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Posted Date: 4 July 2023

doi: 10.20944/preprints202307.0050.v1

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*Article*

# Effects of Abnormal Light/Dark Cycles and Continuous Lighting on Tomato and Eggplant

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**Abstract:** Response of tomato and eggplant to continuous lighting (CL) and abnormal light/dark (L/D) cycles was studied to elucidate the role of the absence of the dark period in the diurnal cycle in the development of leaf injuries under CL. Four light treatments were set: 16/8 h (control), 24/0 h (CL), 6/6 h, and 24/24 h (abnormal L/D cycles). These light treatments provided average daily light integrals of 17.3, 25.9, 13.0 and 13.0 mol $m^{-2}$ $day^{-1}$, respectively. Results of this work have shown that in both tomato and eggplant abnormal L/D cycles caused photoinhibition and leaf injuries similar to those in CL-grown plants. Induced defense mechanisms were not strong enough to contend against oxidative stress caused by light treatments that provided plants with even smaller DLI than 16/8 h photoperiod did. Half time during abnormal L/D cycles were dark periods. Despite these facts, abnormal L/D cycles were injurious. It is concluded that photooxidative stress induced by CL can not be attributed to excessive DLI or the continuity of light itself (the absence of dark periods). Therefore, we believe that the hypothesis suggesting circadian asynchrony to be the main triggering factor of CL-induced leaf injuries is the most plausible among many others proposed.

**Keywords:** photoperiod; continuous lighting; abnormal light/dark cycle; tomato; eggplant

## 1. Introduction

As long ago as in the 1930 it was found that tomato plants exposed to continuous lighting (CL, 24 h photoperiod) develop leaf injury symptoms and eventually die [1]. Then in the 1940$^{th}$ it was observed that low temperature may prevent or alleviate CL-induced injuries [2,3]. It was also shown that even low light can cause injury of leaves if tomato plants are illuminated during 24 h per day [3]. In the 1950$^{th}$Hillman [4] reported that symptoms occurred only in leaves exposed to CL from an early stage in development and plants with 4-7 leaves are injured most quickly while older plants are less sensitive to CL. Hillman also demonstrated that sufficiently wide daily temperature changes can prevent or delay the effects of CL, but he suggested that it is the effect of change, rather than any absolute effect of high or low temperature. Later it was reported that there are other plants sensitive to CL [5–7], while tomato and eggplant appear to be the most sensitive ones. These sensitive species exhibit multiple responses to CL such as leaf chlorosis and necrosis, decreased photosynthetic efficiency, acceleration of leaf senescence [8–10].

This phenomenon attracted attention of the scientists for many reasons. The first one is the remarkable nature of this phenomenon itself. Secondly, as the tomato plant is daylength neutral plant with respect to flowering initiation, it affords an opportunity to study a purely vegetative effect of photoperiod [4]. Thirdly, while some crops exposed to CL displayed photodamages, the others obtained increased yield [8,9]. Thus, CL is considered to be an efficient cultivation strategy, theoretically driving crops to grow better [9–11]. With extension to CL savings possibly could be realized by decreasing the number of light fixtures per unit area and prolonging useful lamp life (no on/off deterioration) [12]. Moreover, it is optimal to use low light intensity and long photoperiods to

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achieve the target daily light integral (DLI)as electricity cost at night is often lower, and heat released by the light fixtures help to meet the heating requirements during the night [13,14]. For plant dry matter production lengthening the photoperiod often is more effective than increasing light intensity [12,15–18].CL is also used in breeding research as it accelerates plant development and therefore shortens the time for crop selection [8]. Moreover, CL is commonly used in the short term as a pre-harvest strategy for the enhancement of crop nutritional value and yield and decrease nitrate content [19–22].

