVit" and treated with BA (Figure 1B). Rootstocks did not exhibit an observable effect on fruit weight or shape index (Figure 1).

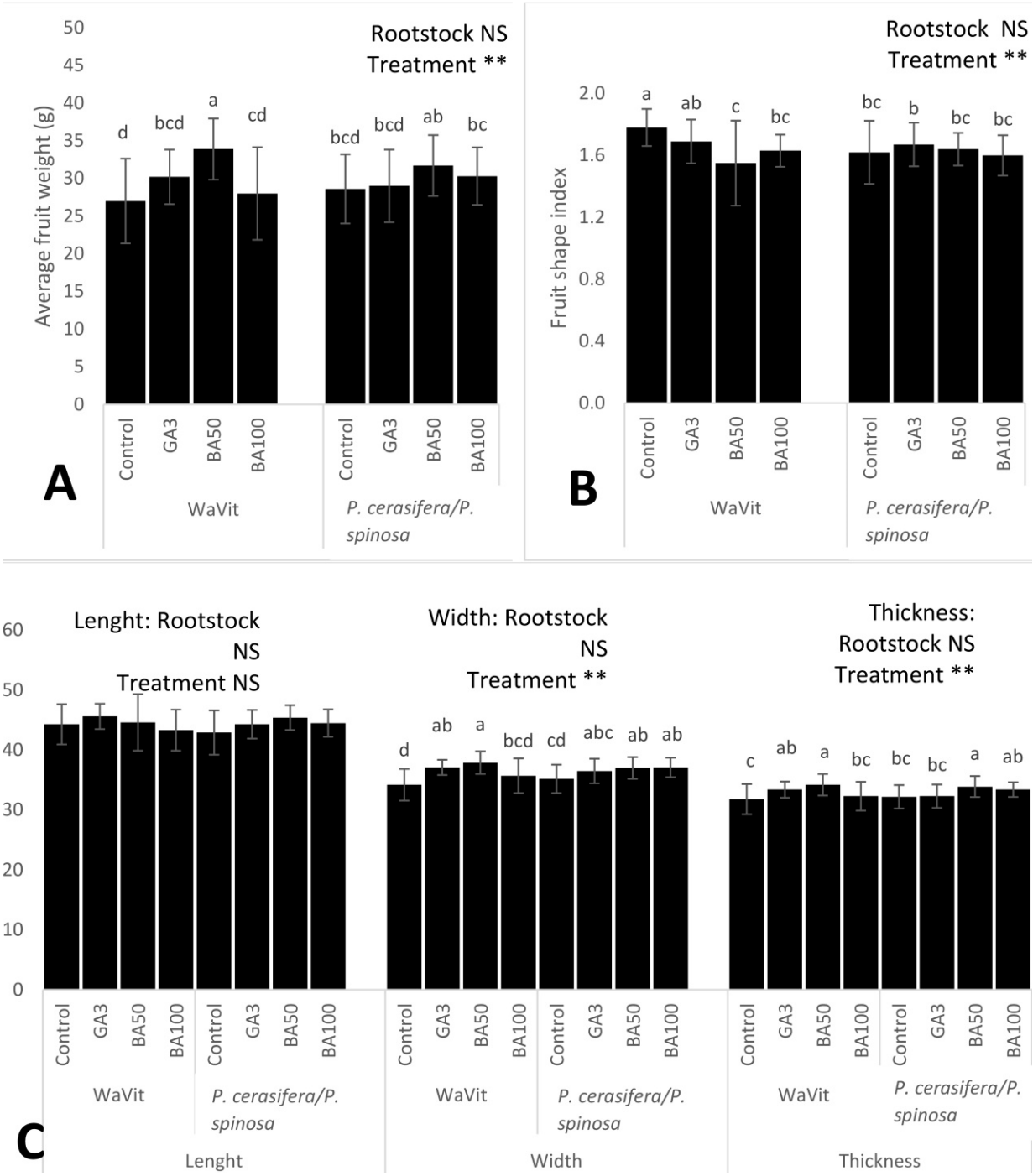

Figure 1. Changes in weight and size of fresh plum, grown on two different rootstocks ("WaVit" and *P. cerasifera*/*P. spinosa*), treated with PGRs. (**A**) Fruit weight (g); (**B**) fruit shape index; (**C**) fruit size (length, width and thickness, mm). PGR treatment: control—untreated plums; GA_3—200 mg L^{-1} of gibberellin A3; BA50—50 mg L^{-1} of 6-benzyladenine; BA100—100 mg L^{-1} of 6-benzyladenine. Marks in one graph followed by the same letter were not significantly different ($p < 0.05$). The main factors are presented, and their significance is annotated by: NS—not significant, **—significant at 0.01. The error bars on the columns represent the standard deviation.

Both at harvest and after cold storage, ethylene production was characterized by an increasing trend regardless of rootstock or PGR treatment, with approximately the same quantities produced regardless of applied treatments (Figure 2A–D). No changes in the respiration rate were observed either. (Figure 2E–H).

