

Article

Biostimulant-Induced Physiological Changes in *Forsythia* × *intermedia* ‘Beatrix Farrand’

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Abstract

In container-grown ornamental shrub production, limited substrate volume can rapidly alter plant water status and affect leaf-level physiological performance. A randomized block experiment was conducted to evaluate the effects of three foliar-applied biostimulants, BiStep, Kelpak, and Yeald Plus, compared with an untreated control in container-grown *Forsythia* × *intermedia* ‘Beatrix Farrand’. Each treatment comprised 25 plants distributed across five blocks. Stomatal density, stomatal length, leaf transpiration, net photosynthesis, total chlorophyll content, and gravimetrically determined daily water loss were measured. Compared with the control, BiStep significantly increased stomatal density, whereas Yeald Plus significantly decreased it. Kelpak and Yeald Plus significantly increased stomatal length compared with the control. Leaf transpiration was significantly lower under Kelpak and Yeald Plus than in the control, while Kelpak and BiStep had significantly higher net photosynthetic rates than Yeald Plus, whereas the control did not differ significantly from any treatment. Total chlorophyll content did not differ significantly among treatments. Whole-plant daily water loss was higher in all biostimulant-treated plants than in the control, with the highest values recorded under BiStep. These results show that the tested biostimulants induced treatment-specific physiological changes rather than a uniform response. Kelpak provided the most favorable leaf-level balance by reducing instantaneous transpiration while maintaining high photosynthetic activity, whereas BiStep promoted a more water-demanding physiological profile. Based on these outcomes, Kelpak may be recommended where improved leaf-level physiological efficiency is required under container-grown nursery conditions, while BiStep should be applied only with adequate irrigation management.

Keywords: container cultivation; gas exchange; water-loss; ornamental shrubs; nursery production



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1. Introduction

Efficient regulation of water use and carbon assimilation is a central challenge in intensive container-based plant production systems, especially under increasingly variable environmental conditions [1]. In ornamental nursery production, container-grown shrubs are important components of urban green infrastructure. Container cultivation has increasingly replaced field production because of its logistical and commercial advantages [2,3]. However, container cultivation also imposes specific physiological constraints. Root-zone volume is limited, water and nutrient supply change rapidly, and substrate temperature can reach extreme values within a short time [4,5]. These factors directly influence canopy functioning, growth performance, and plant quality, all of which are key determinants of marketability in ornamental shrubs [6]. Therefore, practical strategies are needed that improve physiological stability without increasing irrigation or nutrient inputs.

Forsythia × intermedia is widely used in temperate urban green spaces because of its early spring flowering, reliable ornamental value, and tolerance of routine landscape management [7,8]. The cultivar ‘Beatrix Farrand’ is widely used in containerized pre-cultivation because it develops a vigorous, mostly upright habit, forms thicker, well-matured shoots, and, through its well-differentiated buds, has abundant, large-flowered, intensely yellow blooms [8,9]. It is also relevant in local nursery and landscape practice, including Central European production systems, because of these properties. Its vigorous shoot growth and large leaf area may also increase water demand. Under restricted root volume, leaf functioning therefore becomes decisive for plant quality and post-transplant performance [10]. Under container conditions, short-term changes in substrate water availability may rapidly affect stomatal traits, transpiration, photosynthesis, chlorophyll status, and whole-plant water loss [11]. For this reason, these traits should be evaluated together when testing treatments intended to improve the physiological performance of container-grown *Forsythia × intermedia* ‘Beatrix Farrand’.

Biostimulants are increasingly used in container cultivation because they may modify plant physiological functioning without substantially increasing water or nutrient inputs [12,13]. Their effects are most often evaluated through changes in photosynthesis, transpiration, or pigment content. However, these parameters alone do not provide a complete picture of leaf functioning [14]. Gas exchange is determined not only by instantaneous stomatal opening, but also by stomatal density, size, leaf structure, and water transport capacity. Therefore, the effect of biostimulants should be interpreted through the combined evaluation of anatomical and physiological leaf traits [15,16]. Such an integrated approach can help identify whether a treatment improves leaf function or only changes individual physiological variables.

The biostimulants used in this study were BiStep, Kelpak, and Yeald Plus, which represent product groups with different declared compositions and expected physiological effects. Kelpak is a seaweed extract-based product derived from *Ecklonia maxima*, and such formulations may affect growth and water balance through bioactive compounds involved in hormonal regulation, cell division, and stress responses [17,18]. Yeald Plus contains organic components and micronutrients, which may influence nutrient uptake and metabolic processes. BiStep is a multicomponent plant conditioner containing organic and mineral components, and such formulations may affect water retention, osmotic regulation, and cellular-level stress tolerance [19,20]. Because of these compositional differences, the tested treatments may modify leaf structure, gas exchange, and water turnover in different ways. Comparing these products under the same container-grown conditions can therefore clarify whether their physiological effects are treatment-specific.

Leaf functioning can be characterized by the combined assessment of gas exchange, pigment status, stomatal traits, and whole-plant water loss [14,21,22]. Photosynthesis

and transpiration provide direct information on gas exchange intensity and water loss. Chlorophyll content indicates the status of the photosynthetic apparatus [23,24]. Stomatal density and stomatal size characterize the potential gas exchange capacity of the leaf. Anatomical observations provide information on the structural background of internal diffusion and water transport [25]. Gravimetric mass measurement of the plant–container system allows integrated evaluation of daily water loss. This is particularly relevant under container conditions, where water balance can change rapidly [26]. This combined measurement approach allows the comparison of instantaneous leaf-level responses with integrated whole-plant water loss under container-grown conditions [27–29].