Although the phenomenon of CL-induced leaf injury was described more than 90 years ago, its mechanism is not entirely clear yet, although several hypotheses have been proposed. Excessive carbohydrate accumulation, photooxidative pressure, continuous signalling to the photoreceptors and disruption of circadian clock have been suggested [9]. Later it was reported that an unbalanced photosystem I and photosystem II excitation due to downregulation of the type III light harvesting chlorophyll a/b binding protein 13 (CAB-13) gene expression in tomato leaves is responsible for injury development under CL [23]. There were quite many data reported supporting and opposing suggested hypotheses. For instance, there are evidences that hyperaccumulation of carbohydrates cannot be considered as the main and, moreover, the only cause of damage to plant leaves under CL and long photoperiods [24–27]. On the other hand, the role of this factor in the appearance and development of leaf photodamage cannot be excluded. The CL was suggested to induce photooxidative damage in leaves by the generation of harmful reactive oxygen species (ROS) [9,28], but later it was shown that ROS is not directly involved in CL-induced leaf injury, as both ROS production and scavenging was highest in leaves (without chlorotic symptoms) of plants treated by CL under variable temperature (27/17°C) [25]. While the hypothesis concerning endogenous rhythms suggested in 1954 [29] was attractive for explanation of the phenomenon under consideration and valuable for emphasizing its complex, integrated nature, no physiological and biochemical mechanisms fundamental to it were reported.

In the present study, we studied the response of tomato and eggplants to CL and abnormal light/dark (L/D) cycles to elucidate the role of the absence of the dark period in the diurnal cycle in the development of leaf injuries under CL. The L/D cycle is characterized by two factors that are the length of L/D cycle (period) and the ratio of the illumination time to the dark time (L/D ratio). It is called normal L/D cycle when the L/D cycle period is equal to the diurnal cycle period of 24 h, otherwise it is called abnormal L/D cycle [30].

## 2. Results

### *2.1. Photosynthetic pigments*

Total chlorophyll (Chl) content was decreased by 24/0, 6/6 and 24/24 h light/dark cycles in tomato and eggplant leaves, while carotenoids (Car) were little affected in both species with a trend to increase under photoperiods differed from 16/8 h (Table 1). All abnormal light/dark cycles increased Chl *a/b* ratio and decreased Chl/Car ratio compared to photoperiod 16/8 h. Share of Chl in light-harvesting complex II (LHCII) was also decreased by abnormal light/dark cycles.

**Table 1.** Photosynthetic pigment contents and ratios in tomato and eggplant grown under different light/dark cycles.

| Parameter | Tomato | | | | Eggplant | | | |
|---|---|---|---|---|---|---|---|---|
| | 16/8 h | 24/0 h | 6/6 h | 24/24 h | 16/8 h | 24/0 h | 6/6 h | 24/24 h |
| Chl *a+b*, mg/g DW | 15±1.2 A | 11.5±0.6 B | 9.4±1.4 C | 6.2+0.4 D | 10.6±0.6 a | 9.4±0.2 b | 6.7±0.9 c | 7.1±1.3 c |

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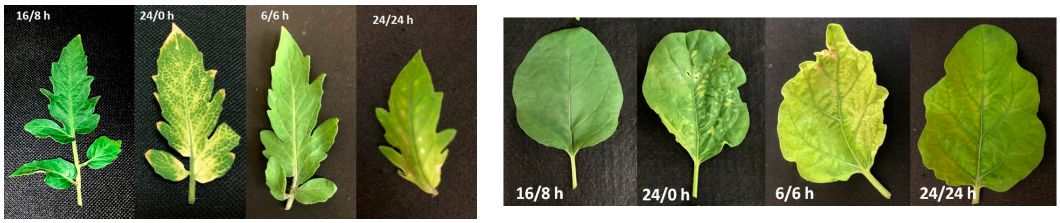
|---|---|---|---|---|---|---|---|---|
| Chl *a*/*b* | 1.5 ±0.1 C | 1.8 ±0.2 C | 2.4 ±0.2 B | 4.5 ±0.2 A | 2.1 ±0.1 c | 2.6 ±0.02 b | 2.4 ±0.2 b | 3.6 ±0.1 a |
| LHCII, % | 87±4 A | 80±3 B | 65 ±4 C | 41 ±1 D | 71±3 a | 62±1 b | 66 ±3 b | 48 ±1 c |
| Carotenoids, mg/g DW | 0.9 ±0.1 B | 0.9 ±0.2 B | 1.1 ±0.1 A B | 1.3 ±0.1 A | 1.0 ±0.1 a | 1.2 ±0.1 a | 1.0 ±0.1 a | 1.2 ±0.2 a |
| Chl/Car | 17.3±1.3 A | 14.4±1.5 B | 8.6 ±0.9 C | 4.9 ±0.2 D | 10.3±0.7 a | 7.7 ±0.2 b | 6.6 ±0.5 c | 5.8 ±0.3 d |

Different letters for each plant species indicate significant differences between the mean values at $p < 0.05$.

