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The Influence of Composite Luminescent Materials Based on Graphene Oxide on the Growth and Development of *Solanum lycopersicum* in Greenhouses

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Abstract: The effect of graphene oxide-based photoconversion covers on the growth and photosynthesis of tomatoes (*Solanum lycopersicum*) was investigated. Two types of photoconversion composite for covers were produced. In the first, only graphene oxide nanoparticles were used as a phosphor, and in the second, the graphene oxide nanoparticles were used jointly with europium oxide nanoparticles. The freshly prepared composites for covers had almost identical photoluminescence spectra: an intense peak in the red region and a minor peak in the blue region. It was revealed that during operation, luminescence in the red region decreased, while in the blue region it increased, probably due to the photothermal reduction of graphene oxide. It was shown that the photoconversion covers increased productivity (25%) and intensified photosynthesis (30–35%) in the tomato plants. It is suggested that the stimulation of plant growth is caused by changes in the light spectrum induced by the photoconversion covers.

Keywords: photoconversion; photoluminescence; graphene oxide; europium oxide; greenhouses; *Solanum lycopersicum*

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1. Introduction

Light is an environmental factor whose characteristics have a strong influence on the growth and development of plants, as well as on their primary and secondary metabolisms [1–4]. The defining characteristics of light are the length of daylight hours and its spectral composition. Changes in daylight hours trigger changes in the phases of plant growth and development, and circadian rhythms. The spectrum of light is also of great importance for plants. Red light promotes plant photomorphogenesis, is critical for the development of the photosynthetic apparatus and the accumulation of sugars, and also regulates the synthesis of phytochemical compounds such as phenols [5]. Blue light regulates stomatal movement, the biosynthesis of chlorophyll and carotenoids, photomorphogenesis, as well as the synthesis of flavonoids and anthocyanins [6,7]. Supplemental illumination with green light under certain conditions can intensify photosynthesis in plants, and lead to an increase in the activity of enzymes of the antioxidant system and the accumulation of aromatic compounds in leaves [8]. Yellow light increases the isoflavonoid content in seedlings [9]. Thus, numerous studies have shown that, for the normal growth and development of plants, a careful selection of the lighting spectrum is necessary [10].

Currently, a variety of approaches are used to create optimal conditions for growing plants in greenhouses, from additional lighting in conditions of a lack of natural light to selective shading in conditions of excessive natural light. At the same time, the high cost of additional lighting reduces the profitability of production. Therefore, approaches aimed at changing the spectrum of sunlight are gaining great popularity [11–14]. Photoselective [15–29] or photoconversion [30–33] coatings are used for these purposes. The first approach is based on reducing the intensity of the spectral component harmful to plants, or on changing the ratio of spectral components in order to influence the plant's receptor of regulatory systems. This approach allows researchers to increase plant productivity as well as create the definite phenotype. The second approach involves the selective absorption of light by phosphors and its re-emission in a different spectral range. This approach allows researchers, on the one hand, to increase PFD in a definite part of the spectrum and, on the other hand, to influence photoreceptors to trigger adaptive changes in the plant itself. At the present time, the light conversion approach is used not only for the acceleration of higher plant growth, but also for microalgae [34,35]. It has been shown that light-converting covers can be used for the regulation of the biomass and lipid productivity of microalgae. Currently, there are numerous phosphors on the market that are, more or less, suitable for creating photoconversion covers. Phosphors created on the basis of organic dyes have an undeniable advantage due to their low cost, ease of use, and high luminescence yield [36–45]; however, constant exposure to light leads to rapid irreversible photodegradation [46]. Another class of compounds are metal-containing nanoparticles [47–52]. Such phosphors have increased stability, but are more difficult to integrate into covers and have a low luminescence quantum yield [53–56]. To date, no photoconversion covers have been created that meet all requirements for greenhouse complexes. However, new materials with improved characteristics are regularly being created and tested.

