

Article

Combined Biostimulant Treatment Is Associated with PSII Energy Partitioning and Leaf Anatomy in Cucumber Leaves and Fruits

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Abstract

Plant biostimulants may alter photosynthetic energy use and leaf structure, but their effects on complementary PSII energy partitioning in both cucumber leaves and fruits remain insufficiently characterized. Using plant material from a commercial greenhouse trial previously reported for agronomic outcomes, we evaluated a combined CropBioLife[®], KeyPlex 120[®], and Key Eco Oil[®] application protocol relative to conventional management. Chlorophyll Content Index (CCI), chlorophyll fluorescence imaging, and leaf anatomical traits were assessed. Under low light (LL; 455 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the combined-treatment greenhouse showed higher ΦPSII than the conventional-control greenhouse by 22% in fruits and 50% in leaves; fruit ΦNPQ was 17% higher, whereas ΦNO was 12% lower in fruits and 26% lower in leaves. Under high light (HL; 973 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), differences in ΦPSII were smaller, while leaf ΦNO remained 17% lower. CCI was 38% higher in fruits and 52% higher in leaves. In leaves, spongy parenchyma thickness increased from 110 ± 3.4 to 135 ± 4.1 μm , palisade parenchyma thickness from 75 ± 2.1 to 78 ± 1.8 μm , total leaf thickness from 230 ± 4.6 to 245 ± 5.2 μm , and intercellular air-space area from 420 ± 18 to 610 ± 22 μm^2 . The parallel physiological and anatomical differences are consistent with a possible functional association, but gas exchange, mesophyll conductance, ROS, and water-use efficiency were not measured in the present dataset. Because the two management regimes occupied separate, non-replicated greenhouses and received different crop-protection inputs, the findings should be interpreted as treatment-associated differences rather than causal effects attributable to individual products.

Keywords: chlorophyll fluorescence imaging; cucumber; energy use; biostimulant; leaf anatomical structure; PSII photochemistry



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1. Introduction

Protected cultivation buffers crops from external weather, but greenhouse canopies still experience strong spatial and temporal variation in irradiance, temperature, humidity, and airflow. High planting density and rapid canopy development can further alter the light environment experienced by individual leaves and fruits. Understanding how photosynthetic energy use and leaf structure vary under these conditions is therefore

relevant to maintaining efficient light use and physiological stability in intensive greenhouse production [1].

Cucumber (*Cucumis sativus* L.) is an important horticultural crop that is widely grown under protected cultivation because of its rapid growth, high productivity, and economic value. Greenhouse cucumber performance is sensitive to changes in light intensity, temperature, water availability, and nutrient supply, all of which can influence photosynthetic function, canopy development, fruit growth, and final crop performance [2–6].

Plant biostimulants are bioactive products of natural, microbial, or biochemical origin that may modify plant physiological processes and structural traits beyond the direct effects of conventional fertilization [7,8]. Studies in several crops indicate that biostimulant applications can alter chlorophyll status, photosynthetic traits, and leaf morphology, including lamina thickness and mesophyll organization [9–12]. In the same greenhouse trial used here, our previous publication evaluated agronomic and physiological endpoints including leaf gas exchange, conventional chlorophyll-fluorescence indices, root and nematode responses, and fruit-quality traits [3]. Those measurements are not repeated in the present study.

Photosynthesis is central to crop growth, and the efficiency with which absorbed light is used or dissipated changes with environmental and cultivation conditions [13,14]. In greenhouse production, rapid changes in irradiance can alter excitation pressure on photosystem II (PSII), making photochemical efficiency and photoprotective energy dissipation useful indicators of short-term physiological status [13,15].

Chlorophyll-a fluorescence is a sensitive, non-destructive approach for evaluating PSII function [14,16]. Imaging-PAM analysis can resolve complementary quantum yields describing the fraction of absorbed light directed to photochemistry (Φ_{PSII}), regulated non-photochemical energy dissipation (Φ_{NPQ}), and non-regulated energy loss (Φ_{NO}), together with photochemical quenching (qP) and non-photochemical quenching (NPQ) [16–19]. These parameters describe PSII energy partitioning but do not, by themselves, directly quantify carbon assimilation, ROS concentration, or photodamage.

Photosynthesis also occurs in chlorophyll-containing non-foliar organs. Cucumber fruits can assimilate atmospheric CO₂ and re-fix internally released CO₂, so fruit photochemistry may contribute to fruit carbon economy even though its photosynthetic capacity differs from that of leaves [20–22]. Assessing PSII energy partitioning in both leaves and fruits can therefore provide a broader view of organ-specific responses within the crop.

Chlorophyll Content Index (CCI) provides a rapid, non-destructive estimate related to tissue chlorophyll content and is widely used as an indicator of pigment status and plant physiological condition [23,24]. Because chlorophyll content and fluorescence describe different components of photosynthetic function, their joint evaluation can provide complementary information, although a direct mechanistic relationship requires paired physiological measurements.

Leaf anatomy can influence light distribution and the physical pathway for CO₂ diffusion within the mesophyll. Palisade and spongy parenchyma thickness, intercellular air spaces, and stomatal distribution are therefore structural traits potentially relevant to photosynthetic function [10,25]. However, anatomical measurements alone cannot establish changes in mesophyll conductance, stomatal conductance, transpiration, or water-use efficiency; those functions require direct gas-exchange or conductance measurements.

A specific knowledge gap remained after the previous study [3]: the same cultivation protocol had not been examined for complementary PSII energy partitioning (Φ_{PSII} , Φ_{NPQ} , and Φ_{NO}) by chlorophyll fluorescence imaging across contrasting actinic light intensities in both leaves and fruits, nor for its association with CCI and detailed leaf anatomical traits. This information is useful for distinguishing photochemical use of absorbed energy

from regulated and non-regulated dissipation and for describing whether physiological differences occur in parallel with structural differences.

Accordingly, the present study tested the hypothesis that cucumber organs sampled from the combined-biostimulant greenhouse would show higher PSII photochemical use and regulated energy dissipation, lower non-regulated energy loss, higher CCI, and measurable differences in leaf mesophyll structure relative to the conventional-control greenhouse. The study reports new Imaging-PAM energy partitioning, CCI, and leaf anatomical data from the previously described trial [3]. Because treatment and greenhouse were not independently replicated, the objective is to characterize treatment-associated differences within this commercial trial rather than to establish greenhouse-independent causality or the contribution of individual products.

2. Materials and Methods

2.1. Plant Material, Greenhouse Conditions, and Biostimulant Treatments

Cucumber plants (*Cucumis sativus* L. cv. Murat) were cultivated under commercial greenhouse conditions in the Marathon region of Attika, Greece, from June to September 2024, until commercial fruit maturity, using the same trial previously described by Ouzounidou et al. [3]. Two physically separate greenhouses (250 m² each) were used. One greenhouse received the conventional-control management regime and the second received the combined-biostimulant regime. Each greenhouse contained 625 plants arranged in six rows, with 40 cm between plants and 80 cm between rows. Uniform 25-day-old plants at the four-leaf stage were transplanted in early June. Importantly, greenhouse was not replicated within management regime and the assignment was not a randomized greenhouse-level design; therefore, the treatment factor is confounded with greenhouse. Individual leaves and fruits sampled from different plants are subsamples within each greenhouse, not independent greenhouse-level treatment replicates.

Greenhouse environmental conditions were automatically controlled during the June–September cultivation period. Recorded temperature ranged from 15.0 to 34.5 °C, with an average of 27.3 °C; average relative humidity was approximately 65%, and average photosynthetic photon flux density (PPFD) at leaf level was $400 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$. The frequency above the threshold was for 20 days in which the duration of temperature above the threshold was approximately 4 h, during the experimental period. The temperature logging frequency was every 15 min. Soil physicochemical characterization from the same trial was reported previously [3]. At the beginning of the experiment, the control and biostimulant greenhouse soils had sand–clay–silt proportions of 78–14–8% and 71–19–10%, respectively; pH values of 7.03 ± 0.04 and 7.12 ± 0.05 ; electrical conductivity of 0.991 ± 0.02 and $0.996 \pm 0.03 \text{ mS cm}^{-1}$; organic matter of $1.12 \pm 0.05\%$ and $1.23 \pm 0.06\%$; CaCO₃ of $5.5 \pm 0.3\%$ and $6.2 \pm 0.4\%$; and total N of $0.30 \pm 0.02\%$ and $0.20 \pm 0.01\%$, respectively [3]. These baseline differences are an additional source of potential confounding.