Continuous lighting (24/0 h) caused typical for tomato and eggplant injuries. Tomato leaves developed interveinal chlorosis and eggplant leaves were chlorotic with necrotic spots (Figure 1). Exposure of plants to other abnormal light/dark cycles also resulted in leaf injuries. They differed by appearance from those induced by continuous lighting, but certainly leaves were not healthy.




(**a**) (**b**)

**Figure 1.** Second true leaf of (**a**) *Solanum lycopersicum* plants; (**b**) *Solanum melongena* plants grown under different light treatments: 16-h light/8-h dark (16/8 h), 24-h light/0-h dark (24/0 h), 6-h light/6-h dark (6/6 h), 24-h light/24-h dark (24/24 h).

*2.2. Photosynthetic activity and transpiration*

Plants treated by abnormal light/dark cycles had significantly lower values of potential quantum yield of photochemical activity of PSII ($F_v/F_m$), net photosynthesis rate ($A_n$) and transpiration rate (Tr) than untreated plants (Table 2). Stomatal conductance ($g_s$) was decreased only by 6/6 h cycle in tomato. The ratio of intercellular to ambient $CO_2$ concentration ($C_i/C_a$) was decreased by 6/6 h cycle in tomato and 24/24 h cycle in eggplant. For tomato, leaf mass per area (LMA) values were much higher in 24/0, 6/6, and 24/24 h plants than in their 16/8 h counterparts. For eggplant, LMA was unaffected by abnormal light/dark cycles except for 6/6 h cycle, which significantly decreased LMA.

**Table 2.** Photosynthetic parameters of tomato and eggplant grown under different light/dark cycles.

| **Parameter** | **Tomato** | | | | **Eggplant** | | | |
|---|---|---|---|---|---|---|---|---|
| | **16/8 h** | **24/0 h** | **6/6 h** | **24/24 h** | **16/8 h** | **24/0 h** | **6/6 h** | **24/24 h** |
| *Fv*/*Fm* | 0.824± | 0.648± | 0.768± | 0.678± | 0.814± | 0.760± | 0.709± | 0.677± |

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|---|---|---|---|---|---|---|---|---|
| | 0.005 A | 0.038 C | 0.030 B | 0.031 C | 0.010 a | 0.012 b | 0.016 c | 0.007 d |
| $A_n$, µmol $m^{-2}$ $s^{-1}$ | 10.5±0.5 A | 9.9±1.6 A | 6.9±0.8 B | 8.9±0.6 A | 11.8±0.4 a | 3.4±0.2 c | 1.9±0.5 d | 7.4±0.5 b |
| $g_s$, mmol $m^{-2}$ $s^{-1}$ | 112.1±7.5 A | 117.3±21.9 A | 43.6±0.78 B | 109.9±9.7 A | 211.2±20.5 a | 143.2±20.2 b | 83.6±0.3 d | 107.0±6.9 c |
| Tr, mmol $m^{-2}$ $s^{-1}$ | 1.55±0.06 A | 1.35±0.07 B | 0.80±0.09 C | 1.30±0.07 B | 10.3±0.7 a | 7.7±0.2 b | 6.6±0.5 c | 5.8±0.3 d |
| $C_i/C_a$ | 0.62±0.02 A | 0.64±0.03 A | 0.22±0.05 B | 0.58±0.04 A | 0.76±0.03 b | 0.88±0.01 a | 0.87±0.03 a | 0.58±0.03 c |
| LMA, mg $cm^{-2}$ | 2.2±0.2 C | 3.6±0.2 A | 3.2±0.3 AB | 2.9±0.2 B | 2.3±0.3 a | 2.5±0.1 a | 1.3±0.1 b | 2.1±0.1 a |

Different letters for each plant species indicate significant differences between the mean values at $p < 0.05$.

*2.3. Oxidative Stress and Antioxidants*

All plants grown under abnormal light/dark cycles (24/0, 6/6, 24/24 h) had higher $H_2O_2$ content compared to 16/8 h photoperiod (Table 3). The intensity of lipid peroxidation determined by the malondialdehyde (MDA) content was also higher in these plants. Besides, they had higher proline and anthocyanin contents.

The activities of antioxidant enzymes ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) were increased by all abnormal light/dark treatments in tomato and eggplants. The activity of catalase (CAT) and superoxide dismutase (SOD) was decreased by 24/0 and 24/24 h cycles and increased by 6/6 h treatment.