A promising alternative to fast-degradation organic dyes and ineffective nanoparticles based on metal compounds may be the use of graphene oxide (GO) nanoluminophores. Due to its properties, graphene oxide has a wide range of applications: electronics, lithium-ion batteries, absorbents, catalysts (including photo- and electrocatalysis), etc. Currently, biocompatible materials have been obtained based on GO [57], which has opened opportunities for their application in biomedicine and tissue engineering. The luminescent properties of graphene oxide are of great interest. Unlike graphene, which is not capable of photoluminescence [58,59], GO-based materials are capable of photoluminescence in a wide range (350 nm–1250 nm), wherein the emission wavelength is determined by the degree of oxidation of graphene [58], which makes it possible to obtain graphene oxide with the determined photoluminescent properties. In this study, we tested the effectiveness of the application of graphene oxide as a phosphor in photoconversion covers for greenhouses. The study was performed using covers containing GO as the sole phosphor (PCC-GO) and GO with the addition of europium oxide composite (PPC-GO-Eu_2O_3).

2. Materials and Methods

2.1. Preparation of Nanoparticles and Study of Their Properties

GO nanoparticles were prepared via ultrasonic treatment of graphene oxide microparticles (prepared using the Hummers method (RusGraphene, Moscow, Russia)) during 1 min with a frequency of 40 kHz under room temperature. Eu_2O_3 nanoparticles were obtained via laser fragmentation of europium oxide powder (Sigma-Aldrich, Stockholm, Sweden, purity 99.99%) with laser (Ekspla, Vilnius, Lithuania), as described previously [60]. The hydrodynamic radius of the obtained nanoparticles was determined via dynamic light scattering using a Malvern Zetasizer ULTRA RED LABEL installation (Malvern panalytical Ltd., Worcestershire, UK) in an aqueous solution with scattering at 174.7° at 25 °C with tenfold measurements.

2.2. The Glass Surface Application of NF

A solution of nanoparticles in acetone (0.8 mg GO/mL and/or 0.6 μg $Eu_2O_3/_{mL}$) was mixed with a liquid component of a fluoroplastic polymer (fluoroplastic-32L, St. Petersburg Paint and Varnish Plant, KRASKI SPB LLC, St. Petersburg, Russia) in a ratio of 1/ 100. The mixture was stirred for 10 min until homogeneous. The nanoparticles were applied to clean, grease-free glass using a spray gun with nozzle No. 4 from a distance of 20 cm and pressure of 2.5–3.0 atm. The consumption of the mixture was 33 mL per m^2 of surface. The covers applied to the glass were resistant to water and detergent solutions.

2.3. Fluorescence of PCC

Three-dimensional fluorescence spectra of the photoconversion covers were obtained using a Jasco FP-8300 Spectrofluorimeter (JASCO Applied Sciences, Victoria, BC, Canada) at room temperature.

2.4. Plant Growing Conditions

The work was carried out using the tomato plants (*Solanum lycopersicum*) determinate cultivar "Balkonnoe Chudo". Seeds were planted in an organomineral plug moistened with nutrient solution as described earlier [60], which was placed under glass coated with a control (without phosphors) or the photoconversion cover. The nutrient solution contained 0.5 mM KNO_3; 0.67 mM $Mg(NO_3)_2$; 4.5 mM $Ca(NO_3)_2$; 2.2 mM K_2SO_4; 1 mM KH_2PO_4; and 2 mM $MgSO_4$. The illumination source was incandescent and UV lamps with a 16 h daylight period and a light intensity of ≈80 μmol photons s^{-1} m^{-2} at 25 °C.

2.5. Calculation of Chlorophyll Content in the Leaves of Plants

The chlorophyll content in the leaves was controlled non-invasively throughout the experiment using a CL-01 chlorophyll meter. To convert the records of the portable chlorophyll content meter to generally accepted units (mg chl × g^{-1} of fresh weight), the equation calculated for tomatoes was used [60].

2.6. Measuring the Kinetics of Photosynthetic Activity in the Plant Leaves

The photosynthetic activity in the plant leaves at 25 days after seed germination was determined by the kinetics of photoinduced changes in chlorophyll a fluorescence, the intensity of transpiration, and assimilation of carbon dioxide, using a DUAL-PAM-100 fluorimeter integrated with a GFS-3000 gas analyzer (Waltz, Eichenring, Effeltrich, Germany) at intensity active light 140 μmol photons s^{-1} m^{-2}, 50% humidity, CO_2 concentration of 200 ppm, and temperature of 25 °C. All measurements were performed in a closed chamber (L × W × H, 1 × 1 × 0.5 cm) and repeated at least three times. Fluorescence and gas exchange parameters were calculated with the DUAL-PAM (v.3.20) and GFS-win software (v.3.79), respectively [61–63].

