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Morphological and Physiological Responses of *Weigela florida* 'Eva Rathke' to Biostimulants and Growth Promoters

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Abstract: Ornamental horticulture and breeding, as well as urban landscape architecture, are facing increasing challenges driven by an intensely changing climate and urbanisation. The expansion of cities should be combined with an overall growth of green spaces, where ornamental plant species and cultivars will have to withstand a diverse range of environmental conditions, whereby they are often exposed to multiple stress factors. One of the most widely used ornamental shrub species *Weigela florida* 'Eva Rathke' was treated with the growth promoters Bistep with humic and fulvic acid, Kelpak® seaweed extract, and Yeald Plus with a high zinc content to test their applicability in a plant nursery. Bistep decreased the physiological parameters (the transpiration rate by 60%, the evapotranspiration rate by 56.5%, and the proline stress enzyme content level by 82.2%), indicating the stress level of the treated plants. The activity of β-glucosidase decreased with all growth-promoting treatments (11.5% for Kelpak and 9.5% for Yeald Plus), as did β-glucosaminidase (22.1% for Kelpak and 9.8% for Yeald Plus), but Bistep treatment reduced the activity of the enzymes less (9.9% for β-glucosidase and 3.3% for β-glucosaminidase). The measured alkaline phosphatase enzyme activity increased with treatment (by 10.7% for Kelpak, 11.7% for Yeald Plus, and 12.63% for Bistep). Based on the results, it was concluded that Bistep and Yeald Plus may be suitable for use in the studied variety, whereas Kelpak® may not be suggested in plant nurseries for growing *W. florida* 'Eva Rathke' plants.

Keywords: enzymes; fulvic acid; green surface; horticulture; humic acid; ornamental plants; rhizosphere; urbanisation

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1. Introduction

Ornamental plants are cultivated and used for their aesthetic appeal, making them a widespread choice for urban green spaces, as their benefits include the provision of ecosystem services supporting the well-being and mental health of the population. Climate change and urbanisation are placing increased pressure on long-established popular species, which are not assured of maintenance and survival in urban environments under conventional growing technology. Cities occupy around 3% of the world's land surface and are currently growing relatively slowly (0.5–0.6% per year). In contrast, peri-urban, urban areas are expanding four times quicker yearly, and maintaining the sufficient quality and quantity of vegetation cover is considered essential [1]. Although the benefits of urban greening are obvious, sustainable implementation still faces problems, as urban areas present multiple levels of challenges for plants, whether in the case of their basic needs or for the blooming ability of ornamental plants [2]. To overcome these challenging conditions, several management strategies can be applied, which include the use of biostimulants [3,4].

The percentage of use of biostimulants and micronutrients is on the increase worldwide, and this tendency is also noticeable in horticultural practices. The main reason for this is that these substances offer natural and sustainable solutions to enhance the growth, health, and resistance of ornamental plants [5,6]. Their vital effects depend on their composition, as they contain various organic and mineral compounds that can be used by plants as metabolites, growth promoters, and nutrients; however, biostimulants are not considered as biofertilisers [7]. Nursery (ornamental horticulture and forestry) professionals are beginning to adopt practices that promote sustainable plant establishments in urban areas in order to achieve higher-volume urban greening outcomes in the future by improving plant vitality [2], which could be achieved by an improved leaf or root uptake [8].

As the prevailing category in the biostimulant segment, marine algae extracts are considered to be the dominant agent group—accounting for more than 33% of the total biostimulant market worldwide and estimated to reach EUR 894 million in 2022 [9]. One of the most commonly used algae species for extract production is *Ecklonia maxima*, which is characterised by high phytohormonal activity [10,11], in addition to containing minimal amounts of mineral elements in a form readily available for uptake by plants [12]. These substances can be applied both through the leaf and root system, but Kachel and Tratwal [13] recommend applications through the leaf. Kisvarga et al. [14] treated a group of *Matthiola incana* 'Varsovia' cultivars with the Bistep biostimulant and found that vegetative parameters were significantly higher than in the untreated group, and in lettuce cultivars, it also increased the polyphenol, vitamin C, and nitrate content in leaves [15]. In addition, they have beneficial effects on root growth and shoot growth [16].

As an example, in container-grown *Photinia fraseri* 'Red Robin' plants, the product has been shown to be unsuitable for both cuttings and container growing [17]. Szabó et al. [18], on the other hand, reported higher rooting rates and higher fresh cutting weights in *Prunus* 'Marianna' plants using Kelpak® (KELP PRODUCTS (PTY) Ltd. 7975 Simon's Town, Blue Water Close, South Africa), while roses showed an improvement in rooting properties [19]. However, *E. maxima* negatively affected branching properties in container-grown *Hydrangea paniculata* [20].

Along with algae extracts, another significant group is the humic substances, which can improve soil properties, such as water retention, through increased soil aggregation and aeration, leading to increased microbial activity. This process enhances the mineralisation of organic matter and increases the bioavailable fractions of micro- and macroelements to the plant [21]. Humic and fulvic acids can contribute to an increase in the leaf area, dry weight, and average fruit weight [22] and affect the leaf chlorophyll content and nitrogen, phosphorus, and potassium levels [23].

In recent years, the use of biostimulant products to counteract abiotic stresses has become a valid technological innovation and is considered as an agronomic tool with great potential for the sustainable development of crop production [24]. The plant stress response involves metabolic pathways such as photosynthesis, sugar synthesis, the tricarboxylic acid cycle, glycolysis, and hormone synthesis, on which biostimulants can have a positive effect [25]. This has been shown to result in an increased chlorophyll content in many herbaceous horticultural cultivars, while maintaining low nitrate levels [26].