Irrigation and basal fertilization were applied according to the commercial production program in both greenhouses. After transplanting, plants received N–P–K fertilizer (20–20–20) every 15 days. Other routine integrated pest-management practices were comparable, except for the nematode-management inputs that defined the two regimes [3]. The conventional-control greenhouse received abamectin at 1 mL L^{−1} together with an iron chelate preparation (EDDHA 5.5% Fe SG) at 1 g L^{−1}. The combined-biostimulant greenhouse did not receive these two conventional nematode-management inputs and instead received the three products described below [3]. Thus, “control” refers to a conventional-management comparator, not to an untreated control.

In the combined-biostimulant greenhouse, CropBioLife[®] (Aussan Laboratories Pty Ltd, Victoria, Australia), KeyPlex 120[®] (KeyPlex, Winter Park, FL, USA), and Key Eco

Oil[®] (KeyPlex, Winter Park, FL, USA) were applied through irrigation and foliar spraying. For soil application, CropBioLife[®] and KeyPlex 120[®] were each applied at 1 mL L⁻¹ and Key Eco Oil[®] at 2.5 mL L⁻¹ in tap water, with soil applications repeated every 15 days; Key Eco Oil[®] was supplied at 200 mL product per application through irrigation. Foliar sprays were applied every 7 days using 100 mL CropBioLife[®], 100 mL KeyPlex 120[®], and 250 mL Key Eco Oil[®] per 100 L water. Consequently, all physiological and anatomical comparisons in this manuscript concern the combined management protocol rather than any single product.

Manufacturer-declared composition reported for the same trial [3] was as follows. Key Eco Oil[®] contained 10% cinnamon oil, 5% thyme oil, 5% rosemary oil, and 5% sesame oil (*w/w*). KeyPlex 120[®] contained Fe (0.20%), Mn (0.11%), and Zn (0.10%) (*w/w*) and contained humic acid. CropBioLife[®] was an activated-flavonoid-based formulation with reported nutrient concentrations of N 1860 ppm, K 1370 ppm, S 482 ppm, and Na 720 ppm. The exact concentrations of the activated-flavonoid fraction and humic acid were not available in the information used for the present manuscript. Application concentrations were selected from preliminary trials and manufacturer recommendations [3].

The experimental design imposes several limitations on inference. First, because each management regime occupied a single greenhouse, greenhouse-specific environmental and soil effects cannot be separated statistically from management effects. Second, the conventional comparator and combined-biostimulant regime differed not only in the three tested products but also in nematode-management inputs, including abamectin and EDDHA iron chelate. Third, the three products were applied together, so their individual contributions and any synergistic or antagonistic interactions cannot be resolved. Finally, KeyPlex 120[®] and CropBioLife[®] provide mineral nutrients in addition to other bioactive components. The results are therefore interpreted as treatment-associated differences within this commercial greenhouse comparison. Future studies should use multiple greenhouse replicates or randomized blocks and factorial single-product and combination treatments.

2.2. Chlorophyll Content Index Measurements

Chlorophyll status was assessed with a CCM-200+GPS Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH, USA) and expressed as Chlorophyll Content Index (CCI). For leaves, the third fully expanded leaf from the top was sampled and the central lamina region was measured. For fruits, mature fruits were sampled from different plants and a thin, flat skin slice (approximately 2–3 mm thick) was removed from the middle region and immediately placed in the sensor window after confirming that the sample was intact and not water-saturated. Ten leaves and ten fruits from different plants were measured per management regime. Fruit-skin CCI was interpreted as a relative index for comparison between the two regimes.

The instrument measures optical transmittance at 653 and 931 nm and compensates for variation in tissue optical properties. CCI was calculated by the instrument as the ratio of percentage transmittance at 931 nm to percentage transmittance at 653 nm.

2.3. Chlorophyll Fluorescence Imaging Analysis

Chlorophyll fluorescence was measured *in vivo* on the third fully expanded leaf from the top and on cucumber fruits of comparable age, size, and developmental stage using a MAX/IMAGING-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany) equipped with blue LEDs ($\lambda < 450$ nm) for measuring light, actinic illumination, and saturation pulses. Five leaves and five fruits from different plants were analyzed per management regime. Samples were dark-adapted for 30 min before fluorescence determination. After dark adaptation, each sample was positioned 18.5 cm from the CCD camera and exposed

to modulated measuring light ($0.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) to determine F_o . Maximum fluorescence (F_m) was obtained using a saturating pulse of $2275 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 0.3 s. Steady-state fluorescence (F_s) was recorded after 5 min of actinic illumination. For the HL measurement, the same organ was dark-adapted again for 30 min before the procedure was repeated. Five areas of interest (AOIs) covering the measured surface were selected per organ and averaged before statistical analysis.

Two actinic light intensities were used. LL was $455 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, which approximated the average greenhouse leaf-level PPFD ($400 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$). HL was $973 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, approximately 2.4-fold higher than the average growth PPFD, and was used as an acute high-light challenge rather than as a second growth condition.

PSII energy partitioning and fluorescence parameters were calculated with Imaging-WinGigE V2.57q46 software using the following equations:

The effective quantum yield of PSII photochemistry, (Φ_{PSII}), estimating the efficiency at which light absorbed by PSII is used for photochemistry, calculated as $(F_m' - F_s)/F_m'$ [17].

The quantum yield of regulated non-photochemical energy loss in PSII (Φ_{NPQ}), that is the quantum yield for dissipation by down regulation in PSII, calculated as $F_s/F_m' - F_s/F_m$ [17].

The quantum yield of non-regulated energy loss in PSII (Φ_{NO}), a loss process due to PSII inactivity, calculated as F_s/F_m [17].

The non-photochemical quenching (NPQ), that reflects heat dissipation of excitation energy, calculated as $(F_m - F_m')/F_m'$ [26].

The photochemical quenching (qP) is a measure of the fraction of open PSII reaction centers, that is the redox state of the plastoquinone pool, calculated as $(F_m' - F_s)/(F_m' - F_o')$ [27].

2.4. Leaf Anatomical and Stomatal Measurements

Leaf anatomical and stomatal observations were performed on fully expanded cucumber leaves collected from comparable canopy positions. Five leaves from different plants were sampled per management regime. Segments were excised from the central lamina while avoiding the midrib and major veins and were processed for light microscopy following Dobrikova et al. [28]. Samples were fixed in 3% paraformaldehyde plus 3% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.0) for 6 h at room temperature, post-fixed in 2% osmium tetroxide in the same buffer for 3 h, dehydrated through an acetone series, treated with propylene oxide, and embedded in Durcupan ACM resin. Semithin transverse sections ($0.5\text{--}2 \mu\text{m}$) were cut with an ultramicrotome using a glass knife, stained with 0.5% toluidine blue O, and observed with a Zeiss Axioplan light microscope equipped with an AxioCam MRc 5 camera [29]. Palisade parenchyma thickness, spongy parenchyma thickness, total leaf thickness, and intercellular air-space area were measured in representative lamina fields that excluded vascular bundles and damaged tissue. Intercellular air-space area was quantified as the summed air-space area within standardized calibrated fields of view ($[220] \times [165] \mu\text{m}$; total field area = $[36,000] \mu\text{m}^2$) and is reported in μm^2 in Table 1; percentage porosity was not included in the final analyzed dataset. Stomatal density was determined separately for the adaxial and abaxial epidermis from paradermal semithin sections. Multiple calibrated fields were counted per leaf and averaged to obtain one organ-level value, so individual fields and sections were treated as technical subsamples rather than independent observations. Image acquisition and measurements used the same magnification and calibration settings for both management regimes, and measurements were performed without reference to treatment identity where feasible.