2.7. Statistical Analysis

One-way analysis of variance (ANOVA) was performed to determine statistically significant differences between the plant groups, followed by post hoc comparisons using Student's *t* test for independent means. The difference was considered statistically significant if $p \leq 0.05$.

3. Results

3.1. Properties of the Nanoparticles and the Photoconversion Covers

In this study, we used nanoparticles obtained by laser fragmentation of a colloid solution of europium oxide in deionized water. In the result of laser fragmentation, the colloid of nanoparticles, which formed aggregates, was prepared. It was shown that the size of the nanoparticles and their aggregates was 16 nm ± 5 nm and 200 nm ± 20 nm, respectively (Figure 1). The graphene oxide nanoparticles were 27 ± 5 nm (Figure 1). Then,

the nanoparticles were transferred from an aqueous solution into acetone, mixed with a fluoroplastic polymer (fluoroplastic-32L), and applied to the glass surface.

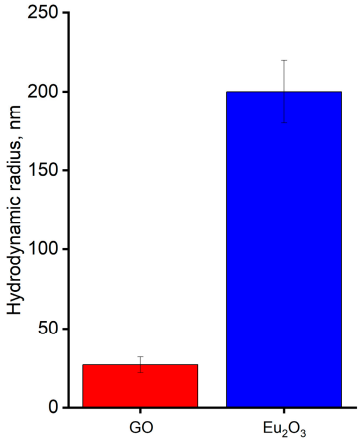

Figure 1. Weighted average particle hydrodynamic radius of GO (red column) and Eu_2O_3 (blue column) at a concentration of 0.8 mg GO/mL and 0.6 µg Eu_2O_3/mL. The measurements were performed in aqueous colloidal solutions of nanoparticles at 25 °C.

The differential ("spectrum of the photoconversion cover with luminophore composites + fluoroplastic 32L" minus "spectrum of common film with fluoroplastic 32L") 3D luminescence matrix of the GO composite applied on the photoconversion cover was obtained before the experiment. It was revealed that the photoconversion cover is characterized by the presence of two luminescence peaks. The first peak is located in the red region (540 nm < λem < 610 nm) and the second peak is located in the blue region of the spectrum (440 nm < λem < 490 nm). The PCC luminescence in these ranges was induced by ultraviolet radiation up to 400 nm (Figure 2). The luminescence spectrum of PCC-GO-Eu_2O_3 was practically the same as for PCC-GO: the covers emitted in the blue (minor band) and red (major band) region under UV excitation. After an experiment with *S. lycopersicum*, we found that the intensity of red luminescence significantly decreased, and luminescence in the blue region, on the contrary, increased (Figure 3). This inversion of the luminescence maxima most likely occurred due to the photothermal reduction of graphene oxide [58].

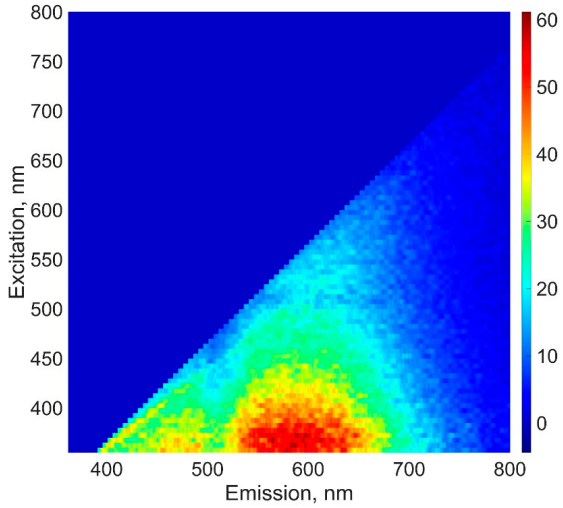

Figure 2. Three-dimensional fluorescence spectrum of the photoconversion cover with GO. The fluorescence intensity is expressed in arbitrary units using a color scale.