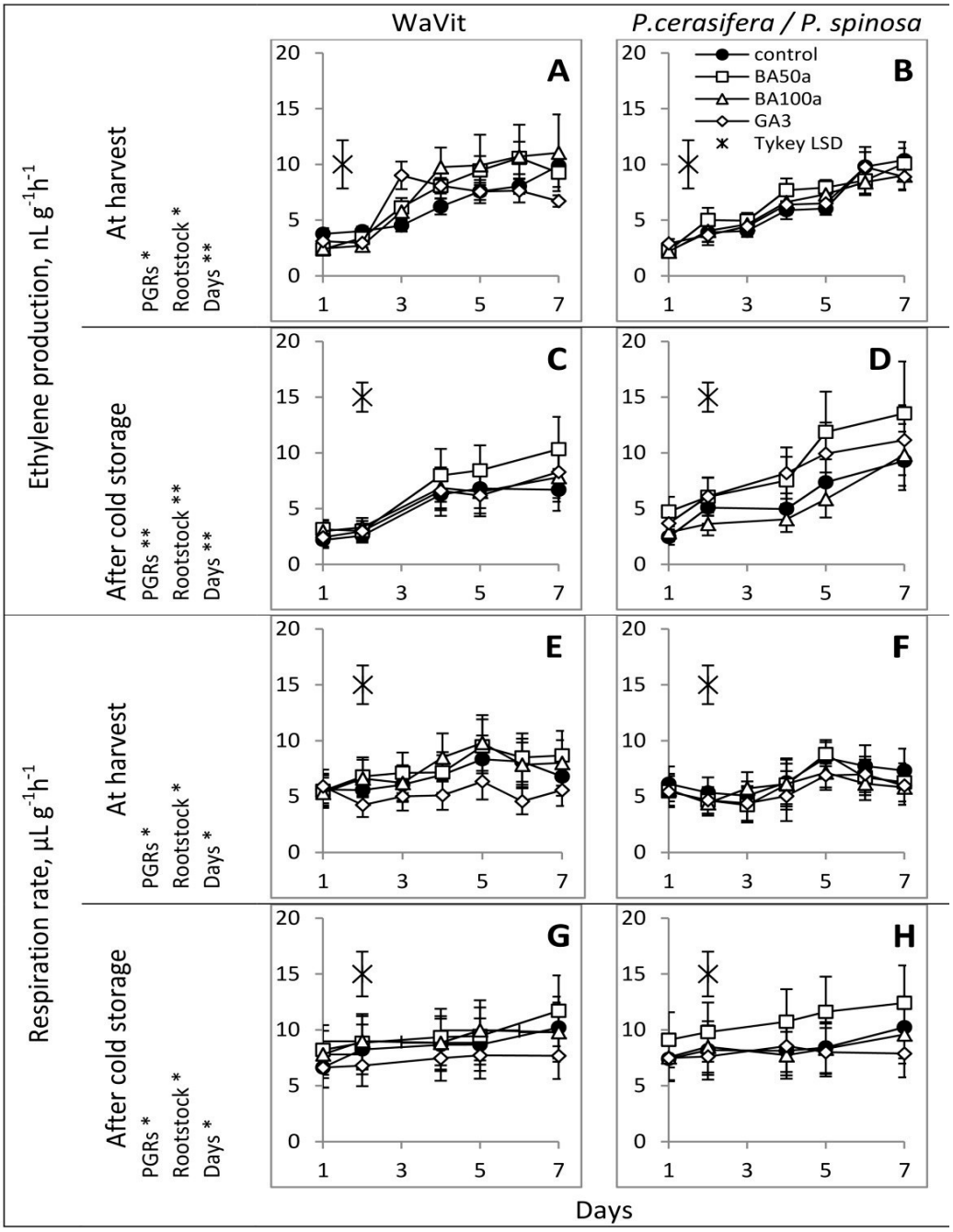

Figure 2. Ethylene production and respiration rate in fresh and stored plums grown on different rootstocks ("WaVit" and *P. cerasifera*/*P. spinosa*) and subjected to PGR treatments. (**A**–**D**) Ethylene production (nL g^{-1} h^{-1}): "WaVit" at harvest (**A**) and after cold storage (**C**). *P. cerasifera*/*P. spinosa* at harvest (**B**) and after cold storage (**D**). (**E**–**H**) Respiration rate (µL g^{-1} h^{-1}): "WaVit" at harvest (**E**) and after cold storage (**G**). *P. cerasifera*/*P. spinosa* at harvest (**F**) and after cold storage (**H**). The main factors are presented, and their significance is annotated by: *—significant at 0.05 and **—significant at 0.01. The error bars represent the standard deviation.

Rootstock type did not exert any effect on the textural properties (Table 1). PGR application induced significant changes, although, at harvest, flesh firmness was significantly lower only in BA100-treated fruits grown on P/P compared to the controls. After cold storage and shelf life, GA_3-treated plums were firmer than the controls, and for plums grown on "WaVit," this difference was statistically significant. BA50 application on fruit grown on "WaVit" increased the skin strength at harvest and after cold storage, while after shelf life, greater skin strength was recorded in fruits grown on P/P and treated with GA_3. Application of BA100 on fruits grown on "WaVit" rootstock significantly reduced fruit skin elasticity after both cold storage and shelf life.

Table 1. Changes in flesh firmness and skin strength, and elasticity of fresh and stored plum grown on two different rootstocks ("WaVit" and *P. cerasifera*/*P. spinosa*) influenced by PGR treatment.