**Table 3.** The malondialdehyde (MDA), $H_2O_2$, anthocyanin, proline contents and catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) enzyme activities in tomato and eggplant grown under different light/dark cycles.

| **Parameter** | **Tomato** | | | | **Eggplant** | | | |
|---|---|---|---|---|---|---|---|---|
| | **16/8 h** | **24/0 h** | **6/6 h** | **24/24 h** | **16/8 h** | **24/0 h** | **6/6 h** | **24/24 h** |
| MDA, µmol $g^{-1}$ DW | 154±14 C | 230±24 B | 293±28 A | 265±25 AB | 126±7 d | 238±38 a | 189±15 b | 143±17 c |

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|---|---|---|---|---|---|---|---|---|
| $H_2O_2$, μmol $g^{-1}$ FW | 0.51±0.08 C | 0.98±0.10 B | 1.19±0.12 B | 2.15±0.31 A | 0.79±0.16 c | 1.39±0.07 a | 0.97±0.28 b | 1.06±0.22 b |
| Anthocyanins, $\Delta A \cdot g^{-1}$ FW | 1.3±0.1 C | 2.5±0.3 A | 2.7±0.2 A | 2.2±0.5 B | 0.33±0.03 c | 0.46±0.08 b | 0.44±0.02 b | 0.69±0.05 a |
| Proline, μmol $g^{-1}$ FW | 5.3±0.6 C | 10.3±2.1 B | 24.3±2.8 A | 29.1+1.7 A | 2.5±0.2 d | 3.4±0.2 c | 4.7±0.7 b | 11.1+0.6 a |
| CAT, μmol $mg^{-1}$ protein $min^{-1}$ | 51.6±5.2 A | 18.4±3.6 B | 61.8±13.1 A | 21.4±2.2 B | 118.7±5.6 b | 37.8±5.3 c | 243.3±25.7 a | 26.0±2.9 d |
| SOD, U $mg^{-1}$ protein | 21.5±3.8 B | 6.8±0.7 C | 34.7±3.6 A | 2.6±0.4 D | 13.7±2.2 b | 6.6±1.1 c | 73.6±12.0 a | 4.2±0.5 d |
| APX, μmol $mg^{-1}$ protein $min^{-1}$ | 101±17 D | 392±15 A | 215±27 B | 144±16 C | 139±13 d | 360±3 a | 216±21 c | 315±10 b |
| GPX, μmol $mg^{-1}$ protein $min^{-1}$ | 52±5 D | 258±15 A | 95±11 C | 130±8 B | 45±9 c | 278±10 a | 58±14 c | 71±13 b |

Different letters for each plant species indicate significant differences between the mean values at $p < 0.05$.

## 3. Discussion

Tomato plant responses to CL are well documented [1,3–5,9,18,23–26,31–42]. There are also some reports on the effects of CL on eggplant [7,28,43,44] and some other species belonging to Solanaceae, Cucurbitaceae, Asteraceae, Brassicaceae, Leguminosae, Convolvulaceae, Laminaceae, Rosaceae, Poaceae, Pinaceae and Taxodiaceae families [22,37,45–50]. In this study we also observed leaf epinasty and the development of interveinal leaf chlorosis in tomato and necrosis in eggplant leaves of CL-grown plants. These plants had lower chlorophyll content, higher chlorophyll *a*/*b* ratio, lower chlorophyll/carotenoid ratio and lower relative chlorophyll content in the light-harvesting complex compared to plants grown under 16/8 h photoperiod. They also had lower values of $F_v/F_m$, $A_n$ and Tr. Both $H_2O_2$ concentration and the intensity of lipid peroxidation estimated by MDA content were increased by CL. The content of antioxidants such as proline and anthocyanins, as well as the activity of some antioxidant enzymes (APX, GPX) were increased. All these changes are aimed to protect photosynthetic apparatus against light stress due to an excess of absorbed light beyond that utilized in photosynthesis. In our experiment CL-treated plants had greater DLI compared to plants grown

 doi:10.20944/preprints202307.0050.v1



under 16/8 h photoperiod (25.9 and 17.3mol $m^{-2}$ $day^{-1}$, correspondingly) and therefore the enhancement of photoprotective mechanisms was rather expected. However, plants grown under abnormal light/dark cycles 6/6 h and 24/24 h also exhibited an entire spectrum of responses that can be regarded as protective and adaptive ones to excessive illumination. These included all aforementioned changes in the pigment complex, PSII efficiency and photosynthesis as well as increased content of $H_2O_2$ and MDA that evidenced oxidative stress in tomato and eggplants. Increased antioxidative capacity was not enough to cope with the oxidative stress caused by abnormal light/dark cycles, which provided plants with even smaller DLI compared to 16/8 h photoperiod (17.3 and 13.0 mol $m^{-2}$ $day^{-1}$, correspondingly). Thus, in our experiments treatments that did not provide excess light energy to plants were injurious for tomato and eggplant causing leaf photodamages similar to those induced by CL.