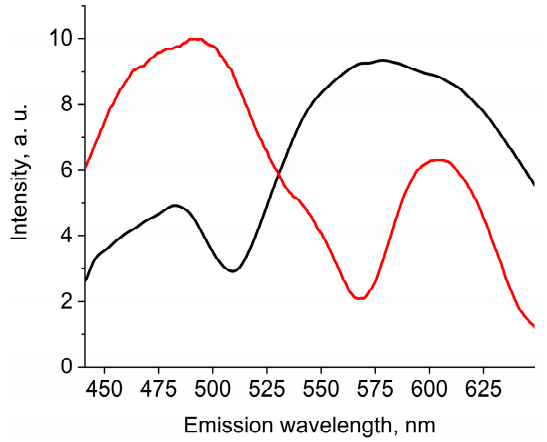

Figure 3. Fluorescence spectrum of the photoconversion cover with GO, excited at 375 nm before (A) and after (B) the experiment with the plants.

Further studies were aimed at testing the effect of the developed covers on the growth of *S. lycopersicum* in laboratory conditions under artificial light, simulating the lack of sunlight in protected soil conditions. The morphology, gas exchange, and photochemistry of the plants were studied.

3.2. Effect of the Photoconversion Covers on Plant Morphology

It was shown that the total leaf surface area was increased in the S. lycopesicum plants grown under PCCs by 26–37% in comparison to the control plants (Figure 4A) (without statistically significant differences between the PPC-GO and PPC-GO-Eu_2O_3 plants). PCCs also increased the number of leaves by 8–15% (Figure 4B). However, PCCs did not affect the length of internodes, the ratio of the leaf fresh weight to dry weight, or the chlorophyll content (Figure 4C) in the studied plants. Thus, the obtained data indicate that the developed graphene oxide-based composites applied on the glass surface have a stimulation effect on the growth of the tomato plants.

3.3. Effect of the Photoconversion Covers on the Gas Exchange Parameters in the Plants

Figure 5 shows the kinetics of light-induced changes in transpiration rates, CO_2 assimilation, and water use efficiency in the *S. lycopersicum* leaves (Figure 5A–C). In the dark, there was no uptake of CO_2 by the tomato leaves, but its release was observed at 0.23 µmol CO_2/m^2 s–0.25 µmol CO_2/m^2 s, without statistically significant differences between the studied groups of plants. This effect was associated with light-independent processes in the leaf tissues, for example, cellular respiration, and processes of the Calvin cycle (Figure 5A). Turning on the light activated CO_2 assimilation in all studied groups of tomato plants, which usually occurs in three phases: (1) a rapid increase in the intensity of CO_2 assimilation in the first minutes of the lighting, associated with the consumption of the reserve of ribulose-1,5-bisphosphate or other intermediate products of the Calvin cycle; (2) a slow growth for 7–10 min of the lighting, when photoactivation of Rubisco by Rubisco activase occurs; and (3) a stationary phase, observed when the maximum intensity of CO_2 assimilation is achieved. The figure shows that during light irradiation of the control plants, the phase of rapid growth of CO_2 assimilation intensity was not observed, while in the plants grown under the experimental covers, the assimilation CO_2 intensity in this phase reaches 1.4 µmol CO_2/m^2 s–1.5 µmol CO_2/m^2 s. The growth rate of CO_2 assimilation intensity in the second phase was similar for all groups of plants. These data may indicate different amounts of Calvin cycle intermediates in the control and experimental plants. At the stationary phase, the maximum intensity of assimilation rate was 25% higher in the plants that were grown under the experimental covers with graphene oxide composites (2.84 µmol CO_2/m^2 s) than in the control plants (2.12 µmol CO_2/m^2 s), probably due to the fact that during the measurement, the intensity of CO_2 assimilation did not manage to reach

maximum values. The intensity of transpiration of H_2O in the dark was the same for all groups of plants, namely, 0.13 mmol H_2O/m^2 s–0.23 mmol H_2O/m^2 s, without statistically significant differences between the studied groups of plants. Turning on the light activated the transpiration of H_2O in the leaves of all plant groups (Figure 5B). However, in the plants grown under the developed photoconversion covers, the activation of transpiration occurs almost immediately, whereas in the control plants, activation begins only after five minutes. The intensity of transpiration at the end of the lighting period was the same in all groups of plants, namely, 0.56 mmol H_2O/m^2 s–0.76 mmol H_2O/m^2 s. Differences in water use efficiency between the control and the experimental plants were observed only in the first minutes of illumination, and are more likely due to the lack of a "fast" assimilation growth phase in the plants grown under control covers (Figure 5C). Figure 5 shows that the changes in transpiration and assimilation rates induced by continuous illumination are accompanied by periodic disturbances. These disturbances arise due to the switching on, every minute, of a saturating flash generated by a PAM-fluorometer operating in tandem with a gas analyzer. Despite the fact that the analysis of flash-induced changes in these parameters was not part of the objectives of this study, we noted that the amplitude of such oscillations was higher in plants grown under PCCs.