A relationship has also been observed between stress enzymes and proline levels [27], which could be of interest for specific measurements, as increased proline levels are associated with increased transpiration and evapotranspiration [28].

Transpiration is a fundamental process in understanding plant ecophysiology, and evapotranspiration is the dominant component of the water cycle in plant life [29], and evapotranspiration is highly dependent on plant conditions and mainly on abiotic influences [30]. Evapotranspiration is not only an important element of the water and energy balance sectors but is also closely related to crop growth, biomass production and yield, and quality [31,32]; it also plays a very critical role in land–atmosphere interactions in the earth system [33]. The magnitude of evapotranspiration is influenced by a number of factors, including meteorological conditions, soil and crop yields, and management and environmental conditions such as water, salinity, and heat stress [34]. A number of factors, including water and salt stress, that influence evapotranspiration have been well described in several studies [34,35]. Transpiration rates showed a decreasing trend during the plant growth period with increasing leaf temperatures and stomatal diffusion resistance [36].

Proline accumulation has been shown in plants exposed to various abiotic stresses, and Tripathi and Gaur [37] suggest that proline protects cells by scavenging free radicals during oxidative stress caused by heavy metals. According to the literature, proline protects plants by acting as a cell osmotic promotery agent between the cytoplasm and the vacuole and by detoxifying reactive oxygen species (ROS), thereby ensuring membrane integrity and stabilizing antioxidant enzymes [38]. The use of *E. maxima* as a biostimulant also helps to counteract abiotic stress effects and has a role in increasing photosynthetic activity. Its beneficial properties have also been described in chicory plants, among others, where it increased the chlorophyll and ascorbic acid content and improved proline levels under drought stress [39].

Furthermore, micronutrients play an important role in enhancing physiological processes. Zinc is an essential micronutrient for plants, regulating the function of many enzymes and hormones, as well as macromolecule metabolism, stabilizing protein structures and affecting gene expressions [40]. The form and content of zinc oxide-based nanoparticles are determined by chemical or environmental techniques and can be produced from various plant components and parts, including the leaf, stem, root, fruit, or seed [41]. In *Pennisetum* and *Miscanthus* species, which are also used in ornamental horticulture, the application of zinc and copper increased the resistance to various stresses [42], while Kumar et al. [43] observed an improvement in the skeletal longevity of *Gladiolus grandiflous* plants under the influence of zinc. In *Freesia hybrida*, also applied as cut flowers, zinc application increased the tuber number as well as the diameter of tubers in individuals [44]. Beneficial effects of zinc on vegetative parameters were also observed in *Rosa* [45], *Celosia argentea var. cristata* [46], *Hydrangea macrophylla*, and *Alcea rosea* species [47].

The rhizosphere is a special environment where there is a strong communication between the plant and microbes [48]. Since plant growth promoters can affect the whole plant, including its root system and rhizosphere, it is considered essential to carry out soil microbiological studies. The enzyme activity associated with microorganisms can answer the question of whether the applied products are beneficial to the plant or its microenvironment. This is an increasingly important question for ecosystem and environmental protection. Alkaline phosphatases are exoenzymes of microbes. Their function is to convert phosphorus in the soil into a plant-available form [1], mainly by the hydrolysis of organophosphate esters in the extracellular space [49]. Soil microorganisms mainly synthesize extracellular enzymes such as β-glucosidase, urease, phosphatase, cellulase, amylase, cyclomaltodextrinase, chitinase, among others [50,51]. The enzyme β-glucosidase is involved in the degradation of organic matter, catalysing the biodegradation of disaccharides to monosaccharides. In this way, the end product of the hydrolysis is glucose, an important source of C for soil microbes. This enzyme is sensitive to pH (soil acidification) and soil management interventions [52,53]. The importance of the β-glucosaminidase enzyme in biological systems has long been recognized, but only recently has its role in microorganisms, plants, and invertebrates begun to be explored. This suggests that the activity of β-glucosaminidase may play a role in the nitrogen-fixing activity of microorganisms [54].

Based on the literature, it was hypothesized that the production of the deciduous ornamental shrub *W. florida* 'Eva Rathke' in plant nurseries could be improved. The key to the popularity of this ornamental shrub is its bright red flowers and compact growth habit, which allows for it to be found in gardens with small footprints, features that have been discussed in the work of Kopeva et al. [55] and Dogadina and Botuz [56].

Therefore, *W. florida* 'Eva Rathke' plants were treated with plant growth promoters and were evaluated through an evaluation of the histological, physiological, and soil microbiological parameters. The aim was to identify a biostimulant or growth promoter that could be successfully applied in horticultural production in the future to increase and maintain the vigour of the *W. florida* 'Eva Rathke' cultivar. As there are no literature results and publications on the use of biostimulants in this cultivar, we want to contribute to the use of plant growth promoters in *W. florida* 'Eva Rathke'.

2. Materials and Methods

2.1. Substances Used in Experiments

Two types of biostimulants were used in this study: humic and fulvic acids and marine algae extracts. In addition, the effects of a plant growth promoter with a high zinc content were investigated in order to better understand the physiological mechanisms in *W. florida* 'Eva Rathke'. These are described below.

Bistep, or Ferbanat L as it is also known commercially, containing humic and fulvic acids, is a biostimulant used in horticultural production. Bistep is a biostimulant, containing extracts of wormwood, microorganisms, and macro- and microelements, the detailed ingredients of which are shown in Table 1.

Table 1. Composition of the substances used.