Biostimulant effects on photosynthesis, transpiration, and stress-related physiological responses have been widely investigated [14,18]. However, less attention has been given to their combined effects on stomatal traits, leaf gas exchange, chlorophyll content, and whole-plant water loss under container cultivation conditions. The aim of the present study was to determine how three foliar-applied biostimulants, BiStep, Kelpak, and Yeald Plus modify stomatal traits, leaf gas exchange, chlorophyll content, and whole-plant water loss in container-grown *Forsythia × intermedia* ‘Beatrix Farrand’. The objective was to compare whether formulations with different declared compositions induce distinct physiological response patterns under the same container-grown conditions. Based on the declared composition and expected physiological effects of the tested products, three hypotheses were formulated. First, the biostimulant treatments were expected to modify stomatal traits and gas exchange parameters compared with the untreated control. Second, the seaweed extract-based Kelpak treatment was expected to improve the balance between carbon assimilation and transpirational water loss. Third, treatments promoting higher physiological activity were expected to be associated with higher whole-plant water loss under container conditions. By evaluating stomatal traits, leaf gas exchange, chlorophyll content, and gravimetrically determined daily water loss together, this study provides an integrated physiological assessment of biostimulant responses at leaf and whole-plant scales. The practical contribution of this study lies in the combined evaluation of stomatal traits, leaf gas exchange, chlorophyll content, and whole-plant water loss in a container-grown ornamental shrub, with relevance to nursery production and urban planting.

2. Results

2.1. Plant Anatomical Indicators

2.1.1. Stomatal Density

Stomatal density differed significantly among treatments (Figure 1). Compared with the control, BiStep increased stomatal density by approximately 8.5%, whereas Kelpak and Yeald Plus decreased it by approximately 7.8% and 21.6%, respectively. Yeald Plus showed the strongest reduction in mean stomatal density compared with the control.

2.1.2. Stomatal Length

Stomatal length was significantly affected by the biostimulant treatments (Figure 2). Compared with the control, Kelpak, Yeald Plus, and BiStep increased stomatal length by approximately 19.7%, 14.9%, and 3.6%, respectively. According to the post hoc comparisons, Kelpak and Yeald Plus formed a statistically similar group and differed significantly from the control and BiStep.

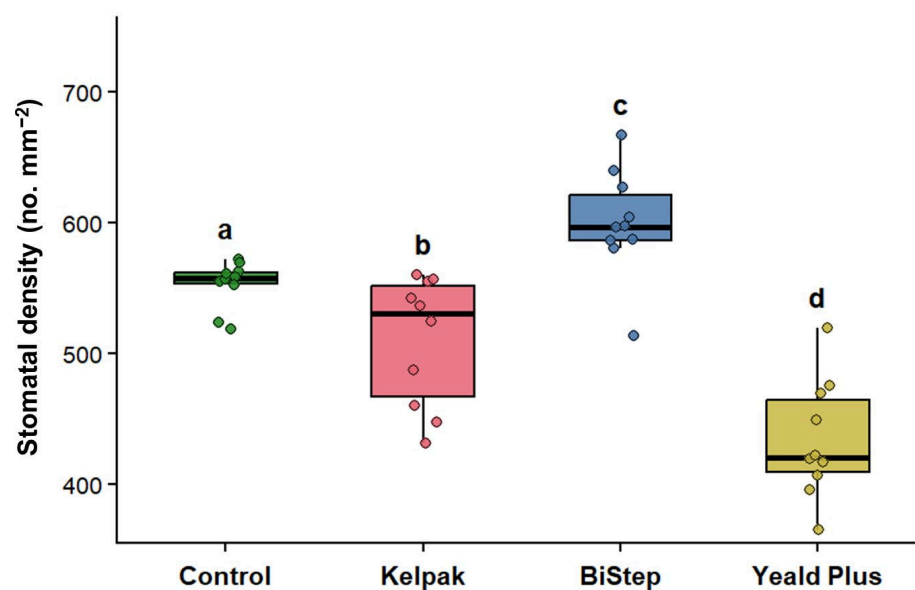


Figure 1. Stomatal density of *F. intermedia* ‘Beatrix Farrand’ leaves under different treatments. Boxplots show leaf-level biological observations ($n = 10$ leaves per treatment; 50 stomata per leaf). The central line indicates the median, boxes represent the interquartile range (25–75th percentiles), and whiskers extend to the most extreme values within 1.5 times the interquartile range. Individual points represent leaf-level means calculated from two microscopic fields of view. Different letters indicate significant differences among treatments according to the Bonferroni post hoc test ($p < 0.05$).

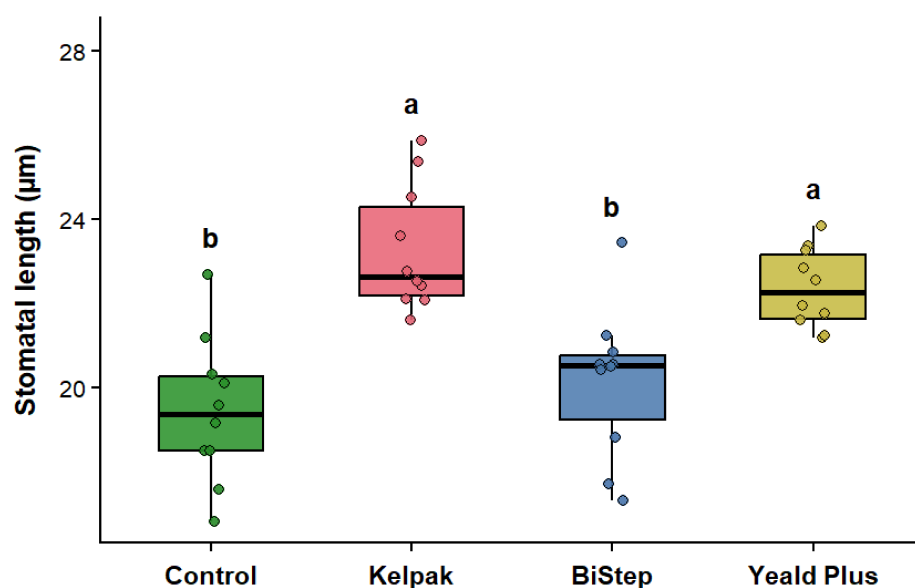


Figure 2. Effect of biostimulant treatments on stomatal length (μm) in *Forsythia* $\times$ *intermedia* ‘Beatrix Farrand’. Boxplots show stomatal measurements nested within leaves ($n = 10$ leaves per treatment). Each leaf-level mean was calculated from 25 stomatal measurements, resulting in 250 stomatal measurements per treatment. The central line indicates the median, boxes represent the interquartile range (25–75th percentiles), and whiskers extend to the most extreme values within 1.5 times the interquartile range. Individual points represent measured stomata. Different letters indicate significant differences among treatments according to the Sidak post hoc test ($p < 0.05$).