Table 1. Anatomical and stomatal characteristics of cucumber leaves sampled from the conventional-control and combined-biostimulant greenhouses. Values are means $\pm$ SE of five leaf samples per management regime, with multiple microscopic fields averaged within each leaf. The leaves are organ-level subsamples within one greenhouse per management regime and are not independent greenhouse-level treatment replicates. Different letters within a column indicate within-trial differences at $p < 0.05$ using one-way ANOVA followed by Tukey's HSD test.

Treatment	Intercellular Air-Space Area (μm^2 ; Standardized Field)	Palisade Parenchyma Thickness (μm)	Spongy Parenchyma Thickness (μm)	Leaf Thickness (μm)	Adaxial Stomatal Density (Stomata mm^{-2})	Abaxial Stomatal Density (Stomata mm^{-2})
Control	420 $\pm$ 18 b	75 $\pm$ 2.1 b	110 $\pm$ 3.4 b	230 $\pm$ 4.6 b	35 $\pm$ 4 b	205 $\pm$ 12 a
Biostimulant-treated	610 $\pm$ 22 a	78 $\pm$ 1.8 a	135 $\pm$ 4.1 a	245 $\pm$ 5.2 a	8 $\pm$ 2 a	202 $\pm$ 15 a

2.5. Statistical Analysis

Statistical analyses were performed in R [30], and data are presented as means $\pm$ standard error (SE). For CCI, fluorescence, anatomy, and stomatal measurements, the sampled leaf or fruit from an individual plant was the organ-level sampling unit. These organ-level samples represent biological subsampling within each greenhouse, but they are not independent treatment replicates because each management regime was applied to only one greenhouse. For fluorescence imaging, the five AOIs selected on each leaf or fruit were technical subsamples and were averaged to generate one value per organ before analysis. Consequently, reported p -values describe within-trial differences among sampled organs and should be interpreted as exploratory evidence associated with the two greenhouse management regimes rather than as greenhouse-replicated causal treatment effects.

For chlorophyll fluorescence parameters, management regime and actinic light intensity were treated as fixed factors. Because the same leaf or fruit was measured under LL and HL, light intensity was treated as a repeated-measures factor. Fluorescence data were analyzed with a mixed-effects model including management regime, light intensity, and their interaction as fixed effects and organ identity as a random factor. Where a management $\times$ light interaction was detected, comparisons between management regimes were examined within each light level using adjusted estimated marginal means; otherwise, main effects were interpreted. Percentage differences reported in the Results provide descriptive effect magnitudes in addition to the p -value annotations shown in the figures.

Anatomical and stomatal traits, which were measured once per sampled leaf, were analyzed by one-way ANOVA with management regime as the fixed factor, followed by Tukey's HSD test when appropriate. Model residuals were checked for approximate normality and homogeneity of variance as part of the original analysis workflow. Differences were considered statistically significant at $p < 0.05$. Because the greenhouse-level treatment factor was not replicated, these tests do not remove the greenhouse-management confounding described above.

The statistical test, organ-level sample size (n), and significance thresholds are stated in the corresponding figure legends and table caption. Asterisks indicate within-light comparisons between management regimes in fluorescence figures, and different letters denote differences in Table 1. Cross-trait correlation analyses were not performed because CCI, fluorescence, and anatomical measurements used different sample sizes and were not documented as a one-to-one paired dataset from the same individual organs; correlating unpaired observations or the two-treatment means would not provide a valid inferential assessment and could compound the pseudoreplication problem. Statistical analyses were performed in R using the lme4 package for model fitting, emmeans for evaluating interaction effects and post hoc comparisons, effect size for estimating effect sizes, and per-

formance for checking model assumptions, including residual normality and homogeneity of variance. Data transformations, such as logarithmic or square-root transformations, were applied when necessary to better satisfy model assumptions.

3. Results

3.1. CCI-Chlorophyll in Fruits and Leaves

CCI differed between the two greenhouse management regimes (Figure 1). Relative to the conventional-control greenhouse, fruit CCI in the combined-treatment greenhouse was 38% higher ($p < 0.01$), while leaf CCI was 52% higher ($p < 0.05$). These comparisons describe organ-level differences within the two-greenhouse trial and should be interpreted in light of the greenhouse-level confounding described in Section 2.1.

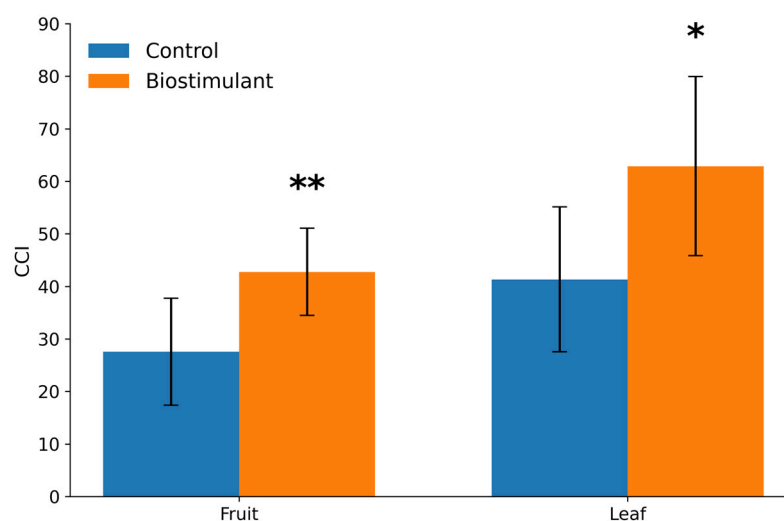


Figure 1. Chlorophyll Content Index (CCI) in cucumber fruits and leaves sampled from the conventional-control and combined-biostimulant greenhouses. Values are means $\pm$ SE of 10 organ-level samples per organ and management regime, with each leaf or fruit sampled from a different plant. These samples are subsamples within one greenhouse per management regime and are not independent greenhouse-level treatment replicates. Asterisks indicate within-trial differences between management regimes within each organ: * $p < 0.05$ and ** $p < 0.01$.

3.2. Chlorophyll Fluorescence Parameters in Fruits and Leaves

PSII-absorbed energy was partitioned among photochemistry (Φ PSII), regulated non-photochemical dissipation (Φ NPQ), and non-regulated energy loss (Φ NO) [14]. Leaves and fruits from the two greenhouse management regimes were measured under LL ($455 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and HL ($973 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

In fruits, Φ PSII was 22% higher in the combined-treatment greenhouse under LL and 16% higher under HL relative to the conventional-control greenhouse; the between-regime difference was statistically significant only under LL ($p < 0.05$; Figure 2a). Φ PSII was lower under HL than LL in both regimes. Fruit Φ NPQ was 17% higher under the combined treatment under LL, with a significant difference at that light level, whereas the HL difference was not significant (Figure 2b). Fruit Φ NO was 12% lower under the combined treatment under LL ($p < 0.05$) and showed no significant between-regime difference under HL (Figure 3a). qP and NPQ were also higher in combined-treatment fruits under LL, including a 40% increase in NPQ, whereas corresponding HL differences were small and non-significant (Figure 3b,c).