The causes of leaf injuries and photoinhibition under CL conditions are still the subject of discussion. Some authors state that energy component of light that drives photosynthesis is responsible for the CL-induced injuries [46,51]. They conclude that photooxidative stress induced by CL can be attributed to excessive DLI. The others [24,26,40] suggest that signaling component of light perceived by several photoreceptors is crucial for triggering injury to CL-grown plants. The results reported here were obtained with plants receiving smaller quantities of light in every 48 h period (except for CL-grown plants) compared to non-injurious for tomato and eggplant 16/8 h photoperiod. Plants grown under 6/6 h and 24/24 h light/dark cycles were injured being subjected to the darkness for half the time. Thus, these results clearly show that the cause of leaf injury is not the continuity of light itself and higher DLI is not needed to cause leaf photodamages. This supports previous research [18,24,40] suggested that continuous light signaling, continuous photosynthesis and continuous high oxidative pressure are not prerequisites to induction of leaf photodamages. In fact, all injurious light/dark cycles (24/0 h, 6/6 h and 24/24 h) provided light to the plants during the subjective night, which implies a circadian asynchrony, i.e. the mismatch between the internal (circadian) biorhythms and the external light/dark cycle. All photoperiods longer than 16-18 h would expose plants to light during advanced stages of the scotophile phase (phase of endogenous daily rhythm in which light is inhibitory or has no promoting effect [52]. Therefore, theoretically predicted and observed effects well agreed as there are evidences of developed chlorosis on leaves of cucumber plants grown under 20 h photoperiod [53]. First it was hypothesized [9] that the mechanism by which circadian asynchrony could cause injury involves a mismatch between a fluctuating circadian-controlled protection against photoinhibition and continuous photo-oxidative stress imposed by light. However, later it was shown that light itself upregulates protection to photoinhibition in a circadian-independent way [26].

## 4. Materials and Methods

### *4.1. Plant Material and Growth Conditions*

The seeds of tomato plants (*Solanum lycopersicum* L., hybrid Verlioka plus F1) and eggplants (*Solanum melongena* L., cv. Almaz) were germinated for 2 days in Petri dishes on filter paper moistened with distilled water in the darkness at 28°C. The germinated seeds were planted in 7×7-cm plastic containers with sand and the plants were grown in a Vötsch growth chamber (Germany) at an air temperature of 25°C and air humidity 70%. Plants were supplied with Hoagland complete nutrient solution (pH 6.2–6.4).

### *4.2. Light Treatments*

Light was provided by fluorescent lamps (F36W/T8 BRITEGRO, Sylvania, Germany). Light intensity was 300 μmol $m^{-2}$ $s^{-1}$ of PPFD. The PPFD value was measured using LI-250A Light Meter (Li-COR Biosciences, USA). All trays were systematically rearranged every day to minimize disproportion in light distribution.

 doi:10.20944/preprints202307.0050.v1



Four light treatments were set: (1) 16-h light/8-h dark (16/8 h), (2) 24-h light/0-h dark (24/0 h), (3) 6-h light/6-h dark (6/6 h), (4) 24-h light/24-h dark (24/24 h). These light treatments provided average DLIs of 17.3, 25.9, 13.0 and 13.0 mol $m^{-2}$ $day^{-1}$, respectively.

*4.3. Growth Measurements*

Fully expanded leaves of six plants from each treatment were sampled on the 30$^{th}$ day after sowing for the measurements.

The values of leaf mass per area (LMA) were calculated as a ratio of a dry mass of the lamina discs to their area. Eight discs were cut from each leaf with an 8-mm in diameter cork borer. The dry weight of the discs was determined after their drying to a constant weight at 105°C.