2.2. Plant Physiological Indicators

2.2.1. Transpiration Rate

The transpiration rate differed significantly among treatments (Figure 3). Compared with the control, Kelpak and Yeald Plus reduced transpiration by approximately 37.1% and 31.9%, respectively, while BiStep showed a smaller decrease of approximately 14.2%.

BiStep did not differ significantly from either the control or the lower-transpiration treatments. Kelpak showed the strongest reduction in mean transpiration rate compared with the control.

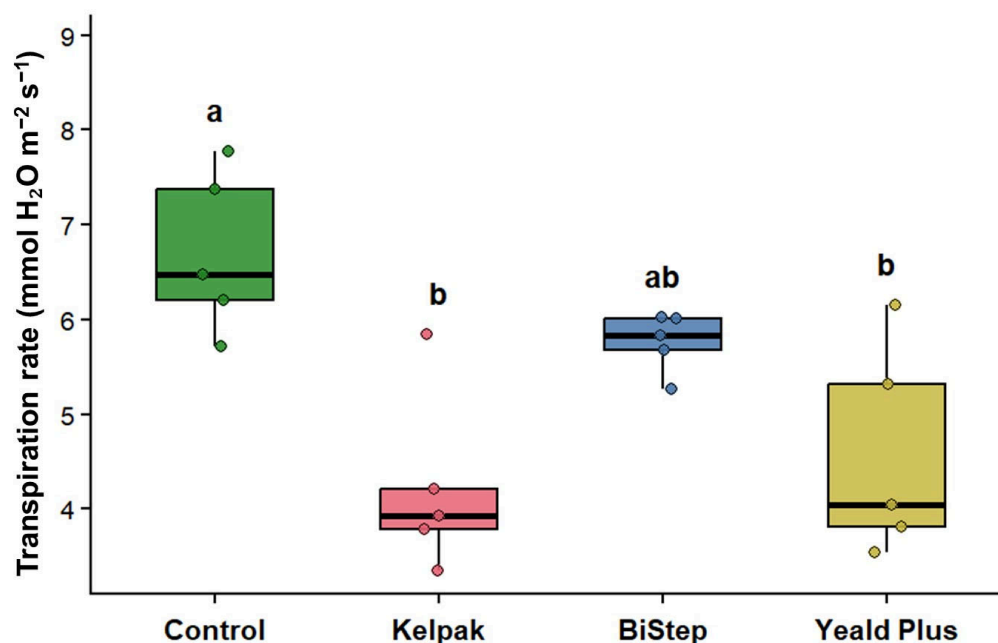


Figure 3. Effect of biostimulant treatments on transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) in *F. intermedia* 'Beatrix Farrand'. Boxplots show leaf-level gas exchange observations ($n = 5$ leaves per treatment). The central line indicates the median, boxes represent the interquartile range (25–75th percentiles), and whiskers extend to the most extreme values within 1.5 times the interquartile range. Individual points represent measured leaves. Different letters indicate significant differences among treatments according to the Bonferroni post hoc test ($p < 0.05$).

2.2.2. Diurnal Patterns of Transpiration and Evapotranspiration

Based on the gravimetric measurements, daily water loss showed clear differences among treatments (Figure 4). The control treatment consistently showed the lowest mean daily water loss at all measurement times. In contrast, all three biostimulant treatments were associated with higher whole-plant daily water loss.

BiStep generally showed the highest mean daily water loss, reaching approximately 1.9–2.1 times the control level depending on the measurement time. Kelpak and Yeald Plus also increased mean daily water loss compared with the control, but their mean responses were generally intermediate between the control and BiStep.

The gravimetric data indicate that the biostimulant treatments increased integrated whole-plant water turnover, even when leaf-level transpiration was reduced in some treatments.

2.2.3. Net Photosynthesis

The net photosynthetic rate differed among treatments (Figure 5). Compared with the control, Kelpak and BiStep increased mean net photosynthetic rate by approximately 67.0% and 50.3%, respectively, whereas Yeald Plus reduced it by approximately 48.2%. According to the Bonferroni post hoc test, Kelpak and BiStep differed significantly from Yeald Plus, whereas the control treatment did not differ significantly from the other treatments.