Storage	Rootstock	Treatment	Flesh Firmness	Skin Strength	Elasticity
At harvest 0 days	***"WaVit"***	Control	12.4 ^{jk}	7.21 $^{d–h}$	3.66 ^{a}
		GA_3	12.3 $^{i–k}$	7.68 $^{f–h}$	3.94 ^{a}
		BA50	13.8 ^{k}	8.17 $^{g–i}$	3.76 ^{a}
		BA100	12.0 ^{ij}	6.72 $^{b–h}$	3.58 ^{a}
	P/P	Control	12.5 ^{jk}	6.90 $^{b–h}$	3.26 ^{a}
		GA_3	12.0 ^{ij}	6.92 $^{b–h}$	3.52 ^{a}
		BA50	13.4 ^{jk}	7.39 $^{e–h}$	3.22 ^{a}
		BA100	10.7 ^{i}	6.58 $^{a–h}$	3.68 ^{a}
Cold storage 28 days	***"WaVit"***	Control	4.94 $^{c–e}$	6.48 $^{a–h}$	5.89 ^{cd}
		GA_3	7.08 $^{f–h}$	6.52 $^{a–h}$	5.75 ^{bcd}
		BA50	6.47 $^{e–g}$	9.95 ^{i}	6.04 ^{cd}
		BA100	6.11 $^{d–f}$	5.95 $^{a–g}$	4.92 ^{b}
	P/P	Control	7.97 ^{gh}	6.51 $^{a–h}$	5.21 ^{bc}
		GA_3	8.60 ^{h}	7.62 $^{f–h}$	5.84 ^{cd}
		BA50	5.31 ^{de}	6.20 $^{a–h}$	6.18 ^{d}
		BA100	5.37 ^{de}	5.83 $^{a–g}$	5.99 ^{cd}
28 days of cold storage + 4 days of shelf life	***"WaVit"***	Control	3.04 ^{a}	4.92 $^{a–d}$	8.29 ^{fg}
		GA_3	3.39 $^{a–c}$	5.66 $^{a–f}$	8.54 ^{g}
		BA50	3.03 ^{a}	4.49 ^{ab}	8.32 ^{fg}
		BA100	3.20 ^{ab}	4.54 $^{a–c}$	7.27 ^{e}
	P/P	Control	2.78 ^{a}	5.04 $^{a–e}$	7.44 ^{ef}
		GA_3	4.73 $^{b–d}$	8.40 ^{hi}	8.30 ^{fg}
		BA50	2.35 ^{a}	4.23 ^{a}	7.30 ^{e}
		BA100	2.77 ^{a}	5.06 $^{a–e}$	7.73 $^{e–g}$
Rootstock			NS	NS	NS
PGR Treatment			**	**	*
Storage			**	**	**
Rootstock × Treatment			NS	*	**
Rootstock × Storage			**	NS	NS
Treatment × Storage			**	*	NS
Rootstock × Treatment × Storage			NS	NS	NS

Values designated by the same letter were not significantly different ($p > 0.05$). Main factors and their interactions are presented, and their significance is annotated as follows: NS—not significant, *—significant at 0.05 and **—significant at 0.01; PGR treatment: control—untreated plums; GA_3—200 mg L^{-1} of gibberellin A3; BA50—50 mg L^{-1} of 6-benzyladenine; BA100—100 mg L^{-1} of 6-benzyladenine; storage: 0—at harvest; 28—28 days of cold storage; 28 + 4—28 days of cold storage followed by 4 days of shelf life; rootstock: WaVit, *P. cerasifera*/*P. spinosa*. Standard deviation of data presented in Tables 1–3 are presented in Supplementary Table S1.

Fruit color analysis revealed that the PGRs and rootstocks exerted a significant impact on lightness (L*) in plum skin and flesh and on the intensity of red (a*) and yellow tone (b*) in the flesh (Table 2, Supplementary Figure S1).

Table 2. Changes in color properties of skin and flesh of fresh and stored plums grown on two different rootstocks ("WaVit" and *P. cerasifera*/*P. spinosa*) influenced by PGR treatment.