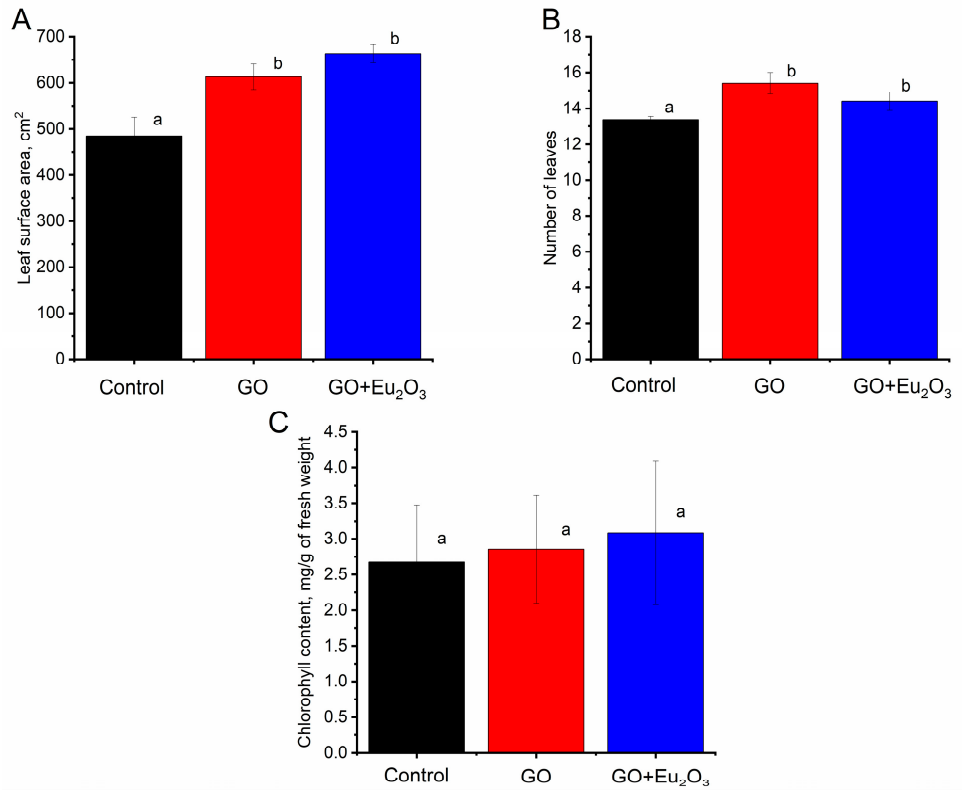

Figure 4. The effect of the photoconversion covers on the leaf surface area (**A**), number of leaves (**B**), chlorophyll content (**C**) of *S. lycopersicum*, measured on the thirty-eighth day after seeding. The data are the result of averaging nine measurements. The letters a, b indicate statistically significant differences between the plant groups ($p \leq 0.05$). PCC-GO are the plants growing under covers containing GO as the sole phosphor, and PPC-GO-Eu_2O_3 are the plants growing under covers with the addition of GO and europium oxide.