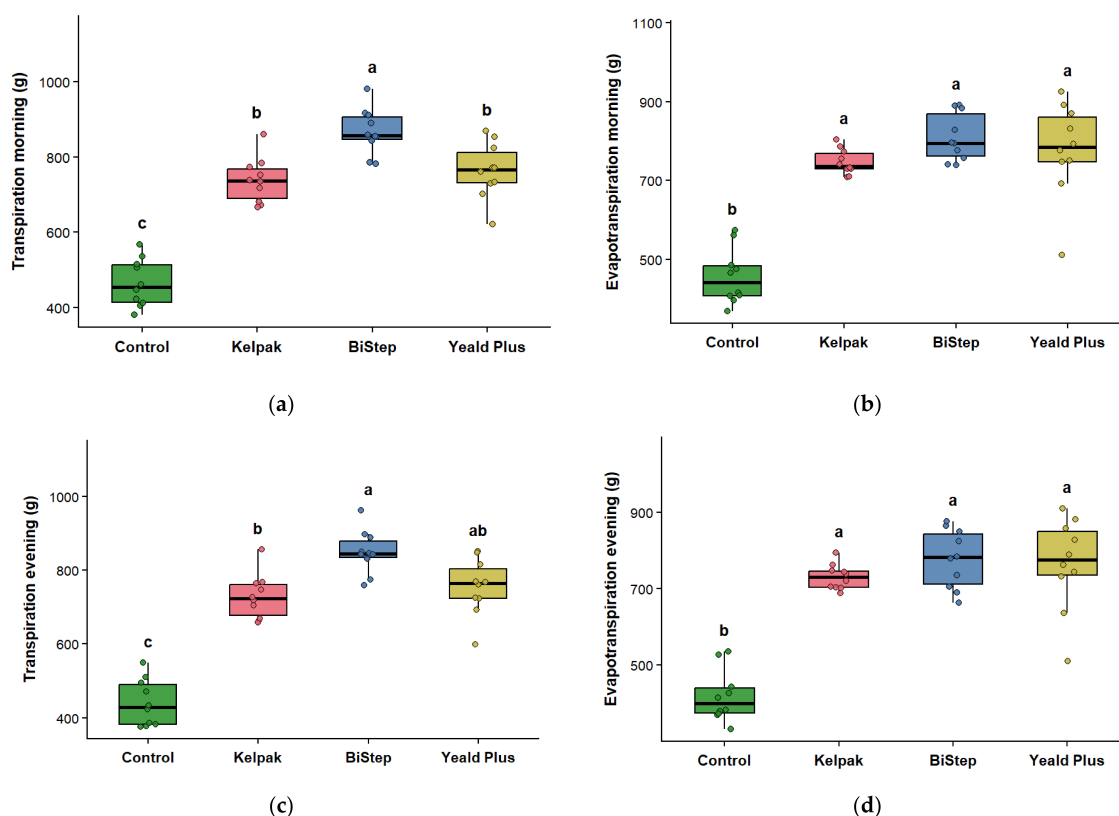


Figure 4. Gravimetrically determined daily water loss in *F. intermedia* ‘Beatrix Farrand’ under different biostimulant treatments at four measurement times: (a) transpiration morning, (b) transpiration evening, (c) evapotranspiration morning, and (d) evapotranspiration evening. Boxplots show plant-level biological observations ($n = 10$ plant-container systems per treatment for each measurement occasion). The central horizontal line represents the median, the boxes indicate the interquartile range (25–75th percentiles), and the whiskers extend to the most extreme observations within 1.5 times the interquartile range. Individual points represent plant-container systems. Different letters indicate significant differences among treatments within each measurement time according to the Bonferroni post hoc test ($p < 0.05$).

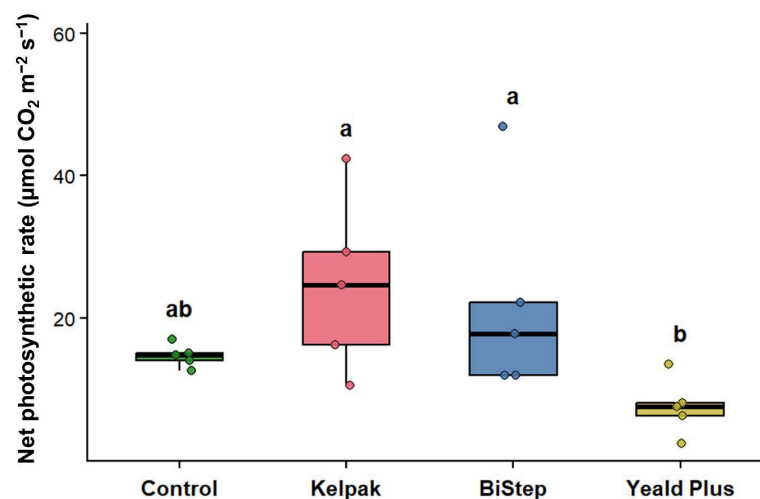


Figure 5. Effect of biostimulant treatments on net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in *F. intermedia* ‘Beatrix Farrand’. Boxplots show leaf-level biological observations ($n = 5$ measured leaves per treatment). The central horizontal line represents the median, the boxes indicate the interquartile range (25–75th percentiles), and the whiskers extend to the most extreme observations within 1.5 times the interquartile range. Individual points represent measured leaves. Different letters indicate significant differences among treatments according to the Bonferroni post hoc test ($p < 0.05$).

2.2.4. Chlorophyll Content

Total chlorophyll content showed moderate numerical variation among treatments, although no statistically significant differences were detected ($p > 0.05$; Figure 6). Compared with the control, BiStep showed a slight numerical increase of approximately 2.3% in mean total chlorophyll content, whereas Kelpak and Yeald Plus showed numerical decreases of approximately 11.0% and 12.8%, respectively. Despite these trends, all treatments belonged to the same statistical group, indicating that the biostimulant applications did not significantly affect total chlorophyll content in *F. intermedia* 'Beatrix Farrand'.