Storage	Rootstock	Treatment	Skin			Flesh		
			L*	a*	b*	L*	a*	b*
At harvest 0 days	**"WaVit"**	Control	34.0 ^{i}	6.49 $^{g–i}$	−2.10 $^{a–c}$	45.8 $^{f–h}$	−0.59 ^{a}	15.6 $^{h–k}$
		GA_3	35.5 ^{jk}	6.82 ^{hi}	0.76 ^{ef}	45.0 ^{fg}	0.46 $^{a–d}$	16.8 $^{j–m}$
		BA50	34.7 ^{ij}	6.00 $^{e–i}$	1.18 ^{f}	51.1 ^{j}	−0.98 ^{a}	19.6 ^{mn}
		BA100	35.3 ^{jk}	7.29 ^{i}	0.66 ^{ef}	50.5 ^{ij}	−0.26 ^{ab}	20.2 $^{k–n}$
	P/P	Control	39.4 ^{l}	6.07 $^{f–i}$	0.64 ^{ef}	45.8 $^{f–h}$	1.12 $^{b–f}$	17.8 $^{k–n}$
		GA_3	35.9 ^{k}	4.87 $^{b–f}$	−2.03 $^{b–d}$	48.1 $^{g–j}$	1.66 $^{c–g}$	20.0 ^{n}
		BA50	28.4 $^{d–f}$	6.42 $^{g–i}$	−0.84 ^{cd}	46.9 $^{f–i}$	−0.39 ^{ab}	19.7 ^{mn}
		BA100	38.5 ^{l}	5.06 $^{b–f}$	−1.56 $^{a–d}$	49.4 $^{h–j}$	0.56 $^{a–e}$	19.0 $^{l–n}$
Cold storage 28 days	**"WaVit"**	Control	29.0 $^{e–g}$	5.20 $^{b–g}$	−1.04 ^{bc}	39.6 $^{c–e}$	0.07 $^{a–c}$	16.0 $^{i–l}$
		GA_3	29.4 ^{fg}	5.34 $^{c–g}$	−1.19 $^{b–d}$	43.1 ^{ef}	2.79 $^{f–j}$	14.2 $^{e–j}$
		BA50	30.5 ^{h}	5.89 $^{d–h}$	−0.34 ^{de}	38.9 ^{cd}	2.06 $^{d–h}$	10.3 ^{cd}
		BA100	29.5 ^{gh}	4.91 $^{b–f}$	−0.90 $^{b–d}$	41.1 ^{de}	2.05 $^{d–h}$	11.2 $^{c–f}$
	P/P	Control	27.2 $^{a–c}$	4.60 $^{b–d}$	−1.25 $^{a–d}$	39.1 ^{cd}	2.26 $^{e–i}$	15.2 $^{g–k}$
		GA_3	28.1 $^{c–e}$	4.62 $^{b–d}$	−1.37 $^{a–d}$	39.0 ^{cd}	3.67 $^{h–l}$	12.0 $^{c–f}$
		BA50	28.3 $^{c–e}$	4.68 $^{b–e}$	−0.99 $^{b–d}$	35.0 ^{b}	1.10 $^{b–f}$	10.2 ^{cd}
		BA100	27.9 $^{c–e}$	5.78 $^{d–h}$	−0.83 ^{cd}	37.0 ^{bc}	1.92 $^{d–g}$	12.4 $^{d–g}$
28 days of cold storage + 4 days of shelf life	**"WaVit"**	Control	26.2 ^{a}	3.83 ^{ab}	−1.58 $^{a–d}$	33.4 ^{b}	4.62 ^{kl}	11.1 $^{c–e}$
		GA_3	27.2 $^{a–c}$	4.20 $^{a–c}$	−2.22 ^{ab}	34.6 ^{b}	4.85 ^{lm}	12.7 $^{d–h}$
		BA50	28.9 $^{e–g}$	4.38 $^{a–c}$	−2.24 ^{ab}	34.1 ^{b}	3.82 $^{ij–l}$	12.6 $^{d–i}$
		BA100	29.0 $^{e–g}$	3.83 ^{ab}	−2.58 ^{a}	34.1 ^{b}	3.95 $^{j–l}$	13.2 $^{d–i}$
	P/P	Control	27.3 $^{b–d}$	4.53 $^{b–d}$	−1.27 $^{a–d}$	25.5 ^{a}	3.12 $^{g–k}$	9.0 ^{bc}
		GA_3	26.8 ^{ab}	3.18 ^{a}	−1.84 $^{a–c}$	34.3 ^{b}	6.21 ^{m}	14.3 $^{f–j}$
		BA50	28.1 $^{c–e}$	3.93 ^{ab}	−1.86 $^{a–c}$	27.0 ^{a}	3.10 $^{g–k}$	7.0 ^{ab}
		BA100	27.5 $^{b–d}$	3.88 ^{ab}	−1.58 $^{a–d}$	34.0 ^{b}	4.10 ^{jk}	5.5 ^{a}
Rootstock			**	**	NS	**	*	*
PGR Treatment			**	NS	NS	**	**	*
Storage			**	**	**	**	**	**
Rootstock × Treatment			**	NS	**	**	NS	*
Rootstock × Storage			**	NS	**	**	NS	**
Treatment × Storage			**	NS	NS	**	NS	**
Rootstock v Treatment × Storage			**	**	**	**	NS	**

Values designated by the same letter were not significantly different ($p > 0.05$). Main factors and their interactions are presented, and their significance is annotated as follows: NS—not significant, *—significant at 0.05 and **—significant at 0.01; PGR treatment: control—untreated plums; GA_3—200 mg L^{-1} of gibberellin A3; BA50—50 mg L^{-1} of 6-benzyladenine; BA100—100 mg L^{-1} of 6-benzyladenine; storage: 0—at harvest; 28—28 days of cold storage; 28 + 4—28 days of cold storage followed by 4 days of shelf-life; rootstock: WaVit, *P. cerasifera*/*P. spinosa*. Standard deviation of data presented in Tables 1–3 are presented in Supplementary Table S1.

At harvest, a darker appearance was noted in the control fruit grown on "WaVit" (Table 2). Applied PGRs reduced the difference in skin L* between fruits grown on different rootstocks. BA50 application on fruits grown on P/P significantly reduced lightness at harvest, while the L* value did not change during cold storage or shelf life. Application of PGRs showed a tendency to increase the lightness of plum flesh at harvest. After cold storage and shelf life, applied PGRs changed the flesh lightness, but their effect was rootstock-dependent. GA_3 application increased red color intensity (a*), while the effect of PGRs and rootstocks on b* did not exhibit a discernible pattern.

Based on the ANOVA analysis results, TSS and TA in plums were significantly affected by all treatments applied in the study: PGRs, rootstocks, as well as cold storage (Table 3). Application of GA_3 resulted in higher TSS regardless of the rootstock type. At harvest, TA in plums treated with PGRs was lower in comparison to the respective controls. However,

the recorded differences were less pronounced after cold storage and shelf life, especially for fruit grown on "WaVit" rootstock.