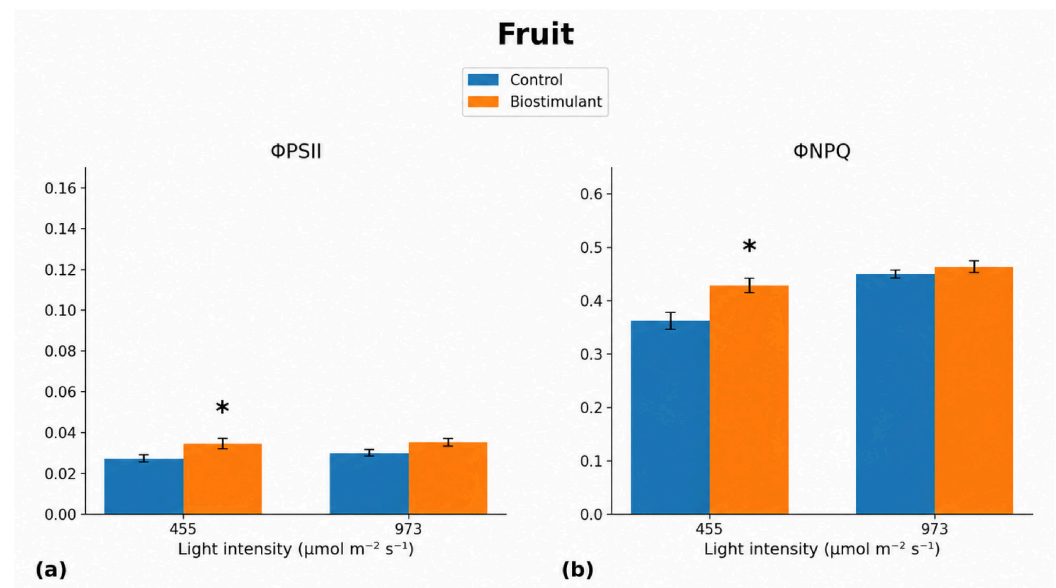


Figure 2. PSII energy partitioning in cucumber fruits sampled from the conventional-control and combined-biostimulant greenhouses under low light (LL; 455 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high light (HL; 973 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). (a) Effective quantum yield of PSII photochemistry, Φ_{PSII} ; (b) regulated non-photochemical energy dissipation, Φ_{NPQ} . Values are means $\pm$ SE of five fruit samples per management regime, with five AOIs averaged within each fruit before analysis. The fruit samples are organ-level subsamples within one greenhouse per management regime. Asterisks indicate adjusted within-light differences between management regimes: * $p < 0.05$.

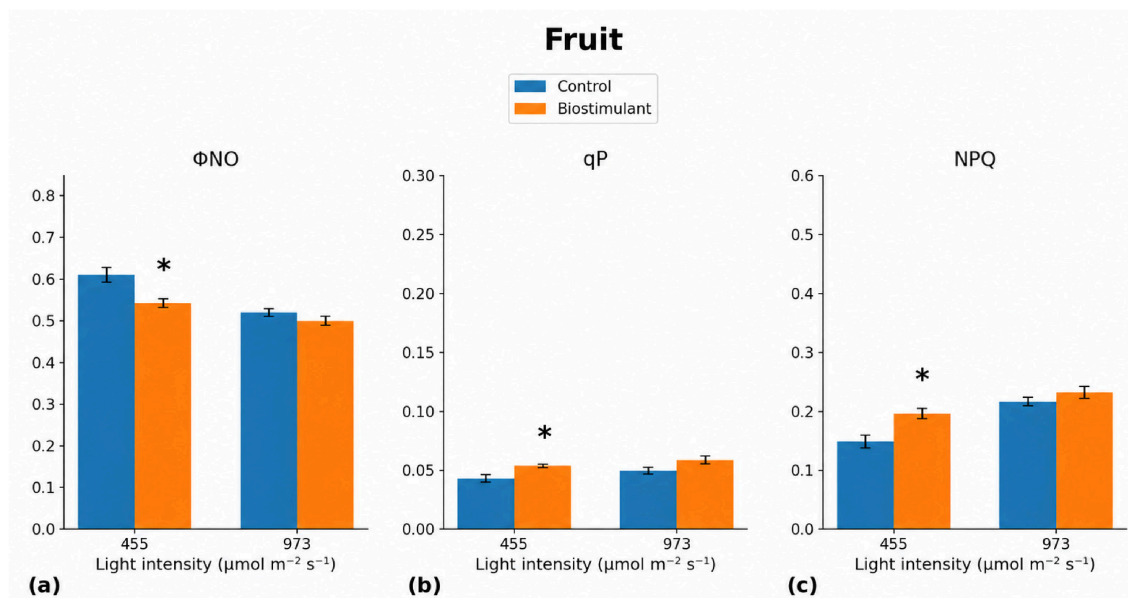


Figure 3. PSII non-regulated energy dissipation and quenching parameters in cucumber fruits sampled from the conventional-control and combined-biostimulant greenhouses under LL and HL. (a) Φ_{NO} ; (b) qP ; (c) NPQ . Values are means $\pm$ SE of five fruit samples per management regime, with five AOIs averaged within each fruit before analysis. The fruit samples are organ-level subsamples within one greenhouse per management regime. Asterisks indicate adjusted within-light differences between management regimes: * $p < 0.05$.

In leaves, Φ_{PSII} was 50% higher under LL in the combined-treatment greenhouse than in the conventional-control greenhouse, whereas the difference under HL was 16% and was not statistically significant (Figure 4a). Φ_{NPQ} was 16% higher under LL and 8% higher under HL, with significance indicated in Figure 4b. Leaf Φ_{NO} was 26% lower under

LL and 17% lower under HL in the combined-treatment greenhouse (Figure 5a). qP was 72% higher under LL and 28% higher under HL, although only the LL between-regime difference was significant (Figure 5b). NPQ was higher in combined-treatment leaves under both light levels, including an approximately 33% increase under HL (Figure 5c).

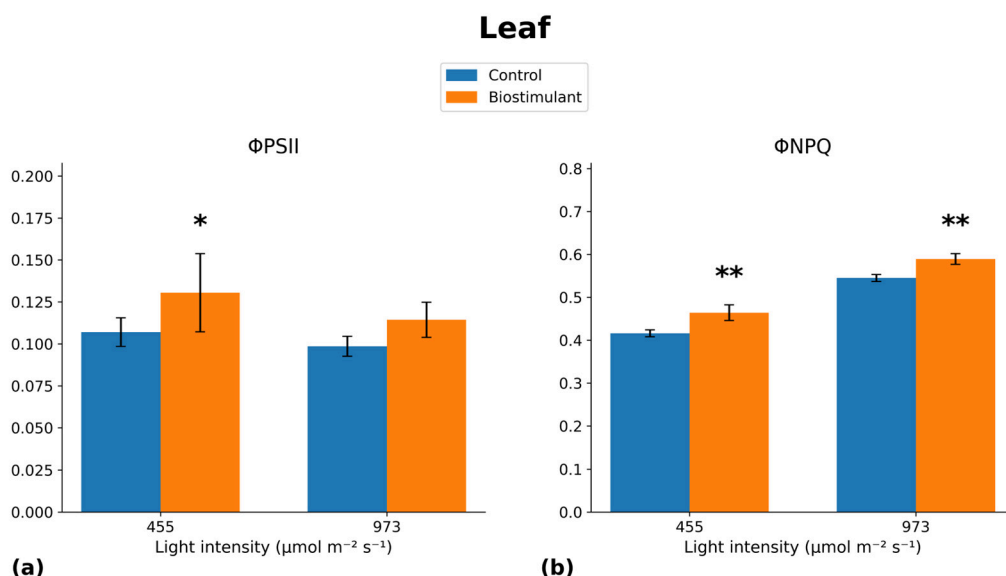


Figure 4. PSII energy partitioning in cucumber leaves sampled from the conventional-control and combined-biostimulant greenhouses under LL and HL. (a) Φ_{PSII} ; (b) Φ_{NPQ} . Values are means $\pm$ SE of five leaf samples per management regime, with five AOIs averaged within each leaf before analysis. The leaves are organ-level subsamples within one greenhouse per management regime. Asterisks indicate adjusted within-light differences between management regimes: * $p < 0.05$ and ** $p < 0.01$.