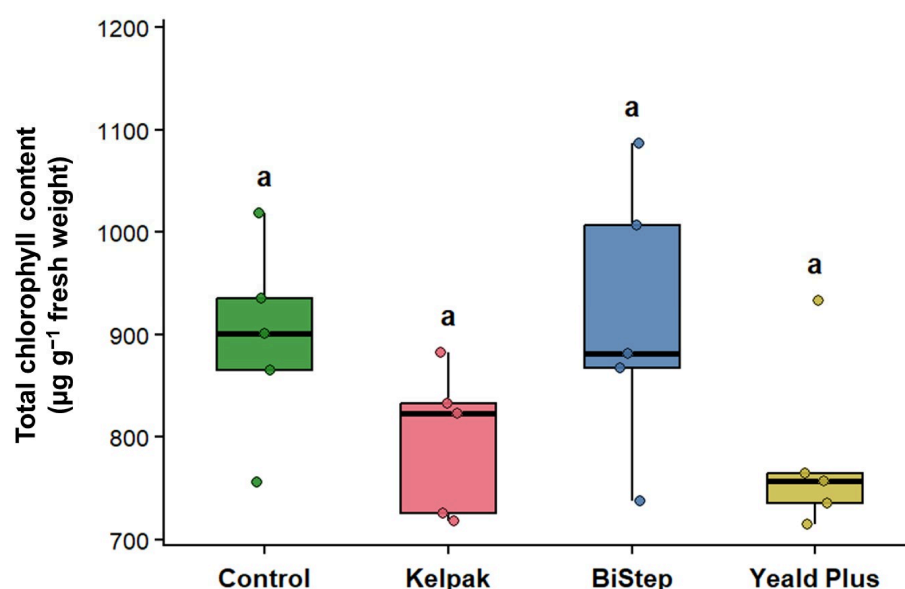


Figure 6. Effect of biostimulant treatments on total chlorophyll content ($\mu\text{g g}^{-1}$ fresh weight) in *F. intermedia* 'Beatrix Farrand'. Leaf-level biological observations ($n = 5$ leaves per treatment). The central horizontal line represents the median, the boxes indicate the interquartile range (25–75th percentiles), and the whiskers extend to the most extreme observations within 1.5 times the interquartile range. Individual points represent measured leaves. Identical letters indicate that no significant differences were detected among treatments ($p > 0.05$).

3. Discussion

3.1. Treatment-Specific Physiological Responses Under Container-Grown Conditions

The present study showed that the three foliar-applied biostimulants induced distinct physiological responses in container-grown *Forsythia* $\times$ *intermedia* 'Beatrix Farrand'. These responses were not uniform across stomatal traits, gas exchange, chlorophyll content, and whole-plant water loss (Figures 1–6). This suggests that, under container-grown nursery conditions, biostimulant effects should not be interpreted on the basis of a single physiological variable. Majsztrik and Owen [1] emphasized that container-grown nursery plants are strongly affected by irrigation and substrate-related constraints, while Tardieu [11] described that water availability directly influences plant water demand, stomatal regulation, and production potential.

Kelpak produced the most favorable leaf-level physiological response. Compared with the control, Kelpak increased net photosynthesis while reducing instantaneous transpiration (Figures 3 and 5). This pattern suggests an improved balance between carbon assimilation and transpirational water loss at the leaf level. Calvo et al. [13] described plant biostimulants as materials that can improve plant growth and physiological functioning through mechanisms beyond direct nutrient supply. Ali et al. [17] reported that seaweed extracts may enhance photosynthesis, stress tolerance, and growth through bioactive com-

pounds, while El Boukhari et al. [18] highlighted that seaweed-based biostimulants can influence hormonal regulation, nutrient uptake, and stress-related plant responses. Our findings are in line with these reports, because Kelpak, as an *Ecklonia maxima*-based seaweed extract, improved photosynthetic performance without increasing instantaneous transpiration.

However, the Kelpak response must be interpreted carefully. Although instantaneous leaf transpiration decreased, gravimetrically determined daily water loss was higher than in the control (Figures 3 and 4). Pieters et al. [26] emphasized that whole-plant transpiration measurements integrate the response of the entire plant system over time, while leaf gas exchange reflects a more instantaneous leaf-scale process. De Clercq et al. [30] also showed in *Hydrangea paniculata* that seaweed-based biostimulant responses depended strongly on drought regime and cultivation conditions. Therefore, Kelpak should not be described as reducing total water consumption.

BiStep showed a different response pattern. It resulted in increased stomatal density and high net photosynthetic activity, but it was also associated with the highest daily water loss (Figures 1, 4 and 5). This pattern indicates a more intensive and water-demanding physiological profile under the present experimental conditions. Berlyn and Sivaramakrishnan [19] reported that organic biostimulants may increase plant growth and stress resistance, while Sani and Yong [20] discussed that multicomponent biostimulatory processes can enhance plant vigor and resilience through combined physiological effects. Yaseen and Takacs-Hajos [31] also found that biostimulants can modify nutrient accumulation and ion balance in lettuce. However, this higher activity was coupled with higher whole-plant water loss, indicating that BiStep cannot be interpreted as a water-saving treatment under the present container-grown conditions.

Yeald Plus showed the least favorable physiological profile in this experiment. Although it reduced stomatal density and instantaneous transpiration, it was also associated with a lower mean net photosynthetic rate (Figures 1, 3 and 5). This suggests that lower transpiration was not accompanied by improved carbon assimilation in this treatment. Stadnik et al. [32] reported that foliar microelement application can affect chlorophyll content, canopy traits, and physiological parameters, but the response depends on the plant system and treatment conditions. Similarly, Yaseen and Takacs-Hajos [31] showed that biostimulant effects on nutrient-related traits are cultivar- and context-dependent. In the present study, Yeald Plus, as a formulation containing organic components and micronutrients, did not induce a balanced physiological response.

Taken together, the different physiological patterns observed among the three products may be related to their declared compositional differences. The response to Kelpak is consistent with previous reports on seaweed extract-based biostimulants. Ali et al. [17] reported that seaweed extracts may enhance photosynthesis, stress tolerance, and plant growth through bioactive compounds, while El Boukhari et al. [18] emphasized their role in hormonal regulation, nutrient uptake, and stress-related physiological responses. De Clercq et al. [30] also showed that seaweed-based biostimulant responses in ornamental plants depend strongly on drought regime and cultivation conditions. These reports may help explain why Kelpak improved net photosynthesis while reducing instantaneous transpiration in the present study (Figures 3 and 5). In contrast, BiStep, as a multicomponent formulation containing organic and mineral components, may have promoted a more intensive physiological status, expressed as increased stomatal density, high photosynthetic activity, and higher whole-plant water loss (Figures 1, 4 and 5). Similar stimulatory effects of organic and multicomponent biostimulants on plant growth, vigor, and stress-related physiological performance have been reported by Berlyn and Sivaramakrishnan [19], Sani and Yong [20], and Rathor et al. [33]. Yeald Plus, which contains organic components and

micronutrients, showed a different response pattern: reduced stomatal density and transpiration were not accompanied by improved photosynthetic activity (Figures 1, 3 and 5). This is consistent with Yaseen and Takacs-Hajos [31], who showed that biostimulant effects on nutrient-related traits can be cultivar- and context-dependent, and with Stadnik et al. [32], who reported that foliar microelement responses depend on the plant system and treatment conditions. These treatment-specific responses suggest that, under container-grown ornamental shrub conditions, seaweed extract-based physiological regulation may be more favorable for improving leaf-level carbon–water balance than treatments primarily associated with generalized stimulation or micronutrient supplementation. However, because the full quantitative composition of the commercial products was not available, these compositional interpretations should be regarded as cautious physiological explanations rather than direct mechanistic evidence.