Table 3. Changes in pigment, sugar and acid content in fresh and stored plums grown on two different rootstocks ("WaVit" and *P. cerasifera*/*P. spinosa*) influenced by PGR treatment.

Storage	Rootstock	Treatment	TSS (%)	TA (%)	Glucose + Fructose (g kg^{-1} FW)	Sucrose (g kg^{-1} FW)	Malic Acid (g kg^{-1} FW)	Succinic Acid (g kg^{-1} FW)	Anthocyanin (mg kg^{-1} FW)	Carotenoid (mg kg^{-1} FW)
At harvest 0 days	**"WaVit"**	control	15.60 ^{bc}	1.37 ^{o}	6.45 ^{ab}	4.07 $^{c–g}$	2.34 $^{c–e}$	1.71 ^{bc}	78.0 ^{a}	3.05 ^{ab}
		GA_3	17.58 ^{g}	1.22 ^{n}	7.16 $^{a–d}$	4.20 $^{d–h}$	2.61 ^{e}	1.80 $^{b–d}$	104.5 $^{a–c}$	1.45 ^{a}
		BA50	15.70 ^{c}	1.36 ^{o}	6.37 ^{a}	4.35 $^{e–i}$	2.52 ^{de}	1.74 ^{bc}	97.5 ^{ab}	0.95 ^{a}
		BA100	16.86 ^{d}	1.22 ^{n}	6.56 ^{ab}	4.34 $^{e–i}$	2.29 $^{b–e}$	1.71 ^{bc}	128.5 $^{b–d}$	6.90 $^{c–e}$
	P/P	control	17.24 ^{f}	1.11 ^{m}	6.93 $^{a–c}$	5.10 $^{i–k}$	2.42 $^{c–e}$	1.71 ^{bc}	107.5 $^{a–c}$	6.25 ^{cd}
		GA_3	18.40 ^{i}	0.83 ^{k}	6.47 ^{ab}	5.20 ^{k}	2.19 $^{a–d}$	1.89 $^{b–e}$	182.0 $^{e–h}$	13.15 ^{hi}
		BA50	17.12 ^{ef}	1.01 ^{l}	6.58 ^{ab}	4.40 $^{e–j}$	2.36 $^{c–e}$	1.98 $^{c–f}$	96.0 ^{ab}	6.15 ^{c}
		BA100	17.08 ^{e}	1.01 ^{l}	6.33 ^{a}	4.77 $^{g–k}$	2.25 $^{b–e}$	1.83 $^{b–d}$	134.5 $^{b–d}$	8.65 ^{ef}
Cold storage 28 days	**"WaVit"**	control	15.12 ^{a}	0.62 $^{f–h}$	8.02 $^{b–f}$	3.19 ^{ab}	1.93 ^{ab}	0.79 ^{a}	81.5 ^{a}	5.05 ^{bc}
		GA_3	18.64 ^{j}	0.59 $^{d–f}$	8.58 $^{d–h}$	4.01 $^{c–g}$	2.14 $^{a–d}$	0.75 ^{a}	131.0 $^{b–d}$	11.40 ^{gh}
		BA50	16.76 ^{d}	0.65 ^{hi}	8.01 $^{b–f}$	2.91 ^{a}	2.13 $^{a–c}$	0.57 ^{a}	135.5 $^{b–d}$	6.75 $^{c–e}$
		BA100	18.02 ^{h}	0.63 ^{gh}	8.31 $^{c–g}$	3.68 $^{b–e}$	2.07 $^{a–c}$	0.77 ^{a}	160.5 $^{d–g}$	10.40 ^{fg}
	P/P	control	17.26 ^{f}	0.54 ^{c}	8.77 $^{e–h}$	3.35 $^{a–c}$	2.05 $^{a–c}$	1.82 $^{b–d}$	113.0 $^{a–c}$	7.35 $^{c–e}$
		GA_3	19.08 ^{k}	0.50 ^{ab}	9.46 $^{f–j}$	4.12 $^{c–h}$	2.08 $^{a–c}$	1.84 $^{b–e}$	195.5 ^{gh}	18.15 ^{j}
		BA50	17.22 ^{ef}	0.60 $^{d–g}$	9.05 $^{f–i}$	3.49 $^{a–d}$	2.20 $^{a–d}$	1.97 $^{c–e}$	123.0 $^{a–d}$	6.20 ^{c}
		BA100	15.54 ^{b}	0.47 ^{a}	7.39 $^{a–e}$	3.36 $^{a–c}$	1.87 ^{a}	1.79 $^{b–d}$	188.0 $^{f–h}$	17.15 ^{j}
28 days of cold storage + 4 days of shelf life	**"WaVit"**	control	16.82 ^{d}	0.60 $^{e–h}$	10.87 ^{jk}	3.92 $^{b–f}$	2.57 ^{e}	2.27 $^{b–d}$	111.5 $^{a–c}$	9.85 ^{fg}
		GA_3	21.18 ^{n}	0.57 ^{de}	10.99 ^{jk}	5.05 $^{i–k}$	2.19 $^{a–d}$	1.96 $^{b–e}$	107.5 $^{a–c}$	11.40 ^{gh}
		BA50	20.34 ^{m}	0.54 ^{c}	10.48 $^{i–k}$	4.89 $^{h–k}$	2.12 $^{a–c}$	1.77 ^{bc}	141.0 $^{b–e}$	8.50 $^{d–f}$
		BA100	19.06 ^{k}	0.63 ^{gh}	9.90 $^{h–k}$	4.66 $^{f–k}$	2.20 $^{a–d}$	1.91 $^{b–e}$	117.5 $^{a–d}$	10.30 ^{fg}
	P/P	control	18.40 ^{i}	0.63 ^{gh}	11.12 ^{k}	4.09 $^{c–g}$	2.34 $^{c–e}$	2.14 ^{ef}	130.0 $^{b–d}$	9.60 ^{fg}
		GA_3	19.18 ^{kl}	0.52 ^{bc}	10.64 ^{jk}	5.42 ^{k}	2.25 $^{b–e}$	2.09 $^{d–f}$	258.5 ^{i}	22.80 ^{k}
		BA50	19.24 ^{l}	0.70 ^{i}	9.95 $^{h–k}$	5.16 ^{jk}	2.27 $^{b–e}$	1.96 $^{b–e}$	150.0 $^{c–f}$	13.90 ^{i}
		BA100	17.96 ^{h}	0.57 ^{d}	9.64 $^{g–k}$	5.21 ^{k}	2.25 $^{b–e}$	1.92 $^{b–e}$	213.0 ^{h}	18.00 ^{j}
Rootstock			**	**	NS	**	NS	**	**	**
PGR Treatment			**	**	**	**	NS	**	**	**
Storage			**	**	**	**	**	**	NS	**
Rootstock × Treatment			**	**	NS	NS	NS	**	**	**
Rootstock × Storage			**	**	NS	NS	NS	**	NS	**
Treatment × Storage			**	**	NS	**	NS	**	**	**
Rootstock × Treatment × Storage			**	**	NS	NS	NS	**	**	**