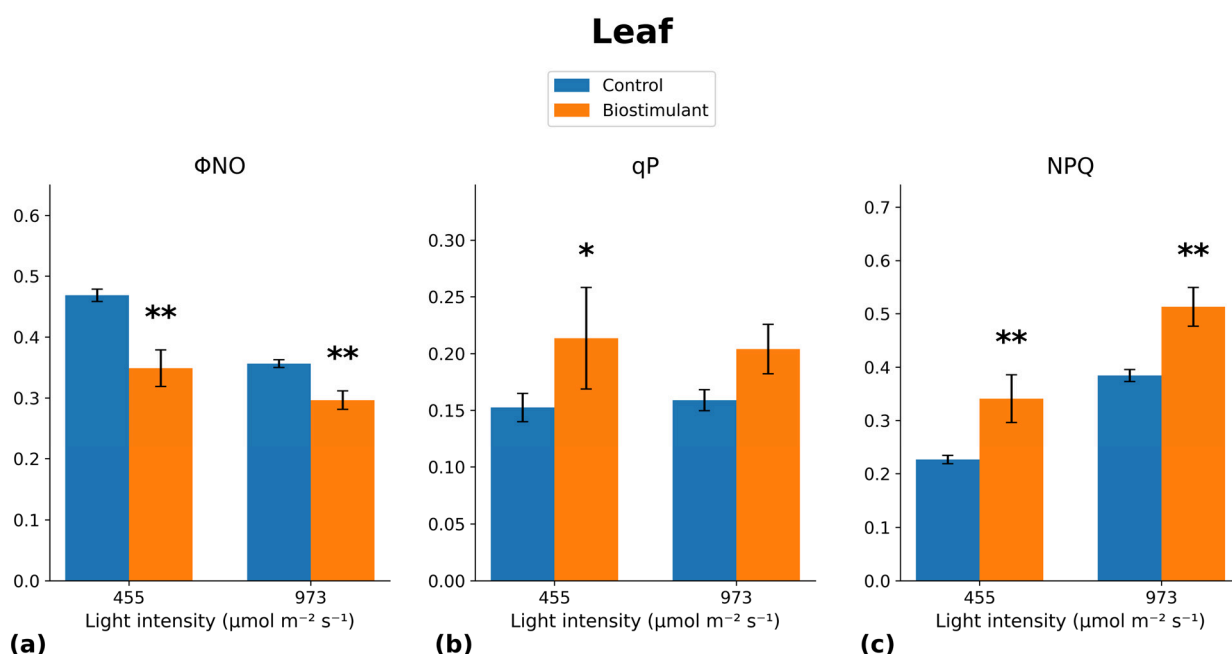


Figure 5. PSII non-regulated energy dissipation and quenching parameters in cucumber leaves sampled from the conventional-control and combined-biostimulant greenhouses under LL and HL. (a) Φ_{NO} ; (b) qP ; (c) NPQ. Values are means $\pm$ SE of five leaf samples per management regime, with five AOIs averaged within each leaf before analysis. The leaves are organ-level subsamples within one greenhouse per management regime. Asterisks indicate adjusted within-light differences between management regimes: * $p < 0.05$ and ** $p < 0.01$.

3.3. Leaf Structural Characteristics

Several anatomical and stomatal traits differed between leaves sampled from the two greenhouse management regimes (Table 1; Figure 6). The combined-treatment greenhouse showed a larger measured intercellular air-space area (610 ± 22 versus $420 \pm 18 \mu\text{m}^2$) and greater spongy parenchyma thickness (135 ± 4.1 versus $110 \pm 3.4 \mu\text{m}$) than the conventional-control greenhouse. These values describe differences in the standardized microscopic fields analyzed and do not constitute a direct measurement of mesophyll conductance.

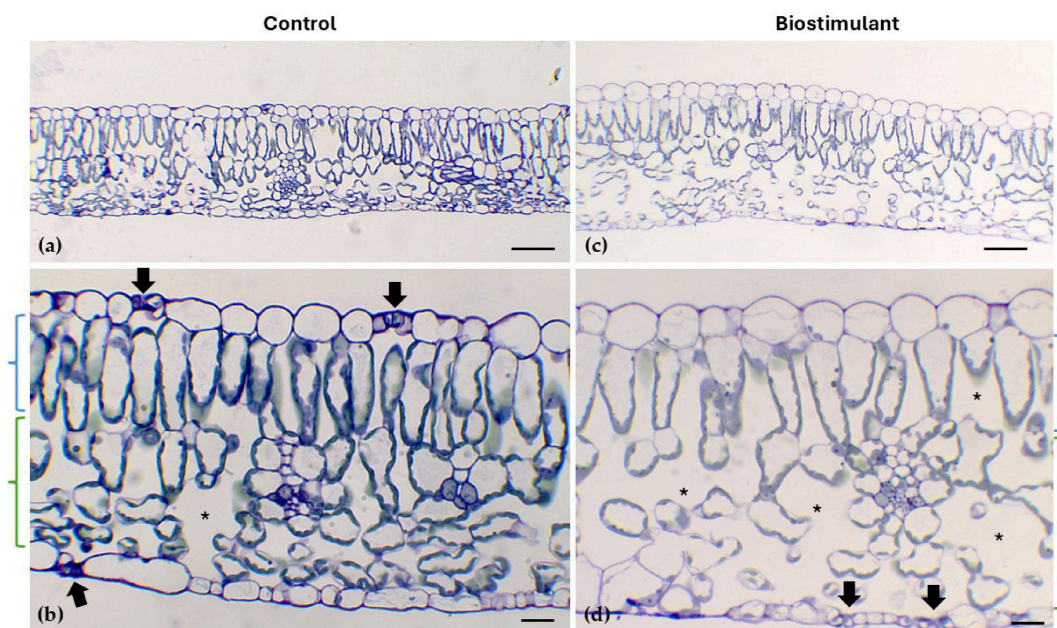


Figure 6. Representative leaf cross-sections from the conventional-control (a,b) and combined-biostimulant (c,d) greenhouses. Arrows indicate stomata, asterisks indicate intercellular air spaces, blue brackets indicate palisade parenchyma, and green brackets indicate spongy parenchyma. Scale bars: 200 μm (a,c), 50 μm (b,d).

Palisade parenchyma thickness was modestly greater in leaves from the combined-treatment greenhouse ($78 \pm 1.8 \mu\text{m}$) than in the conventional-control greenhouse ($75 \pm 2.1 \mu\text{m}$), and total leaf thickness was also greater (245 ± 5.2 versus $230 \pm 4.6 \mu\text{m}$) (Figure 6; Table 1).

Adaxial stomatal density showed a large between-regime difference, with values of 35 ± 4 and $8 \pm 2 \text{ stomata mm}^{-2}$ in the conventional-control and combined-treatment samples, respectively, whereas abaxial stomatal density was similar (205 ± 12 and $202 \pm 15 \text{ stomata mm}^{-2}$) (Table 1). Because stomatal counts were obtained from semithin paradermal sections and the number of leaves was limited ($n = 5$ per regime), the magnitude of the adaxial difference should be interpreted cautiously and should be validated in future work using wider-field epidermal imaging.

4. Discussion

The present study extends the previous evaluation of this greenhouse trial [3] by focusing on complementary PSII energy partitioning in both leaves and fruits and by adding CCI and leaf anatomical measurements. Cucumber fruits showed lower fluorescence-based photochemical performance than leaves, consistent with the lower photosynthetic capacity generally expected for non-foliar organs even though cucumber fruit can contribute to carbon economy through atmospheric CO_2 assimilation and internal CO_2 re-fixation [20,31–35].

Under HL, both management regimes showed lower Φ PSII and qP than under LL, indicating a shift away from photochemical use as excitation pressure increased.

The between-regime difference in Φ PSII was more pronounced under LL than under HL, particularly in fruits. A plausible explanation is that LL was close to the average greenhouse growth irradiance, whereas the acute HL treatment imposed substantially greater excitation pressure on both groups. Under HL, increased engagement of non-photochemical dissipation and partial closure of PSII reaction centers can reduce Φ PSII in both groups and compress between-regime differences [36–39]. This interpretation is consistent with the fluorescence response but should not be taken as evidence that the combined treatment directly prevented photoinhibition, because photodamage and recovery kinetics were not measured.

NPQ and Φ NPQ both describe regulated thermal dissipation but are not interchangeable: NPQ is a quenching coefficient, whereas Φ NPQ represents the quantum yield of regulated non-photochemical energy dissipation [17,38,39]. The generally higher NPQ and/or Φ NPQ values in samples from the combined-treatment greenhouse, together with lower Φ NO in several comparisons, are consistent with a greater relative allocation of absorbed energy to regulated dissipation rather than non-regulated loss. The effect was most consistent under LL in fruits and was evident under both light levels for some leaf parameters.