*4.4. Net Photosynthesis, Transpiration and Stomatal Conductance*

The net photosynthesis ($A_n$) rate, transpiration rate (Tr), stomatal conductance ($g_s$), and ratio of intercellular to ambient $CO_2$ concentration ($C_i/C_a$) were measured on the youngest mature leaf using a portable HCM-1000 photosynthetic system (Walz, Germany) at a leaf temperature of 23°C, air humidity of 65-70%, $CO_2$ concentration of 400-420 ppm, and PPFD of 300 μmol $m^{-2}$ $s^{-1}$. The parameters were measured not earlier than 2 h after the start of a light period.

*4.5. Chlorophyll Fluorescence Measurements*

Chl fluorescence parameters of the plants were measured using a Pulse Amplitude Modulation Fluorometer (MINI-PAM, Heinz Walz, Germany). The values of potential quantum yield of photochemical activity of PSII ($F_v/F_m$) were determined after leaves were dark-adapted for 30 min with leaf clips.

*4.6. Photosynthetic Pigment Content*

Five plants per treatment were randomly selected for the following measurements. Content of Chl*a* and *b* and carotenoids (Car) was measured in 96% ethanol extracts with a SF2000 spectrophotometer (Spectrum, Russia) and calculated according to the known formulas [54]. The percentage of Chl in light harvesting complex II (LHCII) was calculated by accepting that almost all Chl*b* is in LHCII, and that the ratio of Chl*a* and *b* in LHCII is 1.2 [55].

The dynamics of the chlorophyll content was express-monitored with a SPAD 502 Plus chlorophyll meter (Konica Minolta, Japan) in the course of an experiment. We previously demonstrated the applicability of this tool for rapid nondestructive assay of chlorophyll in the leaves with interveinal chlorosis [56].

*4.7. Anthocyanins Content*

Anthocyanins were extracted from leaves according to Kang et al. [57]. Fresh leaves tissues (0.1 g) were homogenized in 4 mL of 95% ethanol-1.5 N HCl- (85:15, v:v). After overnight extraction at 4 °C in darkness, each sample was centrifuged at 10,000× *g* for 5 min. The absorbance of the supernatant was measured at 533 nm (peak of absorption of anthocyanin) and 657 nm (peak of absorption of Chl degradation products). The results were plotted as a difference in absorption at 530 and 657 nm relative to tissue fresh weight ($\Delta A \cdot g^{-1}$ FW) and the formula $\Delta A = A530 - 1/4 A657$ was used to deduct the absorbance contributed by chlorophyll and its degradation products in the extract [58].

*4.8. Malondialdehyde (MDA) Content*

The content of malondialdehyde MDA, the end product of lipid peroxidation, was determined with a standard method based on the reaction of these substances with thiobarbituric acid (TBA) that produces a trimethine complex with an absorption maximum at 532 nm [59]. The value for nonspecific absorption of each sample at 600nm was also recorded and subtracted from the absorbance recorded at 532nm. The concentration of MDA was calculated using an extinction

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coefficient of 155$mM^{-1}cm^{-1}$. The lipid peroxidation levels were expressed as micromoles of MDA per gram of FW.

*4.9. Hydrogen Peroxide Content*

Hydrogen peroxide content was determined according to Velikova et al. [60]. Leaf tissues (0.1 g) were homogenized in ice bath with 2 mL 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000× *g* for 15 min at 4 °C and 0.5 mL of supernatant was added to 0.5 mL potassium phosphate buffer (pH 7.0) and 1 mL 1M KI. The absorbance of supernatant was measured at 390 nm. The content of $H_2O_2$ was calculated by comparison with a standard calibration curve.

*4.10. Proline Content*

Free proline content in the leaf tissues was estimated according to Bates et al. [61]. Fresh leaf samples (0.3 g) were homogenized in 6 mL of 3% sulfosalicylic acid and the homogenate was centrifuged at 5100× *g* for 5 min. Then, 2 mL of the supernatant was mixed with 2 mL freshly prepared ninhydrinreagent (1.25 g ninhydrin in 20 mL of 6M phosphoric acid and 30 mL of glacial acetic acid) and 2 mL glacial acetic acid. The colour reaction developed after incubation of the samples for 1 h in a boiling water bath. After warming to 25°C, the absorbance was measured at 520 nm. The concentration of proline was estimated by referring to a standard curve of L-proline and expressed in μmol $g^{-1}$ FW.