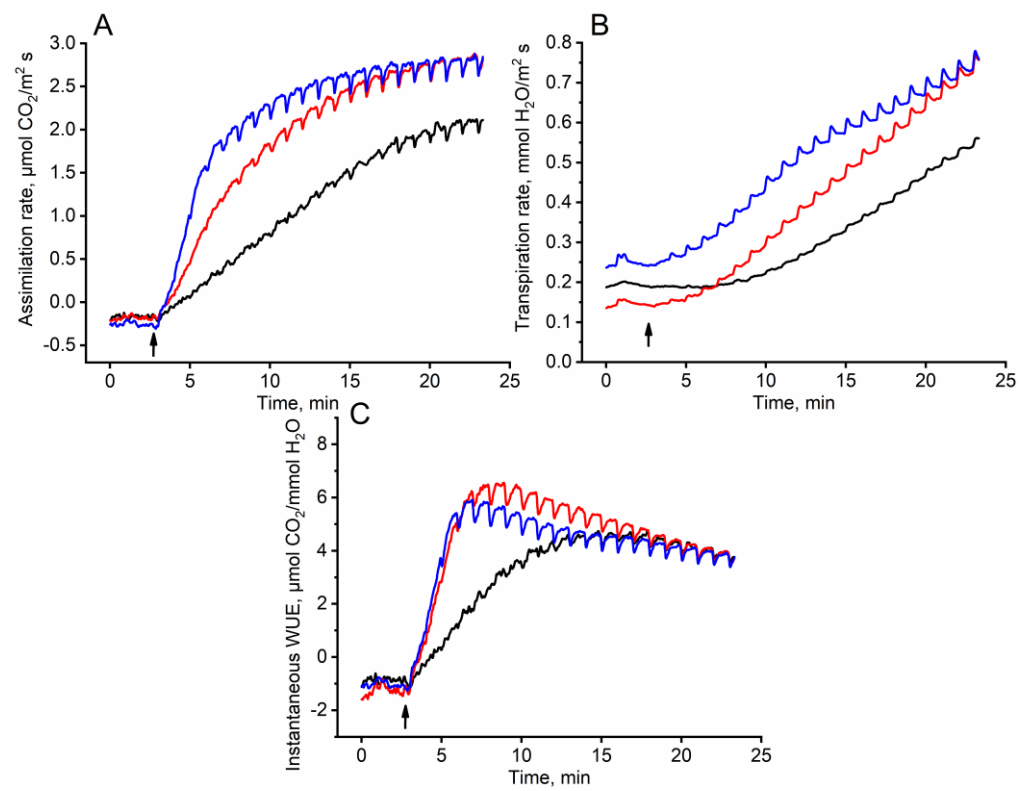

Figure 5. Kinetics of light-induced changes in the intensity of CO_2 assimilation (**A**), transpiration (**B**), and instantaneous water use efficiency (**C**) in the leaves of *S. lycopersicum*. The measurements were performed using plants growing under control covers (black curve), plants growing under PCC-GO (red curve), and plants growing under PCC-GO-Eu_2O_3 (blue curve) at 25 °C, 50% humidity and CO_2 concentration of 200 ppm. ↑—the moment of turning on the acting light (λ = 625 nm, 140 µmol photons m^{-2} s^{-1}). All plants were dark-adapted for 1 h.

3.4. Effect of the Photoconversion Covers on Plant Photochemistry

Further work was devoted to the registration of photoinduced changes in chlorophyll a fluorescence, representing the efficiency of electron transfer in the photosynthetic electron transport chain and related processes. It was revealed that the maximum quantum yield of the photosystem II photochemistry (Fv/Fm) in all groups of plants was the same, namely, 0.80–0.81, without statistically significant differences between the studied groups of plants. However, other parameters of chlorophyll fluorescence were different in the experimental and control groups of plants (Figure 6). The plants grown under the experimental PCCs had increased the effective quantum yield of photosystem II (Y(II)) by 28–36%. Statistically significant differences between the plants grown under PCC-GO and PCC-GO-Eu_2O_3 were not observed (Figure 6A). The PCCs also increased the electron transport rate of photosystem II (ETR(II)) by 26–35%, without statistically significant differences between the experimental groups of plants (Figure 6B). These changes can indicate a more intense dark stage of photosynthesis in experimental plants. The effective quantum yield of the photosystem I (Y(I)) value in the leaves of the experimental plants differed from that in the control plants only in the first few minutes after turning on the light; then, the differences gradually decreased and disappeared after ten minutes of illumination (Figure 6C). Statistically significant differences between the plants grown under PCC-GO and PCC-GO-Eu_2O_3 were not observed during the illumination. The quantum yield of nonphotochemical quenching of chlorophyll a fluorescence (Y(NPQ)) in the control plants, in contrast to Y(II) and ETR(II), was higher than in the plants grown under PCCs, without statistically significant differences between the two groups (Figure 6D).