Higher Φ NO can be associated with conditions in which excitation energy is not efficiently used photochemically or dissipated through regulated protective pathways [40–43]. However, the present study did not measure singlet oxygen, total ROS, lipid peroxidation, antioxidant enzymes, or photodamage. Therefore, the higher Φ NO observed in the conventional-control samples should be interpreted as a fluorescence-based indicator of greater non-regulated energy loss, not as direct evidence of increased $^1\text{O}_2$ generation or ROS accumulation.

CCI was higher in leaves and fruits sampled from the combined-treatment greenhouse. Because chlorophyll content is related to light harvesting and can also reflect nutritional status [44–49], this difference may contribute to the distinct fluorescence responses. Nevertheless, tissue chlorophyll concentration was estimated only by CCI, and leaf mineral concentrations were not measured. It is therefore not possible to attribute the CCI difference specifically to Fe, N, or other nutrients supplied by the combined formulation.

The composition of the three products provides biologically plausible pathways that can be considered as hypotheses rather than demonstrated mechanisms. Fe and Mn are essential components of photosynthetic electron-transfer and water-splitting systems, while Zn contributes to protein and membrane function [50]. Flavonoids have also been associated in other systems with antioxidant signaling and physiological responses to stress [7,25,51–53]. Because the present experiment did not measure nutrient uptake, flavonoid metabolism, ROS, antioxidant enzyme activity, gene expression, or hormone signaling, these literature-supported pathways cannot be assigned as the cause of the observed PSII responses.

This distinction is important because the combined protocol simultaneously changed several inputs. KeyPlex 120[®] supplied Fe, Mn, and Zn; CropBioLife[®] supplied mineral nutrients in addition to an activated-flavonoid fraction; and Key Eco Oil[®] contained botanical oils. The conventional-control greenhouse also received abamectin and EDDHA iron chelate. Thus, the observed differences may reflect the combined management system, including nutrient supply and crop-protection differences, rather than a biostimulant effect in the strict mechanistic sense.

Previous work in tomato has reported associations between exogenous flavonoid treatments and gas exchange, phenolic metabolism, and aquaporin expression [53]. Those

findings provide context for future mechanistic experiments, but aquaporin expression and gas-exchange responses were not determined in the present dataset and are therefore not used here as explanations for the cucumber results.

Similarly, reports that nutrient- and biostimulant-based foliar formulations can improve pigment status or growth in other crops [3,48] support the plausibility of a physiological response but do not identify which component of the present three-product protocol was responsible. Factorial experiments that compare each product alone and in combination are required to separate individual and interactive effects.

The anatomical data shows parallel structural differences between the two management regimes, including greater spongy parenchyma thickness, modestly greater palisade and total leaf thickness, and larger intercellular air-space area in the sampled fields. These traits are relevant to internal light distribution and the geometry of CO₂ diffusion pathways [10,54–57]. However, mesophyll conductance, intercellular CO₂ concentration, and net assimilation were not measured in the present study. Accordingly, the anatomical differences are compatible with altered internal diffusion geometry but do not demonstrate enhanced mesophyll conductance or carbon assimilation.

The large decrease in adaxial stomatal density, with little change on the abaxial surface, may also have functional consequences because stomatal distribution can influence gas exchange and transpiration [29,58]. Nevertheless, stomatal conductance, transpiration, and intrinsic water-use efficiency were not measured in this dataset, so reduced water loss or improved water-use efficiency cannot be concluded. In addition, the adaxial estimate was derived from semithin paradermal sections from five leaves per regime; validation with larger, high-resolution epidermal fields would strengthen confidence in the magnitude of this difference.

The novelty of the present work lies in measurements that were not the focus of the earlier publication from the same trial [3]: Imaging-PAM analysis of complementary PSII quantum yields (Φ PSII, Φ NPQ, Φ NO), qP and NPQ under two actinic light intensities in both leaves and fruits, together with fruit and leaf CCI and quantitative leaf anatomy and stomatal density. The previous study reported other physiological and agronomic endpoints, including leaf gas exchange, conventional fluorescence indices, nematode responses, root anatomy, and fruit-quality traits [3]. The current dataset should therefore be viewed as a complementary characterization of PSII energy allocation and leaf structure rather than a repeated evaluation of those previously published outcomes.

Several limitations constrain generalization. Treatment and greenhouse were confounded because only one greenhouse was used per management regime; baseline soil properties also differed to some extent between greenhouses [3]. The conventional and combined-biostimulant regimes differed in nematode-management inputs, the three commercial products were applied together, and the sample sizes for organ-level measurements were modest ($n = 5$ for fluorescence and anatomy; $n = 10$ for CCI). In addition, the present dataset did not include gas exchange, mesophyll conductance, ROS, antioxidant metabolism, mineral nutrient concentrations, yield, or long-term stress-performance measurements. Future studies should include replicated greenhouses or randomized blocks, factorial product combinations, larger organ-level samples, direct gas-exchange and biochemical measurements, and validation across seasons and greenhouse environments.

5. Conclusions

Within this commercial two-greenhouse comparison, cucumber leaves and fruits sampled from the combined-biostimulant greenhouse showed higher CCI and a shift in several PSII fluorescence parameters toward greater photochemical use and/or regulated energy dissipation, with the clearest between-regime differences generally observed under LL.

Leaves from the combined-treatment greenhouse also showed greater spongy parenchyma thickness, modest increases in palisade and total leaf thickness, larger measured intercellular air-space area, and lower adaxial stomatal density, while abaxial stomatal density was similar between regimes. These anatomical observations describe structural differences but do not by themselves establish changes in CO₂ diffusion, gas exchange, transpiration, or water-use efficiency.

Because each management regime was confined to one greenhouse and the three products were applied as a mixture against a conventional comparator with different nematode-management inputs, the results should be regarded as treatment-associated rather than causal effects of individual biostimulants. Replicated greenhouse experiments with factorial product treatments and direct gas-exchange, nutrient, and biochemical measurements are required for mechanistic and agronomic validation.

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Data Availability Statement: Data supporting the findings of this study, including chlorophyll fluorescence, Chlorophyll Content Index, and anatomical measurements, are available from the corresponding author upon reasonable request.

Conflicts of Interest: The study involved the use of named commercial products, namely CropBioLife®, KeyPlex 120®, and Key Eco Oil®. George Daskas is affiliated with Geogreen Marathon. The authors declare that this affiliation did not influence the experimental design, data collection, statistical analysis, interpretation of the results, or preparation of the manuscript. The commercial products were used only as experimental treatments under greenhouse conditions. No product supplier or commercial entity had any role in data analysis, data interpretation, or the decision to publish the results. The authors declare no conflicts of interest.