Values designated by the same letter were not significantly different ($p > 0.05$). Main factors and their interactions are presented, and their significance is annotated as follows: NS—not significant, **—significant at 0.01; PGR treatment: control—untreated plums; GA_3—200 mg L^{-1} of gibberellin A3; BA50—50 mg L^{-1} of 6-benzyladenine; BA100—100 mg L^{-1} of 6-benzyladenine; storage: 0—at harvest; 28—28 days of cold storage; 28 + 4—28 days of cold storage followed by 4 days of shelf-life; rootstock: "WaVit", *P. cerasifera*/*P. spinosa*. Standard deviation of data presented in Tables 1–3 are presented in Supplementary Table S1.

Regardless of the significance of PGR treatments, no pattern could be established for monosaccharide (glucose + fructose) content in treated plums (Table 3). At harvest, sucrose content was not influenced by PGR treatment either, but after shelf life, it was significantly higher in treated plums. Plums grown on P/P had higher sucrose content than those grown

on "WaVit" rootstock. No significant differences in succinic or malic acid content were observed between the PGR-treated plums and the controls.

The anthocyanins content was the only parameter that was not affected by cold storage but was influenced by both rootstock and PGR treatment type (Table 3). It was notable that the anthocyanin content was higher in the fruits grown on P/P compared to those grown on "WaVit". Additionally, the PGR-treated plums had higher anthocyanin content in comparison to the respective controls. Carotenoid content increased significantly during storage and shelf life and was higher in fruits grown on P/P and in fruit treated with GA_3 and higher BA concentration regardless of the storage period.

3.2. Multivariate Analysis

In order to identify and compare the directions and intensities of rootstock- and PGR-dependent changes, as well as enable comparison of the magnitude of PGR and rootstock influence, multivariate principal component analysis (PCA) was performed (Figure 3).