Parameters	Kelpak	Yeald Plus	Bistep
Manufacturer	KELP PRODUCTS (PTY) Ltd. 7975 Simon's Town, Blue Water Close South-Afrika	De Sangosse Ltd. Hillside Mill Quarry Lane, Swaffham Bulbeck, Cam-bridge CB5 0LU	UAB ALJARA LT-11219 Vilnius, Geniu str. 16–38. Lithuania
pH	4.5	6.5	7.4
N (m/v%)	0.03	6	0.02
P_2O_5 (m/v%)	0.02	-	0.03
K_2O (m/v%)	0.65	-	0.3
MgO (m/v%)	0.02	-	0.02
As (mg/L) max.	10	10	10
Cd (mg/L) max.	2	2	2
Co (mg/L) max.	50	50	50
Cr (mg/L) max.	100	100	100
Cu (mg/L) max.	100	0.25	100
Hg (mg/L) max.	1	1	1
Ni (mg/L) max.	50	50	50
Pb (mg/L) max.	100	100	100
Se (mg/L) max.	5	5	5
B (m/v%)	-	0.03	0.0002
Fe (m/v%)	-	0.25	0.01
Mn (m/v%)	-	0.25	0.007
Mo (m/v%)	-	0.001	0.09
Zn (m/v%)	-	5	0.008
Number of germ (db/cm^3)	-	-	0.8×10^7
Amount of microbes (db/$cm^{3)}$	-	-	1.0×10^2
Ca (mm/m%)	4.5	6.5	7.4
B (m/v%)	0.03	6	0.02

The other biostimulant used in this study was Kelpak®, which is used in several crops, including ornamental horticulture. Kelpak® contains *E. maxima*, according to the information on the company's website, and has an NPK content of 0.05%, which is divided as follows: a nitrogen content of 0.05%, a phosphorus content (in the form of P_2O_5) of 0.03%, and a potassium content (in the form of K_2O) of 0.65% (Kelpak®). This agent has a high auxin content, which stimulates the development and growth of the root system, leading to a better absorption of macro- and microelements [57].

The Yeald Plus (De Sangosse Ltd. Hillside Mill Quarry Lane, Swaffham Bulbeck, Cambridge) growth promoter solution was also used in the experiment. Yeald Plus stimulates auxin synthesis, thus promoting root formation and root mass growth, improving water and nutrient uptake, increasing chlorophyll levels, and accelerating carbohydrate metabolism. Yeald Plus contains 6% of N, 5% of Zn, 1% of K_2O_5, 0.03% of B, 0.25% of Cu, 0.25% of Fe, 0.25% of Mn, and 0.001% of Mo, with acetic acid for a pH adjustment [58]. As a general rule, the younger the plant, the lower the dose needed to treat it [59]. Yeald Plus can be adequately taken up by the plant through the roots and leaves even at lower temperatures (+5–7 °C).

The first treatment was carried out at the same time as planting in the first week of May during the years studied. Thereafter, treatments were carried out every 5 weeks during the growing season until the end of shoot growth (end of July in Hungary), i.e., three treatments per year. The treatments were carried out by spraying. The concentrations applied during the series of measurements were as follows:

- Kelpak®—0.4% solution;
- Yeald Plus—0.3% solution;
- Bistep 0.5%—solution;
- Control group: received only water at the same time as the treatments.

2.2. Experiment Setup

The experiment took place in Zalaszentgyörgy, Hungary, in a nursery with the coordinates 46.87372 N; 16.70235 E. The experiment was conducted between 2016 and 2023. The climate of the landscape is moderately cool to moderately humid. The average annual sunshine duration is 1830–1950 h per year, with a summer daylight duration of around 760 h. The average annual temperature is 9.2–9.8 °C. The frost-free period is between 15 April and 23–25 October. The annual absolute maximum temperature is between 32 and 33 °C, and the winter absolute minimum is around −17 °C.

In this experiment, the aim was to select a widely known deciduous shrub species that is promising for urban applications. A hybrid of *W. florida* and *Weigela praecox* [55,56] fully meets these criteria, as its breeding is an active process, and further new cultivars are expected in the future [60]. Among the hybrids also marketed under the name *W. florida*, 'Eva Rathke' stands out, which was chosen because it is a long-established cultivar with a long history of cultivation, with some studies indicating that it has been grown in the UK since 1893 (Figure 1).

(a) (b) (c)

Figure 1. *W. florida* 'Eva Rathke' experimental specimens at (**a**) basal stand before treatment and potting; (**b**) potted and first-treated plants before transport to the nursery; (**c**) condition after last treatment at the nursery (Kovács, 2023).

New plants were planted each year in 1.5 L containers between 2016 and 2023. During these years, morphological surveys were carried out as well as physiological measurements. The results are shown as an average. The parameters of the planting medium were as follows:

- A total of 30% crushed calcareous black peat (pH, 6.24; humus content, 17.89%);
- A total of 70% crushed white peat (pH, 5.81; humus content, 25.58%).

The medium had a grain size of 10–30 mm and an EC level of 2.0–4.0. The soil analysis report is shown in Table 2. Measurements were made with 25 individuals in 5 replicates per treatment. Plants were uniformly top watered with a sprinkler head. No pruning was applied.

Table 2. Detailed soil analysis of the biostimulants and growth promoters used.

Parameters	Control	Kelpak	Yeald Plus	Bistep
pH (H_2O)	5.37	5.1	5.05	5.3
Organic material (m/m%)	75.9	83	82.5	79.9
P_2O_5 (AL soluble) (mg/kg)	930	1390	1300	1230
K_2O (AL soluble) (mg/kg)	1452	1908	1988	2016
NO_3 + NO_2-N (KCL soluble) (mg/kg)	592	1182	1447	776
Na (AL soluble) (mg/kg)	1680	1228	1444	1392
Cu (EDTA soluble) (mg/kg)	34.6	34.3	29.9	33.7
Mg (KCL soluble) (mg/kg)	936	1200	1144	1060
Ca (EDTA soluble) (mg/kg)	2900	2775	2625	2925
Mn (EDTA soluble) (mg/kg)	170	144	143	153
SO_4^{2-}-S (KCL soluble) (mg/kg)	1625	1666	2066	1528
Zn (EDTA soluble) (mg/kg)	19	23.7	40.9	41.1

2.3. Histological Measurements

2.3.1. Sampling of Plant Material

The sampling was conducted in August, when shoot growth had stopped, and the plants had reached maximum development, but the leaves had not yet started to yellow or anthocyaninise.