3.2. Relationship Between Stomatal Traits, Gas Exchange, and Chlorophyll Content

The results also show that stomatal traits alone were not sufficient to predict gas exchange performance. Harrison et al. [15] emphasized that stomatal morphology and distribution influence photosynthetic gas exchange, but they do not determine actual gas exchange independently of physiological regulation. Zhao et al. [16] also reported that stomatal density and gas exchange responses are strongly affected by soil water deficit. In agreement with these studies, the present results show that stomatal density and stomatal length describe only the structural potential for gas exchange. Realized photosynthesis and transpiration may also depend on stomatal aperture, internal CO₂ diffusion, leaf water status, irradiance, temperature, and vapor pressure deficit.

This separation was clear in the comparison between Kelpak and BiStep. Kelpak had lower stomatal density than the control, but it showed increased net photosynthetic rate and reduced instantaneous transpiration (Figures 1, 3 and 5). BiStep, in contrast, had the highest stomatal density and high net photosynthesis, but also the highest daily water loss. Haworth et al. [34] showed that stomatal traits contribute to gas exchange regulation, but their effect depends on environmental and physiological context. Urban et al. [14] also emphasized that photosynthesis under water deficit should be interpreted through several parameters rather than through gas exchange alone [35–37]. Our results follow this logic: higher stomatal density under BiStep may have increased potential gas exchange capacity, but it was also associated with higher whole-plant water turnover. In contrast, Kelpak showed that lower stomatal density can coexist with high carbon assimilation when physiological regulation is more favorable.

The absence of significant differences in total chlorophyll content is also important for interpretation. Chlorophyll concentration reflects the size of the light-harvesting pigment pool, but photosynthetic rate is controlled by additional stomatal, diffusion-related, biochemical, and environmental factors. Busch et al. [23] emphasized that gas exchange measurements are affected by several physiological and methodological variables, while Massacci et al. [24] showed that photosynthetic responses to drought cannot be explained by pigment status alone. Oivukkamäki et al. [25] also highlighted the value of integrating gas exchange with chlorophyll fluorescence to understand photosynthetic regulation under field conditions. In agreement with these studies, our results show that similar chlorophyll contents may coexist with clearly different photosynthetic rates (Figures 5 and 6).

In Kelpak-treated plants, higher net photosynthesis was not associated with higher chlorophyll content (Figures 5 and 6), suggesting more efficient functional use of the existing photosynthetic apparatus rather than increased pigment accumulation. In BiStep-treated plants, high photosynthesis occurred together with numerically higher chlorophyll content and high daily water loss (Figures 4–6), indicating a more intensive physiological state. In

Yeald Plus-treated plants, low photosynthesis together with numerically lower chlorophyll content suggests weaker physiological performance (Figures 5 and 6). However, because chlorophyll differences were not statistically significant, the decline in photosynthesis cannot be attributed solely to pigment limitation. This interpretation is consistent with Urban et al. [14] and Busch et al. [23], who emphasized that photosynthesis must be evaluated together with stomatal and environmental controls.

3.3. Practical Implications, Novelty, and Limitations

From a practical perspective, the results indicate that biostimulant effects in container-grown ornamental shrubs should be evaluated at both leaf and whole-plant levels. Loconsole et al. [12] reported that biostimulant application can improve the quality and sustainability of ornamental containerized transplants under reduced nutrient supply. De Clercq et al. [30] showed that seaweed-based biostimulant effects in ornamental hydrangea depended on whether plants experienced continuous or periodic drought stress.

Kelpak appears to be the most promising treatment when the production aim is to improve the balance between photosynthesis and instantaneous transpiration at the leaf level (Figures 3 and 5). This agrees with Ali et al. [17] and El Boukhari et al. [19], who reported that seaweed extracts can support stress-related physiological regulation and plant performance. BiStep may be useful when the goal is to promote a more active physiological state, but its association with higher daily water loss means that it should be applied with careful irrigation management (Figure 4). This interpretation is consistent with Sani and Yong [20] and Rathor et al. [33], who described multicomponent and humic-based biostimulants as capable of stimulating plant physiological activity, while also emphasizing that their effects depend on context and plant condition. Yeald Plus did not show a clear physiological advantage in this experiment, because its lower transpiration was accompanied by reduced photosynthetic activity (Figures 3 and 5).

The novelty of the present study lies in the combined interpretation of stomatal traits, leaf gas exchange, chlorophyll content, and gravimetrically determined daily water loss in a container-grown ornamental shrub. Many biostimulant studies focus on crop species or on single physiological indicators, such as photosynthesis, chlorophyll content, or growth [12–14,17,18]. In contrast, fewer studies examine how leaf-level gas exchange and whole-plant water turnover are related in ornamental shrubs intended for nursery production and urban planting. The present results show that treatment-specific physiological responses can differ according to the measurement scale. A treatment may improve leaf-level physiological efficiency without reducing whole-plant water loss. This scale-dependent interpretation is important for both nursery management and post-transplant performance in urban environments.

Whole-plant water loss is not only a water-use variable, but may also influence subsequent growth processes through effects on canopy development, root activity, nutrient uptake, and stress recovery. These downstream responses were not quantified in the present study; therefore, the observed physiological patterns should be interpreted as early functional indicators rather than as direct evidence of long-term biomass accumulation or stress resilience.