*4.11. Antioxidative Enzyme Activity Assays*

For protein and antioxidant enzyme assays, leaf tissues (0.1 g) were ground in 4 mL of 50 mM potassium phosphate buffer (pH 7.8) using a chilled pestle and mortar. The extraction buffer contained 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 14,000× *g* for 20 min at 4°C, and the supernatants thus collected were used for the assays of catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), and guaiacol peroxidase (GPX, EC 1.11.1.7), and protein determinations.

CAT activity was determined using spectrophotometer SF-2000 (OKB Spectr, Russia) by measuring the rate of $H_2O_2$ disappearance at 240 nm [62]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 10.5 mM $H_2O_2$. The reaction was run at 25 °C for 1 min, after adding the enzyme extract and rate of decrease in absorbance at 240 nm (E = 39.4$mM^{-1}cm^{-1}$) was used to calculate the enzyme activity. CAT activity was expressed in μmol $H_2O_2$ per minute *per mg of protein.*

SOD activity assay was based on the measurement of inhibition in the photochemical reduction innitroblue tetrazolium (NBT) spectrophotometrically. The reaction mixture contained 50mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 0.1 μM EDTA, 4 μM riboflavin, and the required amount of enzyme extract. The reaction was triggered by adding riboflavin and placing the tubes underfluorescent lamps for 30 min. A complete reaction mixture without enzyme served as control. A non-irradiated complete reaction mixture served as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction in NBT as monitored at 560 nm according to [63].

APX was assayed by the method described by Nakano, Asada [64]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, and 0.25 mM $H_2O_2$. The reaction was triggered by the addition of $H_2O_2$ after adding the enzyme extract. The decrease in absorbance at 290 nm for 1min was recorded and the amount of APX was calculated from the extinction coefficient 2.8 $mM^{-1}cm^{-1}$.

The activity of GPX was determined spectrophotometrically by measuring the increase in absorbance at 470 nm [65Maehly, Chance, 1954]. The reaction mixture contained 80 mmol/L guaiacol and 10 mmol/L $H_2O_2$ in 0.066 mol/L phosphate buffer (pH = 7.4). The enzymatic reaction was started by adding 0.05 mL of the extract to 2 mL of reaction mixture. The SOD, APX, and GPX *activity* values are expressed as unit U per mg of protein.

The concentration of protein was determined according to Bradford [66] using bovine serum albumin (BSA) (Dia-M, Russia) as a standard.

### *4.12. Data Analysis*

The experiment was performed twice overtime for each species and data were pooled across replications. The figures show mean values and standard errors. Significant differences between the means were revealed at $p< 0.05$ using one-way ANOVA analysis (the least significant difference test).

## 5. Conclusions

Results of this work with sensitive to CL tomato and eggplants have shown that abnormal light/dark cycles (6/6 h and 24/24 h) caused photoinhibition and leaf injuries similar to those in CL-grown plants. Induced defense mechanisms were not strong enough to contend against oxidative stress caused by light treatments that provided plants with even smaller DLI than 16/8 h photoperiod did. Half time during abnormal light/dark cycles were dark periods. Despite these facts, these light/dark cycles were injurious. It is concluded that photooxidative stress induced by CL can not be attributed to excessive DLI or the continuity of light itself (the absence of dark periods). Therefore, we believe that the hypothesis [26] suggesting circadian asynchrony to be the main triggering factor of CL-induced leaf injuries is the most plausible among many others proposed. However, the underlying reason of why a light interruption during the scotophile phase is inhibitory remains to be uncovered.

**Author Contributions:** Conceptualization, T.G.S.; methodology, T.G.S. and E.G.S.; formal analysis, investigation, T.G.S., E.G.S., E.N.I. and A.V.M.; data curation, T.G.S., E.G.S., E.N.I. and A.V.M.; writing—original draft preparation, T.G.S.; writing—review and editing, T.G.S., A.V.M., E.G.S., E.N.I., and A.F.T.; funding acquisition, A.F.T. and T.G.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** Support for this study was provided by RNF 23 23-16-00160.

**Acknowledgments:** This research was carried out using the equipment of the Core Facility of the Karelian Research Center of the Russian Academy of Science.