2.3.2. Histological Analysis

Since the products and active substances used can modify a number of morphological parameters, it was considered important to investigate the treated plants at the tissue level. Histological analyses were carried out in each year of the experiment—these results were summarised, and the sections that best described and represented them were selected. Cross-sectional images of leaf samples were used for the measurements to show any tissue changes. Tissue samples were taken from the areas adjacent to the midrib of the leaf discs of the treated plants.

- Microscope: Euromex bScope BS.1153-PLi biological microscope (Euromex Microscopen B.V., Arhem, Netherlands);
- Camera: Euromex CMEX-5f 5 Mp Camera (DC.5000f);
- Lens: due to the sectioning procedure, oil immersion lens blocks cannot be used; therefore, due to the nature of the sections, the PLi Lens PLi 4/0.1 was used, and because of the type of samples, no oil immersion was applied. Magnification: 40 × 4/0.1. Magnification: 40×;
- Ocular: WF120×/20 type and size.

The samples were not painted. The images were post-corrected using GIMP 2.10.34 (property of Spencer Kimball, Peter Mattis).

2.4. Physiological Measurements

2.4.1. Transpiration and Evapotranspiration Analysis

Evapotranspiration is the transfer of water from the earth's surface to the atmosphere, involving a phase change of water from liquid to water vapour [61]. In parallel, plant transpiration is the transfer of water from the plant surface to the atmosphere, and leaf transpiration is the amount of water transferred from leaves to the atmosphere, which can be a good indicator of morphological and stress physiological relationships [62]. An assessment of the physiological status is essential to study the effects of growth promoters. To determine this, transpiration and evapotranspiration values were measured using the Kern 572-45 laboratory balance. To carry out the survey, the containers were uniformly filled with water the day before. In order to measure only evaporation through the canopy, the containers were covered with a waterproof material to prevent other types of water loss from the canopy. Measurements were then taken the following morning and evening. The difference between the two data gave the evapotranspiration rate, i.e., the amount of water evaporated through the canopy and the surface of the medium. The difference between the morning and evening data was the transpiration rate.

2.4.2. Proline Content Determination

The proline content was determined by the spectrophotometric method based on Chinard [63] and was modified according to Ábrahám [64]. At acid pH, proline forms a red product with ninhydrin. The method requires 100 mg of the plant sample. The plant sample was homogenised with 3% sulphosalicylic acid (5 mL/mg of fresh weight), stored frozen, and centrifuged for 5 min at room temperature. The reaction mixture consisted of 100 mL of 3% sulfosalicylic acid, 200 mL of cubic meters of acetic acid, 200 mL of acid ninhydrin, and 100 mL of the samples. This was poured into test tubes and sealed with aluminium foil. Afterwards, it was kept in a drying oven at 96 °C for 1 h. The reaction was stopped in cold water, then was dissolved in 1.5 mL of toluene, stirred for 20 s, and allowed to stand for a further 5 min. The absorbance of the supernatant was measured at 520 nm in a glass cuvette and compared with the L-proline standard.

2.4.3. Rhizosphere Measurements

For collecting the rhizosphere soil samples, plant roots with the adhered soils were transferred to the laboratory in a cooling box after terminating the plants. Soil samples from the surface of the roots were collected carefully according to the method of Tong et al. [65]. The remained plant particles were picked up with forceps. Samples were stored at −20 °C until the analysis. The β-glucosidase enzyme activity was calculated by measuring the p-nitrophenol concentration released from p-nitrophenyl-β-D-glucoside spectrophotometrically at 405 nm [66]. Since the pH of the used planting medium was around pH 6, the alkaline phosphatase activity was measured, because this type is not secreted by plant roots [67], and, therefore, it is suitable for studying the microbial processes in the rhizosphere. Alkaline phosphatase activity was measured by the method of Tabatabai and Bremner [68], where p-nitrophenyl phosphate was used as a substrate. The released p-nitrophenol was measured spectrophotometrically at 405 nm. The p-nitrophenyl-N-acetyl-β-D-glucosaminide was the substrate for the measurement of β-glucosaminidase activity. The released p-nitrophenol was measured photometrically at a wavelength of 405 nm [69].