References

1. Fanourakis, D.; Makraki, T.; Ntanasi, T.; Giannothanasis, E.; Tsaniklidis, G.; Tsitsigiannis, D.I.; Ntatsi, G. Climate-Driven Pest and Disease Dynamics in Greenhouse Horticulture. *Horticulturae* **2026**, *12*, 415. [\[CrossRef\]](#)
2. Sun, W.; Ma, N.; Huang, H.; Wei, J.; Ma, S.; Liu, H.; Zhang, S.; Zhang, Z.; Sui, X.; Li, X. Photosynthetic Contribution and Characteristics of Cucumber Stems and Petioles. *BMC Plant Biol.* **2021**, *21*, 454. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Ouzounidou, G.; Antarakis, N.-S.; Anagnostou, A.; Adamakis, I.-D.S. Combined Effects of Vegetable Oil-, Micronutrient-, and Activated Flavonoid-Based Biostimulants on Photosynthesis, Nematode Suppression, and Fruit Quality of Cucumber (*Cucumis sativus* L.). *Plants* **2026**, *15*, 274. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Sallam, B.N.; Lü, T.; Yu, H.; Li, Q.; Sarfraz, Z.; Iqbal, M.S.; Khan, S.; Wang, H.; Liu, P.; Jiang, W. Productivity Enhancement of Cucumber (*Cucumis sativus* L.) through Optimized Use of Poultry Manure and Mineral Fertilizers under Greenhouse Cultivation. *Horticulturae* **2021**, *7*, 256. [\[CrossRef\]](#)
5. Zhang, Y.; Sun, J.; Wang, J.; Li, X.; Wang, L. Advances in Physiological and Molecular Mechanisms of Cucumber Stress Tolerance. *Horticulturae* **2025**, *11*, 1268. [\[CrossRef\]](#)
6. Jones, J.T.; Haegeman, A.; Danchin, E.G.J.; Gaur, H.S.; Helder, J.; Jones, M.G.K.; Kikuchi, T.; Manzanilla-López, R.; Palomares-Rius, J.E.; Wesemael, W.M.L.; et al. Top 10 Plant-Parasitic Nematodes in Molecular Plant Pathology. *Mol. Plant Pathol.* **2013**, *14*, 946–961. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Di Sario, L.; Boeri, P.; Matus, J.T.; Pizzio, G.A. Plant Biostimulants to Enhance Abiotic Stress Resilience in Crops. *Int. J. Mol. Sci.* **2025**, *26*, 1129. [\[CrossRef\]](#) [\[PubMed\]](#)

8. Kumari, M.; Swarupa, P.; Kesari, K.K.; Kumar, A. Microbial Inoculants as Plant Biostimulants: A Review on Risk Status. *Life* **2023**, *13*, 12. [CrossRef] [PubMed]
9. Khetsha, Z.P.; Sedibe, M.M.; Pretorius, R.J.; Van Der Watt, E. Biostimulants Improve the Leaf Micro-Morphology and Essential Oil Biosynthesis of Simulated Hail-Damaged *Pelargonium graveolens* L'Hér. *Acta Hortic.* **2023**, *1372*, 283–294. [CrossRef]
10. Roupahel, Y.; De Micco, V.; Arena, C.; Raimondi, G.; Colla, G.; De Pascale, S. Effect of *Ecklonia maxima* Seaweed Extract on Yield, Mineral Composition, Gas Exchange, and Leaf Anatomy of Zucchini Squash Grown under Saline Conditions. *J. Appl. Phycol.* **2017**, *29*, 459–470. [CrossRef]
11. Vitale, E.; Velikova, V.; Tsonev, T.; Ferrandino, I.; Capriello, T.; Arena, C. The Interplay between Light Quality and Biostimulant Application Affects the Antioxidant Capacity and Photosynthetic Traits of Soybean (*Glycine max* L. Merrill). *Plants* **2021**, *10*, 861. [CrossRef] [PubMed]
12. Abou Jaoudé, R.; Luziatelli, F.; Ficca, A.G.; Ruzzi, M. Effect of Plant Growth-Promoting Rhizobacteria Synthetic Consortium on Growth, Yield, and Metabolic Profile of Lettuce (*Lactuca sativa* L.) Grown under Suboptimal Nutrient Regime. *Horticulturae* **2025**, *11*, 64. [CrossRef]
13. Sharma, A.; Kumar, V.; Shahzad, B.; Ramakrishnan, M.; Sidhu, G.P.S.; Bali, A.S.; Handa, N.; Kapoor, D.; Yadav, P.; Khanna, K.; et al. Photosynthetic Response of Plants under Different Abiotic Stresses: A Review. *J. Plant Growth Regul.* **2020**, *39*, 509–531. [CrossRef]
14. Moustaka, J.; Moustakas, M. Early-Stage Detection of Biotic and Abiotic Stress on Plants by Chlorophyll Fluorescence Imaging Analysis. *Biosensors* **2023**, *13*, 796. [CrossRef] [PubMed]
15. Chauhan, J.; Prathibha, M.D.; Singh, P.; Choyal, P.; Mishra, U.N.; Saha, D.; Kumar, R.; Anuragi, H.; Pandey, S.; Bose, B.; et al. Plant Photosynthesis under Abiotic Stresses: Damages, Adaptive, and Signaling Mechanisms. *Plant Stress* **2023**, *10*, 100296. [CrossRef]
16. Baker, N.R. Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. *Annu. Rev. Plant Biol.* **2008**, *59*, 89–113. [CrossRef] [PubMed]
17. Kramer, D.M.; Johnson, G.; Kiirats, O.; Edwards, G.E. New Fluorescence Parameters for the Determination of QA Redox State and Excitation Energy Fluxes. *Photosynth. Res.* **2004**, *79*, 209–218. [CrossRef] [PubMed]
18. Ouzounidou, G.; Moustakas, M.; Strasser, R.J. Sites of Action of Copper in the Photosynthetic Apparatus of Maize Leaves: Kinetic Analysis of Chlorophyll Fluorescence, Oxygen Evolution, Absorption Changes and Thermal Dissipation as Monitored by Photoacoustic Signals. *Aust. J. Plant Physiol.* **1997**, *24*, 81–90. [CrossRef]
19. Sperdouli, I.; Ouzounidou, G.; Moustakas, M. Hormesis Responses of Photosystem II in *Arabidopsis thaliana* under Water Deficit Stress. *Int. J. Mol. Sci.* **2023**, *24*, 9573. [CrossRef] [PubMed]
20. Sui, X.; Shan, N.; Hu, L.; Zhang, C.; Yu, C.; Ren, H.; Turgeon, R.; Zhang, Z. The Complex Character of Photosynthesis in Cucumber Fruit. *J. Exp. Bot.* **2017**, *68*, 1625–1637. [CrossRef] [PubMed]
21. Simkin, A.J.; Faralli, M.; Ramamoorthy, S.; Lawson, T. Photosynthesis in Non-Foliar Tissues: Implications for Yield. *Plant J.* **2020**, *101*, 1001–1015. [CrossRef] [PubMed]
22. Henry, R.J. Pathways of Photosynthesis in Non-Leaf Tissues. *Biology* **2020**, *9*, 438. [CrossRef] [PubMed]
23. Liu, C.; Liu, Y.; Lu, Y.; Liao, Y.; Nie, J.; Yuan, X.; Chen, F. Use of a Leaf Chlorophyll Content Index to Improve the Prediction of Above-Ground Biomass and Productivity. *PeerJ* **2019**, *6*, e6240. [CrossRef] [PubMed]
24. Nile, W.; Lu, J.; Zhou, Y.; Yang, Y.; Cao, W.; Zhu, Y.; Tian, Y.; Yao, X.; Cheng, T.; Zhu, Y.; et al. Inversion of Leaf Chlorophyll Content in Different Growth Stages of Rice Based on Multi-Source Data and Hybrid Optimization Algorithms. *Remote Sens.* **2025**, *17*, 572. [CrossRef]
25. Baltazar, M.; Correia, S.; Guinan, K.J.; Sujeeth, N.; Bragança, R.; Gonçalves, B. Recent Advances in the Molecular Effects of Biostimulants in Plants: An Overview. *Biomolecules* **2021**, *11*, 1096. [CrossRef] [PubMed]
26. Bilger, W.; Björkman, O. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.* **1990**, *25*, 173–185. [CrossRef] [PubMed]
27. Genty, B.; Briantais, J.M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* **1989**, *990*, 87–92. [CrossRef]
28. Dobrikova, A.; Apostolova, E.; Hanć, A.; Yotsova, E.; Borisova, P.; Sperdouli, I.; Adamakis, I.-D.S.; Moustakas, M. Tolerance Mechanisms of the Aromatic and Medicinal Plant *Salvia sclarea* L. to Excess Zinc. *Plants* **2021**, *10*, 194. [CrossRef] [PubMed]
29. Bertolino, L.T.; Caine, R.S.; Gray, J.E. Impact of Stomatal Density and Morphology on Water-Use Efficiency in a Changing World. *Front. Plant Sci.* **2019**, *10*, 225. [CrossRef] [PubMed]
30. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2024. Available online: <https://www.R-project.org/> (accessed on 28 April 2026).
31. Hibberd, J.M.; Quick, W.P. Characteristics of C4 Photosynthesis in Stems and Petioles of C3 Flowering Plants. *Nature* **2002**, *415*, 451–454. [CrossRef] [PubMed]
32. Hu, Y.; Zhang, Y.; Li, W.; Oguchi, R.; Chow, W.S.; Luo, H.; Zhang, W. Important Photosynthetic Contribution from the Non-Foliar Green Organs in Cotton at the Late Growth Stage. *Planta* **2012**, *235*, 325–336. [CrossRef] [PubMed]