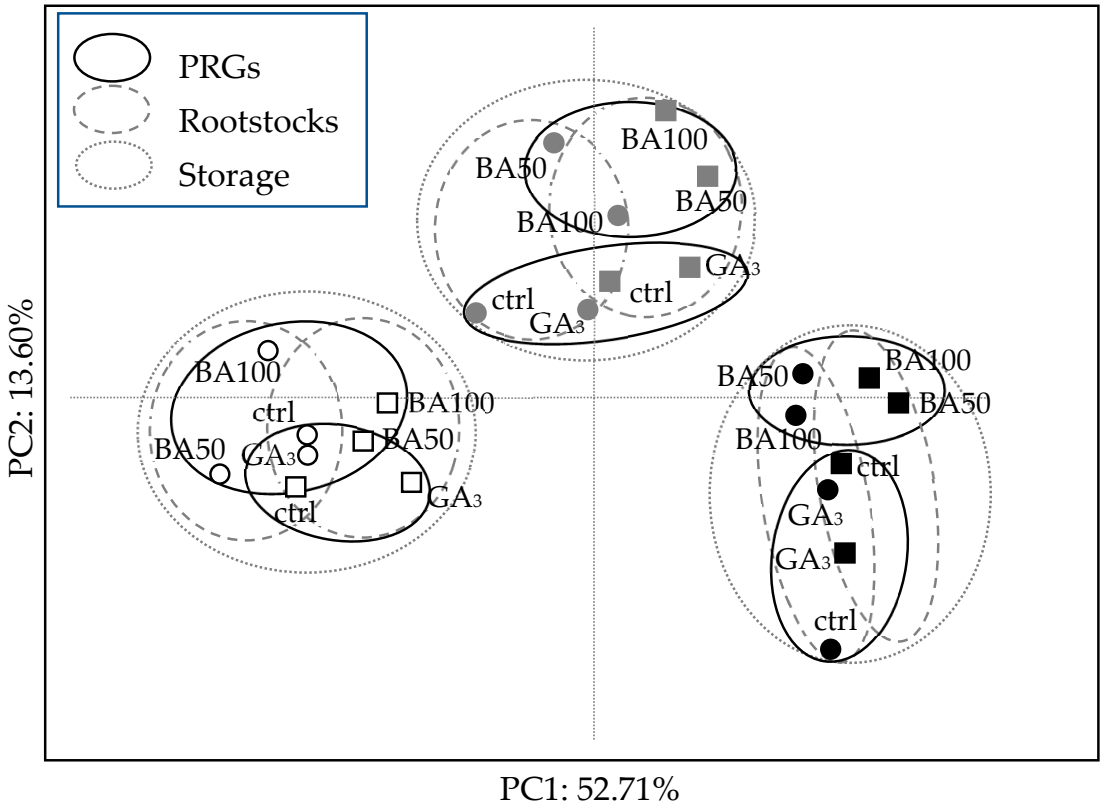

Figure 3. Principal component analysis: bi-plot of plum physicochemical properties with corresponding treatments. Legend: **Rootstock:** circles—"WaVit", squares—*P. cerasifera*/*P. spinosa*. **Storage duration:** ○□—at harvest; ●■—after 28 days of cold storage; ●■—after 4 days of shelf life. **Treatments:** ctrl—control; GA_3—200 mg L^{-1} of gibberellin A3, BA50—50 mg L^{-1} of 6-benzyladenine, BA100—100 mg L^{-1} of 6-benzyladenine.

The findings indicate that the first two principal components jointly explain >65% of the variability, with 52.71% attributed to the first and 13.60% to the second principal component. Clear segregation of the samples with respect to the first principal component in relation to the storage duration can also be noted, differentiating fruits analyzed at harvest, after cold storage and after shelf life. Moreover, fruits after cold storage can be distinguished from other samples concerning the second principal component. In each cluster of samples stored for different time periods, differentiation based on the rootstock type can also be made for the first principal component. The first principal component is highly correlated with monosaccharide content (with a 0.93 correlation coefficient obtained

for both fructose and glucose), texture properties (skin strength −0.86, fruit firmness −0.96 and skin elasticity 0.96) as well as color properties (skin L*, a* and b*, −0.81,−0.87 and −0.66, respectively, and flesh L*, a* and b*, −0.93, 0.86 and −0.83, respectively).

Differentiation of non-treated fruits from those treated with PGRs, particularly with BA, within clusters of samples stored for different time periods can also be made with respect to the second principal component, confirming the physicochemical properties and composition of the studied fruit are influenced by PGR treatment. Still, in comparison to the changes induced by rootstock type, and particularly by prolonged cold storage, this influence is minor (PC1 = 52.71%; PC2 = 13.60%). The second principal component is negatively correlated with sucrose (−0.68) and acid (−0.73 and −0.68 for malic and succinic acid, respectively) content, as well as positively correlated with anthocyanin and carotenoid content (0.63 and 0.32, respectively).

4. Discussion

Gibberellins and cytokinines are involved in the regulation of numerous plant development processes. These plant growth regulators (PGRs) accumulate in the early phases of fruit development, during which cell division and cell expansion occur. Empirical evidence further indicates that higher concentrations of these compounds during the fruit ripening phase inhibit the ripening process [46]. Gibberellins are known to increase cell elongation, while cytokinines influence cell division [57]. Thus, their application in the early phase of fruit development, as was done in the present study, induces beneficial changes to the morphological properties of the fruit (fruit weight, size, diameter, dimensions and volume) and thus to fruit production [45,46,58–62]. However, several authors have challenged this assertion, arguing that these effects are not significant and are dependent on the production season or variety [35,59,63].

In our investigation, the application of cytokinine (BA) and gibberellin (GA_3) induced changes in at least one of the measured fruit shape parameters, indicating modifications in fruit morphology. These findings confirm that the expected alterations occurred in the fruit development phase due to increased concentration of PGRs (Figure 1). The most prominent effects were exerted by BA50, as fruit width and thickness changed irrespective of the rootstock used. The observed differences in the morphological properties of fruit grown on different rootstocks are consistent with those published in pertinent literature, where the influence of rootstock on plum yield, fruit and stone dimensions and weight is well documented for both European and Japanese plum [49,51,64–71]. Numerous findings confirm that GA_3 and BA significantly impact the physical properties and chemical composition of harvested fruit. Still, the majority of extant research focusing on the effects of PGR application on plums was carried out on Japanese plums [4,45,58–61,63], while the European plums are rarely studied [46,47].

The majority of plum varieties, with rare exceptions, are categorized as climacteric [72]. In these fruits, the ripening processes, accompanied by the changes in fruit composition that leads to decay, are closely related to increased ethylene production [28,73,74]. Recent findings [75] related to the effects of both gibberellins and cytokinins on the ripening process in different climacteric fruit species emphasize the inhibitory role of higher concentrations of these PGRs, which may be associated with the inhibition of ethylene production. However, the underlying molecular mechanisms which control the ripening processes, the complete set of ripening-related genes and signal transduction have yet to be fully discovered and understood [75]. Our results indicate that ethylene production increased in fresh fruit as well as after prolonged cold storage. Thus, it can be assumed that the inhibiting mechanisms of GA_3 or BA on ethylene production were not triggered in our study, probably due to the PGR application in the early phase of fruit development, i.e., long before the ripening stage.