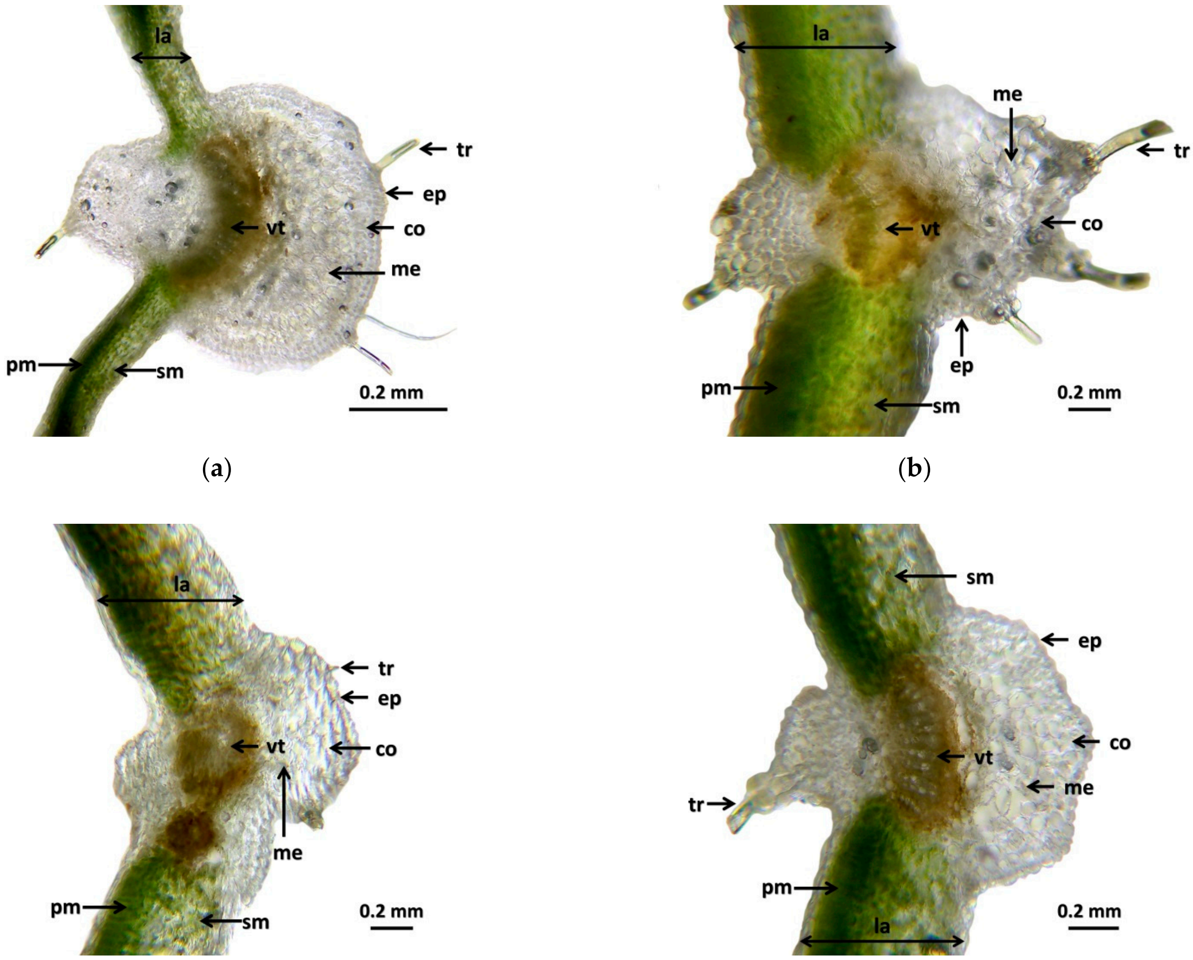
2.5. Statistical Evaluation

Data were organised into a suitable format for statistical evaluation using the Excel (Office 2021 Professional Plus) data management software package and then analysed by a one-way analysis of variance (ANOVA), followed by Tukey's test at a 95% significance level, using the IBM SPSS Statistics 29.0.1.0. software package.

3. Results

3.1. Histology Analysis of Investigated Plants

The aggregated results of the microscopic images show that the effect of biostimulants and growth promoters is also reflected in the leaf tissue (Figure 2). In the control group, the transport tissue system is uniform, the cells of the intestinal tissue show a uniform pattern, and the intestinal tissue and epidermis cells are uniform (Figure 2a). The leaf plate is uniform but thin, and the cells are ordered. In comparison, in the leaves of individuals treated with the Kelpak® biostimulant, the transport tissue system is undeveloped, with a diameter smaller than the thickness of the leaf plate, which also indicates an inefficient functioning of the biostimulant (Figure 2b). The epidermis cells of the leaf plate are uniform. In several cases of plants treated with the growth promoter Yeald Plus, an amorphous transport tissue system was observed, which was paralleled by, and resulted in, a less developed leaf blade. The spongy and columnar parenchyma did not show a uniform pattern (Figure 2c). The most developed and strongest leaves were observed in plants treated with Bistep. Here, the transport tissue system is not as long and uniform as in the control group, but it is well developed, with the ligule and wood elements separated from each other. The leaf plate is thick, and the epidermis is uniform. The leaf plate tissues are well developed and distinct (Figure 2d). No detectable and uniform results for trichomes were found between the groups studied. Histological measurements testify that treatment with the Bistep biostimulant in *W. florida* 'Eva Rathke' greatly improves and enhances the physical characteristics, thus allowing the plant to withstand environmental stresses to a greater extent, which is also highlighted by the increasing urbanisation effects.



(c) (d)

Figure 2. Microscopic images of leaf tissue and transport rays in *W. florida* 'Eva Rathke' as a result of treatments. (**a**) Control; (**b**) Kelpak; (**c**) Yeald Plus; (**d**) Bistep. The abbreviations shown in the pictures mean the following: ep—epidermis; co—collenchyma; vt—vascular tissue; la—lamina; me—mesophyll; ph—phloem; tr—trichome; sm—spongy mesophyll; pm—palisade mesophyll.

3.2. Physiology

3.2.1. Transpiration and Evapotranspiration Assessment

Differences in transpiration and evapotranspiration values were indicative of plant stress levels, as higher evapotranspiration under the same conditions may suggest higher stress levels [70]. Examining these results (Figure 3), lower evapotranspiration values were measured for all treatments applied compared to the evapotranspiration results of the control group (88 g), indicating more a favourable water balance. Among the values, the Bistep biostimulant had the lowest evapotranspiration value (37 g). The transpiration results showed similar results. The values measured for the control group were the highest (81.7 g). In comparison, the values of Kelpak® (61.1 g) and Yeald Plus (53.2 g) were statistically lower, while Bistep (35.6 g) had the lowest transpiration value. Based on the results of transpiration values per leaf, the highest values were measured for the control group (1.25 g), while the lowest values were measured for the biostimulant Bistep (0.54 g).