33. Xu, H.L.; Gauthier, L.; Desjardins, Y.; Gosselin, A. Photosynthesis in Leaves, Fruits, Stem and Petioles of Greenhouse-Grown Tomato Plants. *Photosynthetica* **1997**, *33*, 113–123. [\[CrossRef\]](#)
34. Hetherington, S.E.; Smillie, R.M.; Davies, W.J. Photosynthetic Activities of Vegetative and Fruiting Tissues of Tomato. *J. Exp. Bot.* **1998**, *49*, 1173–1181. [\[CrossRef\]](#)
35. Carrara, S.; Pardossi, A.; Soldatini, G.F.; Tognoni, F.; Guidi, L. Photosynthetic Activity of Ripening Tomato Fruit. *Photosynthetica* **2001**, *39*, 75–78. [\[CrossRef\]](#)
36. Zhu, X.G.; Long, S.P.; Ort, D.R. Improving Photosynthetic Efficiency for Greater Yield. *Annu. Rev. Plant Biol.* **2010**, *61*, 235–261. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Moustaka, J.; Ouzounidou, G.; Sperdouli, I.; Moustakas, M. Photosystem II Is More Sensitive than Photosystem I to Al³⁺ Induced Phytotoxicity. *Materials* **2018**, *11*, 1772. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Kromdijk, J.; Glowacka, K.; Leonelli, L.; Gabilly, S.T.; Iwai, M.; Niyogi, K.K.; Long, S.P. Improving Photosynthesis and Crop Productivity by Accelerating Recovery from Photoprotection. *Science* **2016**, *354*, 857–861. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Ruban, A.V. Nonphotochemical Chlorophyll Fluorescence Quenching: Mechanism and Effectiveness in Protecting Plants from Photodamage. *Plant Physiol.* **2016**, *170*, 1903–1916. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Ouzounidou, G.; Zervakis, G.I.; Gaitis, F. Olive Mill Wastewater Triggered Changes in Physiology and Nutritional Quality of Tomato (*Lycopersicon esculentum* Mill.) Depending on Growth Substrate. *J. Hazard. Mater.* **2008**, *158*, 523–530. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Posch, S.; Bennett, L.T. Photosynthesis, Photochemistry and Antioxidative Defence in Response to Two Drought Severities and with Re-Watering in *Allocasuarina luehmannii*. *Plant Biol.* **2009**, *11*, 83–93. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Klughammer, C.; Schreiber, U. Complementary PSII Quantum Yields Calculated from Simple Fluorescence Parameters Measured by PAM Fluorometry and the Saturation Pulse Method. *PAM Appl. Notes* **2008**, *1*, 27–35.
43. Moustaka, J.; Sperdouli, I.; Moustakas, M. Light Energy Use Efficiency in Photosystem II of Tomato Is Related to Leaf Age and Light Intensity. *Crops* **2024**, *4*, 623–635. [\[CrossRef\]](#)
44. Croft, H.; Chen, J.M.; Luo, X.; Bartlett, P.; Chen, B.; Staebler, R.M. Leaf Chlorophyll Content as a Proxy for Leaf Photosynthetic Capacity. *Glob. Change Biol.* **2017**, *23*, 3513–3524. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Mohammed, A.A.; Söylemez, S.; Sarhan, T.Z. Effect of Biofertilizers, Seaweed Extract and Inorganic Fertilizer on Growth and Yield of Lettuce (*Lactuca sativa* var. *longifolia* L.). *Harran Tarım Ve Gıda Bilim. Derg.* **2022**, *26*, 60–71. [\[CrossRef\]](#)
46. Chaudhary, P.; Singh, S.; Chaudhary, A.; Sharma, A.; Kumar, G. Overview of Biofertilizers in Crop Production and Stress Management for Sustainable Agriculture. *Front. Plant Sci.* **2022**, *13*, 930340. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Gupta, S.; Choudhary, M.; Patel, J.S.; Srivastava, P.K.; Singh, H.B. Growth Promotion and Zinc Biofortification in Lettuce (*Lactuca sativa* L.) by the Application of *Talaromyces* Strain as a Biostimulant. *Sci. Hortic.* **2024**, *323*, 112534. [\[CrossRef\]](#)
48. Turan, M.; Yildirim, E.; Ekin, M.; Argin, S. Effect of Biostimulants on Yield and Quality of Cherry Tomatoes Grown in Fertile and Stressed Soils. *HortScience* **2021**, *56*, 414–423. [\[CrossRef\]](#)
49. Martínez, D.E.; Guiamet, J.J. Distortion of the SPAD 502 Chlorophyll Meter Readings by Changes in Irradiance and Leaf Water Status. *Agronomie* **2004**, *24*, 41–46. [\[CrossRef\]](#)
50. Hawkesford, M.J.; Cakmak, I.; Coskun, D.; De Kok, L.J.; Lambers, H.; Schjoerring, J.K.; White, P.J. Functions of macronutrients. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Academic Press: Amsterdam, The Netherlands, 2012; pp. 135–189.
51. Martins, T.; Barros, A.N.; Rosa, E.; Antunes, L. Enhancing Health Benefits through Chlorophylls and Chlorophyll-Rich Agro-Food: A Comprehensive Review. *Molecules* **2023**, *28*, 5344. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Mishra, N.; Jiang, C.; Chen, L.; Paul, A.; Chatterjee, A.; Shen, G. Achieving Abiotic Stress Tolerance in Plants through Antioxidative Defense Mechanisms. *Front. Plant Sci.* **2023**, *14*, 1110622. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Martinez-Alonso, A.; Garcia-Ibañez, P.; Bárzana, G.; Carvajal, M. Leaf Gas Exchange and Growth Responses of Tomato Plants to External Flavonoids Application as Biostimulators under Normal and Salt-Stressed Conditions. *Agronomy* **2022**, *12*, 3230. [\[CrossRef\]](#)
54. Flexas, J.; Ribas-Carbó, M.; Diaz-Espejo, A.; Galmés, J.; Medrano, H. Mesophyll Conductance to CO₂: Current Knowledge and Future Prospects. *Plant Cell Environ.* **2008**, *31*, 602–621. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Tomás, M.; Flexas, J.; Copolovici, L.; Galmés, J.; Hallik, L.; Medrano, H.; Ribas-Carbó, M.; Tosens, T.; Vislap, V.; Niinemets, Ü. Importance of Leaf Anatomy in Determining Mesophyll Diffusion Conductance to CO₂ across Species: Quantitative Limitations and Scaling up by Models. *J. Exp. Bot.* **2013**, *64*, 2269–2281. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Earles, J.M.; Thérout-Rancourt, G.; Roddy, A.B.; Gilbert, M.E.; McElrone, A.J.; Brodersen, C.R. Beyond Porosity: 3D Leaf Intercellular Airspace Traits That Impact Mesophyll Conductance. *Plant Physiol.* **2018**, *178*, 148–162. [\[CrossRef\]](#) [\[PubMed\]](#)

57. Evans, J.R. Leaf Anatomy Enables More Equal Access to Light and CO₂ between Chloroplasts. *New Phytol.* **1999**, *143*, 93–104. [[CrossRef](#)]
58. Wall, S.; Vialet-Chabrand, S.; Davey, P.; Van Rie, J.; Galle, A.; Cockram, J.; Lawson, T. Stomata on the Abaxial and Adaxial Leaf Surfaces Contribute Differently to Leaf Gas Exchange and Photosynthesis in Wheat. *New Phytol.* **2022**, *235*, 1743–1756. [[CrossRef](#)] [[PubMed](#)]

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