Some limitations should also be considered. Vapor pressure deficit, relative humidity, and air temperature were not continuously monitored throughout the full experimental period, although these variables strongly influence gas exchange. In addition, biomass accumulation, canopy development, leaf area, and root growth were not quantified, so the contribution of plant size to daily water loss could not be directly separated from leaf-level transpiration intensity. Future studies should therefore combine gas exchange and gravimetric water-loss measurements with direct indicators of plant water status, canopy

area, biomass production, root development, and chlorophyll fluorescence. This would help clarify how different biostimulant formulations affect the relationship between carbon assimilation, water use, and whole-plant performance during container cultivation and after transplanting into urban environments.

4. Materials and Methods

4.1. Study Site, Plant Material, and Experimental Layout

The experiment was conducted under outdoor nursery conditions at Kovács Tree Nursery, Zalaszentgyörgy, Hungary, from 15 April to 31 August 2025. The trial was established in a container yard used for ornamental shrub production. Plants were placed on a level surface covered with mechanically fixed UV-stable horticultural agrotextile. A 20 × 20 cm grid was used to maintain uniform plant spacing and row alignment within the experimental area.

One-year-old, vegetatively propagated *Forsythia* × *intermedia* ‘Beatrix Farrand’ plants were used. All plants originated from the same propagation cycle. Before treatment assignment, plants were visually inspected, and damaged, diseased, pest-affected, or poorly developed individuals were excluded. Plants were then sorted by size to reduce initial heterogeneity.

The plants were transplanted into identical 1.5 L rigid black plastic containers before the start of the experiment. The growing medium was a peat-based substrate composed of 30% ground calcareous black peat and 70% ground white peat, supplemented with perlite and granulated clay to improve aeration and water retention. The same container type, substrate, and cultivation surface were used for all treatments.

The experiment followed a randomized block design with four treatments: untreated control, Kelpak, Yeald Plus, and BiStep. Five blocks were established, and each block contained all four treatments. Each treatment was represented by five plants per block, resulting in 25 plants per treatment and 100 plants in total.

Plants were irrigated using the nursery sprinkler irrigation system. Irrigation water was well water with pH 7.1 and electrical conductivity of 0.9 mS cm^{−1}. Irrigation timing was controlled automatically, and during the summer period two irrigation events per day were typically applied. The same irrigation regime was used for all treatments.

4.2. Biostimulant Formulations and Foliar Application

Three commercially available biostimulant formulations were selected to represent different product types used in nursery practice: a seaweed extract-based formulation, a micronutrient- and organic component-containing formulation, and a multicomponent plant conditioner. Product composition was described on the basis of manufacturer and distributor information [38–40]. Because the full quantitative composition of commercial biostimulant products is partly proprietary, the formulations were characterized according to their declared active ingredient groups.

Kelpak is a seaweed extract-based biostimulant derived from *Ecklonia maxima* and contains naturally occurring compounds associated with seaweed extracts, including auxin- and cytokinin-like substances, amino acids, carbohydrates, and other organic components [38]. Yeald Plus is a formulation containing organic components and micronutrients, with zinc indicated among the declared elements [39]. BiStep is a multicomponent plant conditioner containing organic and mineral components [40].

The biostimulants were applied as foliar sprays at two-week intervals between 15 April and 31 August 2025, resulting in ten applications during the growing season. The experimental factor was the biostimulant formulation; therefore, the application method and timing were identical for all treated groups. The applied concentrations followed the

product-specific nursery application rates provided by the manufacturers and distributors [38–40]. Different concentrations were used because the products differed in formulation type, declared composition, and recommended application dose. Kelpak was applied at 0.4%, Yeald Plus at 0.3%, and BiStep at 0.5%. Control plants were sprayed with water at the same dates and using the same procedure as the treated plants. Because the exact quantitative composition of some commercial formulations is proprietary, the products were characterized according to their declared active ingredient groups rather than by complete chemical composition.

4.3. Sampling Strategy and Measured Variables

The study focused on leaf anatomical traits, leaf gas exchange, chlorophyll content, and whole-plant water loss. Fully developed, healthy, intact leaves were selected from the middle part of current-year shoots to ensure comparable developmental stage and exposure. The same sampling principle was followed for anatomical, gas exchange, and chlorophyll measurements.

The main response variables were stomatal density, stomatal length, transpiration rate, net photosynthetic rate, total chlorophyll content, and gravimetrically determined daily water loss. Plant-level measurements were interpreted at the level of individual plants. For stomatal density, individual leaves were used as biological observational units. For stomatal length, repeated stomatal measurements were treated as nested observations within leaves.

4.4. Stomatal Anatomical Measurements

Stomatal anatomical measurements were performed on the abaxial epidermis of fully developed leaves. Ten leaves were sampled per treatment, resulting in 40 leaves in total. Epidermal impressions were prepared using clear nail polish. After drying, the impression layer was removed with transparent adhesive tape and mounted on microscope slides. Samples were examined without staining.

Stomatal density was determined from two microscopic fields of view per leaf. The mean of the two fields was used as the leaf-level value. A consistent counting rule was applied: stomata touching the left and upper borders of the field were counted, whereas stomata touching the right and lower borders were excluded. The field of view area was 1.449 mm^2 , and stomatal density was expressed as number of stomata per mm^2 .

Stomatal length was measured using Fiji (ImageJ, version 1.54t; latest available version at the time of analysis). Ten images were recorded per leaf under standardized imaging settings. Five stomata were measured on each image, giving 50 stomatal measurements per leaf, 500 per treatment, and 2000 in total. Stomatal length was measured along the long axis of the guard cell pair. The image scale was calibrated using a calibration slide, with 10 divisions corresponding to 1.0 mm.