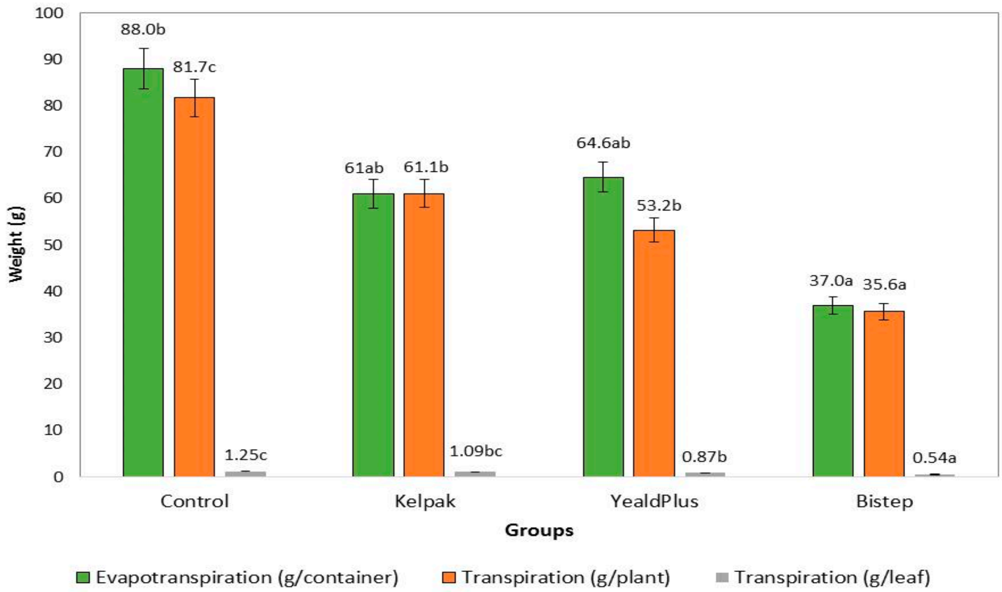


Figure 3. Evapotranspiration and transpiration results in *W. florida* 'Eva Rathke' cultivars under the influence of biostimulators and growth promoters. To compare differences, one-way ANOVA with Tukey's test was performed ($p < 0.05$). Different letters indicate different statistical groups.

3.2.2. Proline Content Analysis

The theoretical background for measuring proline levels is that it is an amino acid that positively influences the response of plants to stress. It also functions as an osmolyte, with three other main roles in stress mitigation, such as a metal chelator, antioxidant defence molecule, and signal transduction molecule. The literature suggests that environmental stress is associated with elevated proline levels in plants, which helps to increase tolerance by maintaining the osmotic balance and stabilising membranes to prevent electrolyte leakage. Through these processes, it helps to keep the concentration of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative bursts in plants [71]. Proline levels can increase significantly under salt stress [72]. The results of the polynucleic acid measurement are summarized in Figure 4, showing that the Kelpak® biostimulant-treated individuals had the highest stress level (33.3 $mg \cdot g^{-1}$), and the control group (26.7 $mg \cdot g^{-1}$) and Yeald Plus (13.3 $mg \cdot g^{-1}$) did not differ significantly from this value. The lowest proline level was observed in the Bistep biostimulant (5 $mg \cdot g^{-1}$), which also indicates the best stress defence capabilities of the biostimulant.

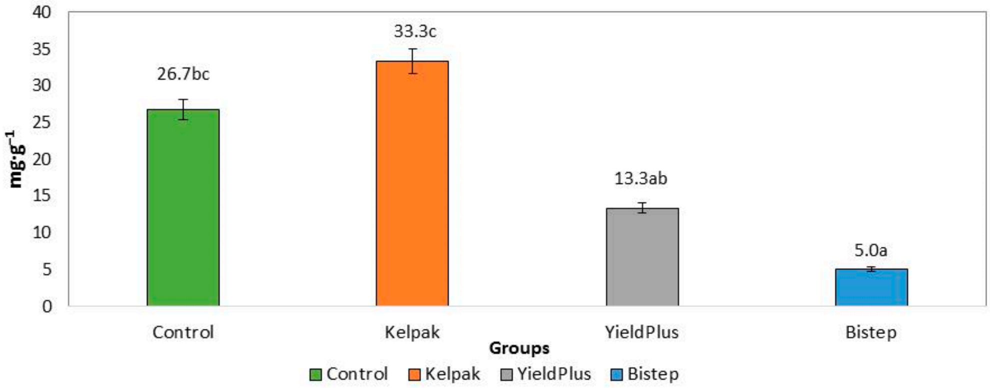


Figure 4. Proline level in *W. florida* 'Eva Rathke' cultivars under the influence of growth promoters and biostimulators. To compare differences, one-way ANOVA with Tukey's test was performed ($p < 0.05$). Different letters indicate different statistical groups.

3.2.3. Rhizosphere Measurements

An increase in enzyme activity in the rhizosphere of the plant species under study was hypothesized, as the enzymes alkaline phosphatase, β-glucosidase, and β-glucosaminidase help plants to take up macroelements, but significant differences were found between the effect of biostimulants and enzyme activity. For the alkaline phosphatase activity produced only by microbes, a significant increase in treatments was observed compared to the control. The applied biostimulants modified the activity of rhizosphere enzymes, including a statistically verifiable increase in the activity of the enzyme alkaline phosphatase compared to the control group for all treatments, while the activity of β-glucosidase and β-glucosaminidase decreased, as summarized in Figure 5.