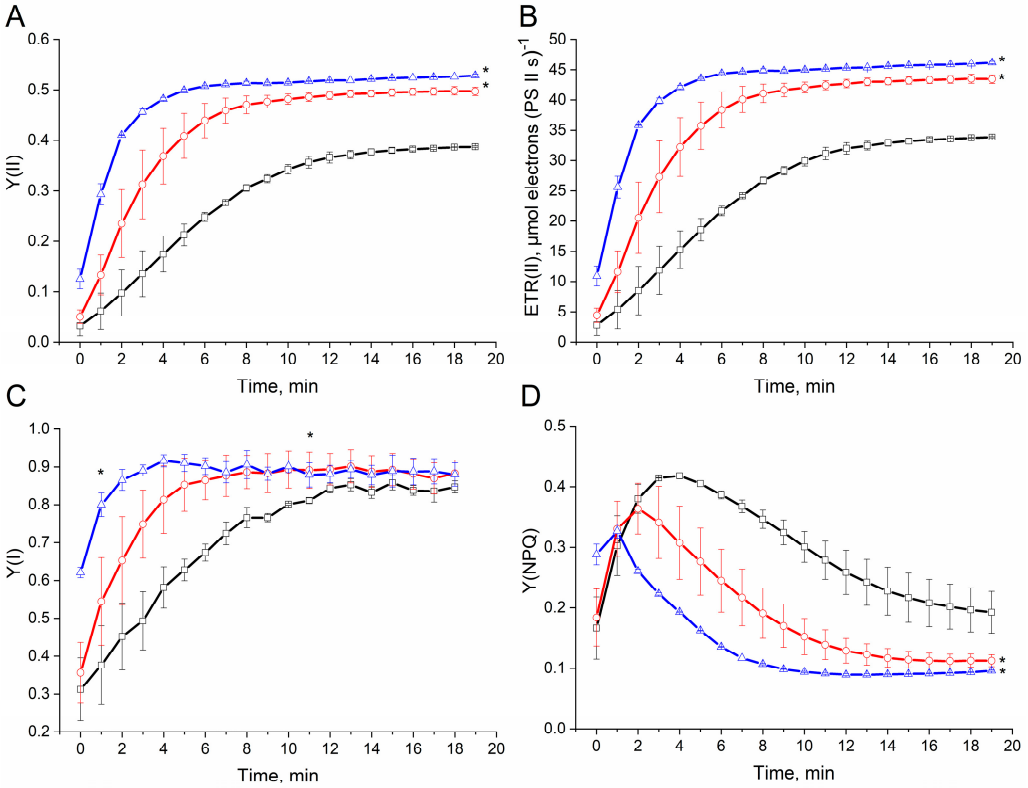

Figure 6. Light-induced changes in the parameters of the effective quantum yield of PSII (**A**), the rate of linear electron transport per PSII reaction center (**B**), the effective quantum yield of PSI (**C**), and the quantum yield of light-induced non-photochemical quenching of chlorophyll a fluorescence (**D**) in the leaves of *S. lycopersicum*. The measurements were performed using plants growing under control covers (black curve), plants growing under PCC-GO (red curve), and plants growing under PCC-GO-Eu_2O_3 (blue curve). Before the measurements, the plants were adapted in the dark for 1 h at 25 °C. The intensity of the 300 ms saturating light pulses was 12,000 µmol photons m^{-2} s^{-1}. * denotes statistically significant differences between the experimental and control plant groups ($p \leq 0.05$).

Thus, the present data indicate that an intensification of photosynthesis in the plants grown under the PCCs led to an increase in plant growth.

4. Discussion

Graphene oxide consists of graphene particles modified at the edges or inside the carbon network with oxygen-containing functional groups in the form of epoxy, hydroxyl, phenolic, carboxyl, ether, and other groups [64]. The mass fraction of oxygen atoms in the GO can vary from 3% to 40%. GO is effectively used in many fields of science, from biomedicine to energy [65–69], as well as in agriculture [70–76]. It is important that nanosized GO particles have now been obtained that are highly biocompatible with mammalian and plant cells [77–80]. At the same time, studies on the influence of GO on plants have focused on the response of the plants to the addition of graphene oxide along with a nutrient solution or when sprayed.