Microscopic images were captured using a Euromex bScope BS.1153-PLi biological microscope equipped with a Euromex CMEX-5f 5 Mp camera (Euromex Microscopen BV, Duiven, The Netherlands). A PLi 4/0.1 objective and WF12 20×/20 eyepiece were used. Image correction was performed using GIMP 2.10.34.

4.5. Gravimetric Measurement of Whole-Plant Daily Water Loss

Whole-plant water loss was determined by measuring the mass change of the plant-container system with a Kern 572-45 laboratory balance (KERN & SOHN GmbH, Balingen, Germany). Before measurement, containers were irrigated to saturation and allowed to drain gravitationally to establish comparable initial water status. The upper surface of the containers was covered with a waterproof vapor-tight cover to reduce soil-surface

evaporation. Under this setup, mass loss was interpreted primarily as canopy-related water loss.

Measurements were carried out in the morning and evening using the same protocol. Daily water loss was calculated as the difference between the initial and final mass of the plant–container system and expressed in grams per plant. This gravimetric approach was used to compare treatment-related differences in integrated whole-plant water loss under container-grown nursery conditions.

4.6. Leaf Gas Exchange Measurements

Leaf gas exchange was measured with a portable infrared gas analysis system, LCI, LCI portable infrared gas analysis system (ADC BioScientific Ltd., Hoddesdon, Hertfordshire, UK). The main variables evaluated in the present study were net photosynthetic rate (P_n) and transpiration rate (E). Stomatal conductance, leaf-level photosynthetically active radiation, and leaf surface temperature were recorded by the instrument and used to characterize the immediate measurement conditions.

Measurements were conducted on 12 measurement days during the growing season. On each measurement day, measurements were taken at two-hour intervals. For each measurement, one fully developed, intact, well-illuminated leaf was selected per plant. Because the measurements were carried out under outdoor nursery conditions, short-term variation in irradiance and leaf temperature was treated as part of the production environment.

The gas exchange data were used to evaluate treatment-related differences in leaf-level physiological performance. Net photosynthesis and transpiration were interpreted together to assess the coordination between carbon assimilation and water loss at the leaf scale.

4.7. Plant Physiological Measurements

Total chlorophyll content was determined as the sum of chlorophyll a and chlorophyll b according to Arnon [41]. Fully developed, healthy leaves were sampled from the middle part of current-year shoots. Fresh leaf tissue of 150 mg was homogenized in a mortar cooled below 4 °C with quartz sand and a small amount of Na_2CO_3 . Pigments were extracted with 80% acetone.

The extract was adjusted to a final volume of 10 mL with 80% acetone and centrifuged at 4 °C and 1000 rpm for 10 min using an Eppendorf 5418 R centrifuge. Absorbance of the supernatant was measured with a GeneSys VIS-10 spectrophotometer at 663 and 644 nm. Total chlorophyll content was expressed on a fresh weight basis and calculated using the following equation:

$$\mu\text{g chlorophyll g}^{-1} \text{ fresh weight} = (20.2 \times A_{644} + 8.02 \times A_{663}) \times V/w$$

where A is absorbance at the given wavelength, V is the final extract volume, and w is the fresh sample weight.

4.8. Statistical Analyses

Statistical analyses were performed in R version 4.5.1 using RStudio version 2026.06.0 (Posit Software, PBC, Boston, MA, USA). Treatment was considered the main fixed factor. The experimental design, sampling structure, and level of repeated measurements were considered when selecting the statistical approach.

For variables measured once at the plant or leaf level, treatment effects were evaluated using analysis of variance. Pairwise comparisons among treatments were performed using post hoc multiple-comparison tests at $p < 0.05$. For stomatal length, a linear mixed-effects

model was applied because multiple stomata were measured within the same leaf. In this model, treatment was included as a fixed effect and leaf identity as a random factor.

For variables measured at multiple time points, treatment comparisons were interpreted within the relevant measurement occasion or summarized response variable. Because the experiment was conducted under outdoor nursery conditions, the results were interpreted as comparative treatment-related physiological response patterns within the applied nursery production environment.

Results are presented as boxplots. In the boxplots, the central line represents the median, boxes show the interquartile range, whiskers represent values within 1.5 times the interquartile range, and individual points represent measured observations. Significant differences among treatments are indicated by different letters.

5. Conclusions

The present study demonstrates that foliar-applied biostimulants can modify the physiological preconditioning of container-grown *Forsythia × intermedia* ‘Beatrix Farrand’ before landscape use. Biostimulant suitability in ornamental nursery production should therefore be judged not only by reduced transpiration or stimulated photosynthesis, but by the coordination between stomatal traits, gas exchange, pigment status, and whole-plant water turnover. Under the present conditions, Kelpak produced the most favorable leaf-level coordination, suggesting that seaweed extract-based preconditioning may help maintain carbon assimilation while moderating instantaneous water loss. BiStep induced a more intensive but water-demanding physiological state, whereas Yeald Plus did not provide a comparable functional advantage. These contrasting responses suggest that Kelpak’s seaweed extract-based composition may have contributed to the more favorable photosynthesis–transpiration balance, while the multicomponent organic and mineral composition of BiStep may have promoted broader physiological stimulation with higher whole-plant water turnover. The weaker response of Yeald Plus indicates that organic components and micronutrients alone did not induce the same coordinated leaf-level carbon–water response under the present container-grown conditions. These findings are relevant for the urban use of *F. intermedia* ‘Beatrix Farrand’, where shrubs often face restricted rooting volume, discontinuous water supply, high evaporative demand, and heat-load episodes. The results suggest that nursery-stage biostimulant treatments may act not only as growth-supporting inputs, but also as physiological conditioning tools for improving establishment potential and functional stability under climate-stressed urban conditions. Future studies should test whether these leaf-level advantages persist after transplanting and improve establishment, canopy retention, flowering performance, and recovery after repeated drought and heat events. For climate-adaptive ornamental plant production, nursery preconditioning should be integrated with cultivar-specific physiological screening and post-transplant evaluation under realistic urban stress conditions.

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