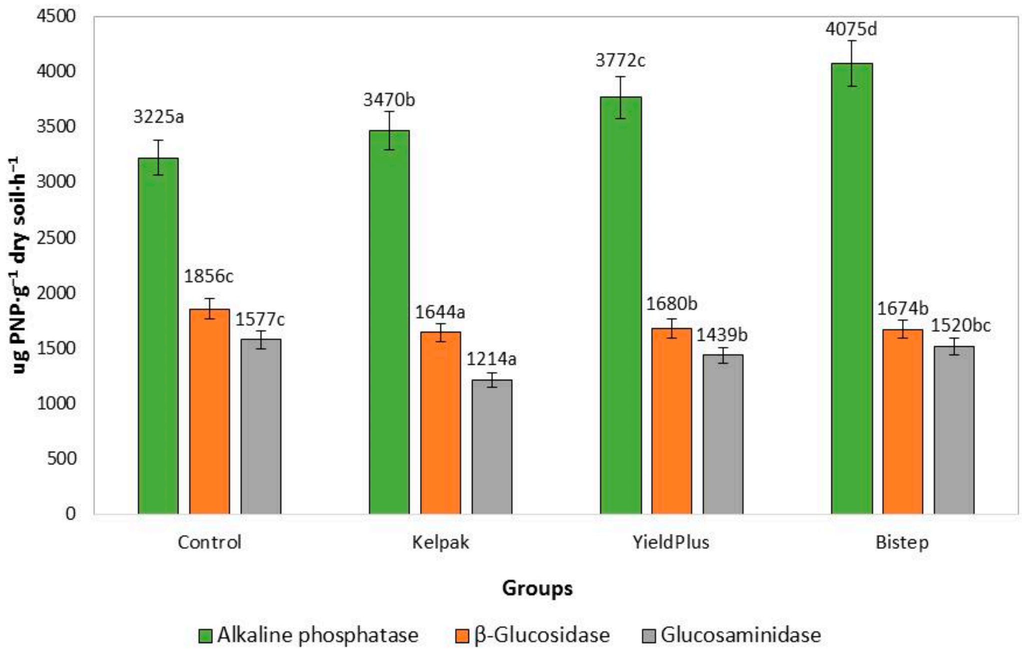


Figure 5. Activity of some enzymes of the rhizosphere during the treatment of *W. florida* 'Eva Rathke' with biostimulators. One-way ANOVA was used to evaluate the treatment effects. Different letters indicate different statistical groups of means according to the results of Tukey's test ($p < 0.05$).

Alkaline phosphatase enzyme: The control group (3225 μg PNP·g^{-1} dry soil·h^{-1}) resulted in the lowest enzyme activity, and the results were significantly different between all treatments. The highest alkaline phosphatase enzyme levels were obtained by the group treated with the Bistep biostimulant (4075 μg PNP·g^{-1} dry soil·h^{-1}).

β-glucosidase enzyme: In the rhyzosphere soil of *W. florida* 'Eva Rathke', the β-glucosidase enzyme was detected in the control group in the highest amount (1856 µg PNP·g^{-1} dry soil·h^{-1}), while the enzyme activity results were significantly lower in all other treated groups. The lowest activity was measured in the Kelpak® treatment (1644 µg PNP·g^{-1} dry soil·h^{-1}).

β-glucosaminidase enzyme: Similar to *β*-glucosidase, the activity of the *β*-glucosaminidase enzyme was the highest (1577 µg PNP·g^{-1} dry soil·h^{-1}) in the control treatment. The results of the group treated with the Bistep biostimulant (1520 µg PNP·g^{-1} dry soil·h^{-1}) did not differ from this statistically, but the result of the Yeald Plus promoter treatment was significantly different (1439 µg PNP·g^{-1} dry soil·h^{-1}). The lowest level of the *β*-glucosaminidase enzyme was found in the Kelpak® biostimulant-treated group.

4. Discussion

W. florida 'Eva Rathke' has been an important urban planting species for many years due to its aesthetic appeal, although it is also a species that may be vulnerable to the changing climatic conditions. This has led to a strong interest in exploring the potential impact of biostimulants and growth regulators on plant development. The sustainable cultivation of established ornamental plant cultivars is dependent on adapting to current challenges, such as climate change, as mentioned above, whilst also taking into account the increasing urbanisation. The use of biostimulants and growth regulators could be a new way to improve and support urban tolerance in ornamental plants. Consequently, there is a need for a greater understanding and appreciation of their possible use in combination with their practical benefits. This is needed because the urban environment, as described by Guerra et al. [1], is a highly stressed environment in itself.

When used in urban and urbanised environments, Bistep biostimulant treatment can greatly improve the resistance and physical properties of the variety (stronger, thicker leaves), which is also an outstanding ornamental feature. These results are similar to results measured with other shrub species [17–20]. The results of Werner and Lefi [10,11] showed that a high phytohormonal activity was observed for the Kelpak® algal product. The results with *Photinia* 'Red Robin', commonly used in urban environments, are similar to those with *W. florida* 'Eva Rathke', where [17] found no suitability for Kelpak® in ornamental nursery containers. The results are similar to those obtained with *Hydrangea paniculata*, also an ornamental shrub [20].

4.1. Histology

Based on the results of the tests carried out, it was concluded that the growth promoters used played a role in modifying the parameters studied, a hypothesis analogous to the research hypothesis that the key to sustainable crop production in the city is the strengthening of plants [2]. The histological results show that the leaf architecture of individuals treated with the Bistep biostimulant was more developed, and cell walls in the epidermis, leaf tissue, and vasculature were more structured and much stronger than those of Kelpak®, the control, and Yeald Plus, as also shown by Guo et al. [25] for growth promoters—increased stronger cells and tissues may indicate enhanced photosynthesis. These results are closely correlated with the results of humic and fulvic acid applications [21,22], showing that humic and fulvic acids contribute to the increase in physico-histological and physiological parameters. The use of Yeald Plus with a high zinc content may also contribute to the growth of *W. florida* 'Eva Rathke', as the results presented by Ramzan et al. [41] are similar to these measurements, which determined the changes induced by applied zinc from different plant parts, in our case, roots.