The intensity of fruit respiration, as a series of biochemical processes resulting in the oxidation of sugars to CO_2 [76], is another factor contributing to the postharvest quality losses [77]. Increased CO_2 production shortens shelf life and leads to the degradation of traits that determine fruit acceptability for consumers [78–80]. As in the present study, PGR

application during the early fruit development phase did not affect the respiration rate of ripe fruit after harvest. The differences in respiration cannot be correlated with PGR use.

The main plum composition parameters affected by PGR application—as indicated by the PCA findings (Figure 3)—were pigment content (including both anthocyanins and carotenoids), as well as sugar (i.e., sucrose) and acid content. Higher sucrose, anthocyanin and carotenoid contents were noted in PGR-treated plums at harvest, and these values increased after prolonged cold storage and shelf life (Table 3). These findings indicate that PGR application might have significant positive effects on the fruit's sensory properties, visual appeal and taste, as well as the nutritional profile (in terms of higher content of bioactive compounds) during the postharvest period when most fruit is consumed. A significant increase in the polyphenol (especially anthocyanin) content in stored fruits was also reported for multiple treatments of plums with salicylates [81]. In the present study, anthocyanin and carotenoid content increased irrespective of the rootstock type; however, higher values were obtained for both pigments on fruits grown on P/P.

Due to the high sugar content in soluble solids, plums can be stored at low temperatures, close to 0 °C. These storage conditions slow down postharvest ripening and decrease biochemical processes, allowing successful preservation of fruit quality for up to five weeks [77]. The changes after cold storage, regardless of the applied PGR treatment or rootstock, are followed by the expected postharvest fruit behavior, described in pertinent literature for both Japanese and European plums [27,32,33,77]. Thus, the main focus of our discussion will not be on the postharvest ripening process but rather on the differences between the fruit treated with PGRs and non-treated plums in the postharvest period.

Our findings showed that, following GA_3 and BA application, the TA values declined at harvest in comparison to non-treated fruits (Table 3). Moreover, GA_3-treated plums in all investigated stages had higher TSS content at harvest when compared to the relevant controls. Additionally, present findings suggest that PGRs impact the sucrose, anthocyanin and carotenoid contents compared to the controls. While the observed increase in TSS, carotenoid and anthocyanin, along with the decrease in TA in PGR-treated fruits, could be attributed to increased fruit ripening, the noted improvements in texture do not support this assumption. Thus, it is likely that PGR application in the early stages of development targets specific biochemical pathways rather than the whole ripening process.

Based on the results reported in this work, it can be concluded that the application of BA and GA_3 impacts texture, flesh color, TSS, TA, sucrose and carotenoid content (Tables 1–3). The data reported in extant literature confirm that PGRs presence can induce changes in the fruit quality parameters (including TSS, TA and fruit firmness) at harvest. However, the findings obtained by other authors are inconsistent. For example, several reports on different varieties of *P. domestica* and *P. salicina* indicate that TSS increases due to GA (GA_3) treatment [45,47,60,62], while TA decreases [59,62,63], and fruit firmness remains unaffected [61]. On the other hand, an ample body of evidence suggests that fruit firmness increases as a result of GA_3 application [45,46,58–62], which has no influence on TSS [58,59,61,63].

The rootstock-induced differences in tree productivity and fruit properties at harvest could be the reason for the different responses of the grafted cultivar "Čačanska rodna" to PGR treatments at harvest and during the postharvest period [82,83]. *P. spinosa* is used as the interstock with *P. cerasifera* as the rootstock for apricots and plums to reduce the trunk diameter and tree growth and enable high-density planting [50,84]. It induces early bearing, wide soil adaptation, frost resistance, larger fruit size, and better coloration [85]. On the other hand, "WaVit" is a rootstock selected from seedling populations of "Wangenheim" (*P. domestica*) with excellent uniformity. It reduces tree growth, induces early cropping, accelerates fruit ripening, and increases fruit size while producing high and regular yields [86,87].

Plum cultivar "Čačanska lepotica" did not significantly differ in terms of tree vigor when grown either on "WaVit" or *P.cerasifera*/*P.spinosa*, while the higher yields and yield efficiency were recorded on "WaVit" rootstock [50]. The rootstock did not affect

fruit size in "Čačanska lepotica" or in the present research in "Čačanska rodna", while *P.cerasifera/P.spinosa* consistently induced the lower titratable acidity in fruits compared to "WaVit" rootstock.

5. Conclusions

Although rootstock type and PGR application impact the overall fruit quality at harvest as well as after cold storage and shelf life, these factors affect different quality parameters.

The effects of early application of BA and GA_3 at harvest manifest through positive changes (in terms of consumer acceptance) in fruit weight and shape, the color of skin and flesh, and TSS, TA and pigment content. However, changes to fruit weight and shape are rootstock-dependent. The initial differences between PGR treatments and rootstocks in terms of TSS, TA, and pigment content persist after cold storage. Still, additional differences in fruit texture emerge while the color difference diminishes. After shelf life, TSS and TA remain treatment- and rootstock-dependent, and the same difference starts to emerge in sucrose and pigments. Based on the obtained results, it can be surmised that PGR application in the early phases of fruit development results in different postharvest fruit biochemistry.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae8070621/s1, Figure S1: Changes of plum fruit after cold storage and subsequent shelf life, Table S1: Standard deviation of data presented in Tables 1–3 (flesh firmness, skin strength, elasticity, skin and flesh L*, a*, b*, TSS, TA, glucose + fructose, sucrose, malic and succinic acid, anthocyanin and carotenoid content).

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