Graphene oxides have very diverse fluorescent properties [81–88]. Depending on the conditions, pre-treatments, and synthesis method, GO can fluoresce in the red and infrared regions (typical of suspensions of freshly synthesized GO particles), in the entire visible range of the spectrum (graphene sheet after treatment with oxygen plasma), or in the blue region (after exposure to ultraviolet light, chemical reducing agents, or thermal annealing in an inert environment), wherein the quantum yield varies on average from 4% to 30% [89–93].

The nanosized GO in our study has intense luminescence in the red and weak luminescence in the blue part of the spectrum, which allowed us to study the effect of

photoconversion covers based on graphene oxide on the growth and photosynthetic activity of tomato plants. Unfortunately, we were unable to find other examples of the application of graphene oxide to create photoconversion covers for greenhouses. This may be the first time this has been done in research.

The effect of the inversion of the luminescence maxima of the photoconversion covers can be explained by the effect of the photothermal reduction of graphene oxide. It was previously shown that the illumination of GO with a xenon lamp leads to an increase in the proportion of carbon atoms linked by sp^2 bonds (C=C) as a result of deoxygenation from 25% to 69% for 3 h, which is accompanied by a shift of the luminescence maximum from the red to the blue region of the spectrum [58]. Thus, we have shown that in the first days of the experiment, the luminescent properties of covers based on graphene oxide were unstable. On the 25th day of the experiment, the short-wavelength luminescence (about 450 nm) increased and the red peak decreased, as a result of which the graphene oxide in the covers became a blue phosphor (Figure 3).

It is known that graphene oxide nanoparticles tend to aggregate and assemble into sheets, wherein the addition of various nanoparticles, including lanthanide oxides such as europium oxide, prevents agglomeration [94]. Thus, we hypothesized that europium oxide nanoparticles could improve the photoconversion properties of the developed graphene oxide-based covers both by improving the structural properties of graphene oxide nanoparticles and adding luminophores, which was effective in the photoconversion cover [60]. However, our assumption was not confirmed, because there were no significant changes in the morphology, physiology, and photochemistry of the tomatoes (Figures 4–6). We also noted that the addition of the Eu_2O_3 nanoparticles to a colloid containing GO nanoparticles practically does not change the luminescent properties of the colloid. Probably, when mixed, GO nanoparticles are adsorbed on the aggregates of the Eu_2O_3 nanoparticles, preventing the excitation and quenching the luminescence of the latter. Previously, in studies on the adsorption of europium ions on graphene oxide, it was found that Eu(III) interacts with the carbonyl, carboxyl, epoxy, and alkoxy groups of graphene oxide [95–97].

The increased solar energy absorption efficiency observed in the tomato plants grown under PCC-GO and PCC-GO Eu_2O_3 (Figure 6) leads to an increased CO_2 assimilation (Figure 5A), which ultimately increases plant productivity (Figure 4). The result obtained may be due to several changes in the lighting spectrum at once. Firstly, the photoconversion covers effectively absorb ultraviolet radiation, which can have a harmful effect on the plants [98,99]. On the other hand, a decrease in UV intensity is accompanied by an increase in PAR intensity (in the red or blue regions at the beginning or end of the experiment, respectively). It is known that plants exhibit maximum photosynthetic activity in red and blue light, and it is red and blue light that most effectively stimulate photosynthesis under low light conditions [100]. Red and blue light are involved in regulating the opening of stomata, thereby increasing the intensity of CO_2 assimilation. However, the exact mechanism of the stomatal movement in response to the red light is still unknown. An increase in the amount of red light can lead to a change in the red light/far-red light ratio and subsequently affect the functioning of the phytochrome system, which can regulate plant growth and influence its resistance when exposed to stress factors [101].

Thus, photoconversion covers based on graphene oxide as a phosphor were created, which have a positive effect on plant growth and photosynthesis. It was revealed that during the experiment, graphene oxide nanoparticles undergo modifications, turning from red to blue, without losing their efficiency.

Author Contributions: Conceptualization, A.V.S. and S.V.G.; methodology, A.V.S., S.V.G. and D.V.Y.; investigation, D.V.Y., M.O.P., R.V.P., I.V.G. and D.V.K.; writing—original draft preparation, M.O.P. and D.V.Y.; writing—review and editing, D.V.Y. and S.V.G.; visualization, M.O.P.; funding acquisition, S.V.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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