Improved physical characteristics (histological results) may indicate higher quality physiological functions, as shown by the results of ornamental grasses [42], showing that zinc application increased the resistance to stress effects and had beneficial effects on physical parameters. This was also found to be the case for *W. florida* 'Eva Rathke' by the histological parameters examined.

4.2. Transpiration and Evapotranspiration

The evapo- and transpiration results show that the plants were more equilibrated as a result of the treatment, the evaporation rate was slowed down, and the physiological parameters became more equilibrated, which was confirmed by the stress level measurements. The findings of Rajametov et al. [28] are in agreement with our results in terms of measured respiration variables—the evapotranspiration and transpiration and evaporation per leaf were highest in the control group plants, while the lowest values were found in the Bistep biostimulant-treated groups. The decrease in the transpiration rate compared to the results of Surendar et al. [36] suggests that at lower transpiration values, stress cell resistance is higher.

The extent of evapotranspiration and transpiration may be prominently influenced by stress [34], which correlates with our results that lower levels of proline indicate lower stress, as indicated by the lower evapotranspiration and transpiration results. Additional beneficial effects of Kelpak® and Yeald Plus were observed in the tested individuals, but often not pronounced, and no significant difference was clearly detectable (transpiration and evapotranspiration results), which contradicts the results of Szabó et al. [18].

4.3. Proline Enzyme

Proline regulates cellular responses by preventing water loss through osmotic regulation, which, in addition to higher proline levels, may explain the higher transpiration values of the culture strain studied. It was hypothesized that an increase in proline levels indicates a higher stress level, following the results of Bohnert and Jensen [38], which could be explained by the beneficial effect of biostimulants on stress responses.

Measurements of proline, however, showed a contradiction, which also contradicts the measurements of [39], who observed a prominent effect of *E. maxima* and increased proline levels. Lower proline levels were detected by *W. florida* 'Eva Rathke', confirming the claim of Ozden [27] et al. for grapevines. In *W. florida* 'Eva Rathke', a negative effect was observed in both the control and treated plants. A negative effect was also observed for the proline measurement, where Kelpak® had the highest proline levels. Yeald Plus and Bistep, on the other hand, showed an outstanding reduction in proline levels, clearly demonstrating the beneficial effects of the products against biotic and abiotic stress effects.

4.4. Microbilogical Activity

These results are also consistent with the results of the rhizosphere measurements. All biostimulants and growth promoters used contained micronutrients and/or organic matter and nitrogen but no phosphorus (P), so the increased P requirement of plants in the rhizosphere caused an increased alkaline phosphatase activity, which is consistent with the results of Li et al. [73]. The addition of nutrients (e.g., Ni nitrogen) to the soil with biostimulants can reduce β-glucosidase and β-glucosaminidase activity [74], as observed in the Kelpak and Yeald Plus treatments. However, the main effects of humic substances, which have been documented for Bistep, are a reduction in stress levels or tissue strengthening [75]. These effects of Bistep may be enhanced by an increased enzyme activity in the rhizosphere. The tested alkaline phosphatase, β-glucosidase, and β-glucosaminidase enzymes responded differently, although the alkaline phosphatase enzyme levels were significantly higher than the control group in all treatments.

These results may justify the answer to the target question, which is the ability of the applied growth promoters to withstand and tolerate changing weather conditions in the face of urbanisation. The answer is no for Kelpak®, despite the positive effects observed for some parameters, but the proline growth rate shows that their use is not recommended for this variety. This is in line with the results of Loconsole et al. [17] for *Phoetinia fraseri* 'Red Robin' and De Clerq [20] for *Hydrangea paniculata* but is in contrast to the results of Szabó et al. [76] for *Prunus* 'Marianna'. However, the plant growth enhancers Yeald Plus and Bistep are suitable for use in *W. florida* 'Eva Rathke', which may make the cultivar more suitable to withstand urban conditions by beneficially altering the physiological effects and making the tissue structure more resistant, thus making cultivation easier and more cost-effective.

5. Conclusions

Sustainable ornamental horticulture faces a number of challenges as urbanisation takes off. The use of commercially available biostimulants and growth promoters has had a significant impact on the development of the popular ornamental plant *W. florida* 'Eva Rathke'. Among the preparations, Bistep and Yeald Plus have been shown to improve leaf tissue structures, positively influence evapotranspiration and transpiration rates, and reduce abiotic stress effects. Nevertheless, negative effects such as the adverse effects of Kelpak® have also been observed in this study. In summary, the best biostimulant and growth promoter for *W. florida* 'Eva Rathke' was the Bistep biostimulant containing humic and fulvic acids, which showed statistically higher values of physiological parameters than the control, Kelpak®, and Yeald Plus treatments. The use of Bistep can improve the efficiency of growing *W. florida* 'Eva Rathke'.

The use of Bistep and Yeald Plus and their incorporation into cultivation technology can provide long-term benefits for treated *W. florida* 'Eva Rathke' plants, which can respond to environmental challenges with a higher degree of tolerance and contribute to sustainable horticultural development, also contributing to the development of urbanized environments. The novelty of this research lies in the fact that biostimulators and growth promoters have only been used to a lesser extent in ornamental shrubs, thus presenting a novel way to demonstrate the sustainable development of ornamental horticulture in the context of urbanisation. Future research may reveal the long-term effects of Bistep and Yeald Plus on the growth, flowering, and overall performance of the variety under field conditions.

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