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

## References

1. Arthur, J.M.; Guthrie, J.D.; Newell J.M. Some effects of artificial climates on thde growth and chemical composition of plants plants. *Amer. J. Bot*. **1930,** 17, 416.
2. Roodenburg, J.W.M. Das Verhalten von Pflanzen in Verschieden Farbigem Licht. *Recueil des Travaux Botaniques Néerlandai* **1949**, *37*, 303-374.
3. Withrow, A.P.; Withrow, R.B. Photoperiodic chlorosis in tomato. *Plant Physiol.* **1949**, *24*, 657–663.
4. Hillman, W.S. Injury of tomato plants by continuous light and unfavorable photoperiodic cycles. *Am. J. Bot.* **1956**, *43*, 89–96.
5. Arthur, J.M. Plant growth in continuous illumination. In *Biological effects of radiation;*Ed. Duggar, B.M.; McGraw-Hill Book Company: New York; 1936; Volume 2, pp. 715–725.
6. Stutte, G.W.; Yorio, N.S.; Wheeler, R.M. Interacting effects of photoperiod and photosynthetic photon flux on net carbon assimilation in potato leaves. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 264-268.
7. Murage, E.N.; Watashiro, N.; Masuda, M. Influence of light quality, PPFD and temperature on leaf chlorosis of eggplants grown under continuous illumination. *Sci. Hortic.* **1997**, *68*, 73–82.
8. Sysoeva, M.I.; Markovskaya, E.F.; Shibaeva, T.G. Plants under continuous light: a review. *Plant Stress* **2010**, *4*, 5–17.
9. Velez-Ramirez, A.I.; van Ieperen, W.; Vreugdenhil, D.; Millenaar, F.F. Plants under continuous light. *Trends Plant Sci*. **2011**, *16*, 310-318.
10. Liu, W.; Zha, L.; Zhang, Y. Growth and nutrient element content of hydroponic lettuce are modified by LED continuous lighting 482 of different intensities and spectral qualities. *Agronomy* **2020**, *10*, 1678.

11. Velez-Ramirez, A.I.; Heuvelink, E.; van Ieperen, W.; Vreugdenhil, D.; Millenaar, F. Continuous light as a way to increase green-468 house tomato production: expected challenges. *ISHS Acta Hortic.* **2012**, *956*, 51-57.
12. Koontz, H.V.; Prince, R.P. Effect of 16 and 24 hours daily radiation (light) on lettuce growth. *Hort. Sci.* **1986,** *21,* 123–124.
13. Hao, X.; Guo, X.; Lanoue, J.; Zhang, Y.; Cao, R.; Zheng, J.; Little, C.; Leonardos, D.; Kholsa, S.; Grodzinski, B.; Yelton, M. A review on smart application of supplemental lighting in greenhouse fruiting vegetable production. *Acta Hortic*. **2018**, *1227*, 499-506.
14. Lanoue, J.; Zheng, J.; Little, C.; Thibodeau, A.; Grodzinski, B.; Hao, X. Alternating red and blue light-emitting diodes allows for injury-free tomato production with continuous lighting. *Front. Plant Sci*. **2019**, *10*, 1114.
15. Kitaya, Y.; Niu, G.; Kozai, T.; Ohashi, M. Photosynthetic photon flux, photoperiod, and $CO_2$ concentration affect growth and morphology of lettuce plug transplants. *Hort. Sci*. **1998,** *33,* 988-991.
16. Ohyama, K.; Omura, Y.; Kozai, T. Effects of air temperature regimes on physiological disorders and floral development of tomato seedlings grown under continuous light. *HortSci.* **2005**, *40*, 1304-1306.
17. Matsuda, R.; Ozawa, N.; Fujiwara, K. Leaf photosynthesis, plant growth, and carbohydrate accumulation of tomato under different photoperiods and diurnal temperature differences.*Sci. Hortic. (Amsterdam)* **2014**, *170*, 150-158.
18. Shibaeva, T.G.; Mamaev, A.V.; Sherudilo, E.G.; Titov, A.F. The role of photosynthetic daily light integral in plant response to extended photoperiods. *Russ. J. Plant Physiol.* **2022**, *69,7.*
19. Zhou, W.L.; Liu, W.K.; Yang, Q.C. Quality changes of hydroponic lettuce under pre-harvest short-term continuous light with different intensity. *J. Hortic. Sci. Biotechnol.* **2012**, 87, 429–434.
20. Bian, Z.-H.; Cheng, R.-F.; Yang, Q.-C.; Wang, J.; Lu, C. Continuous light from red, blue, and green light-emitting diodes reduces 477 nitrate content and enhances phytochemical concentrations and antioxidant capacity in lettuce. *J. Amer. Soc. Hort. Sci.* **2016**, *141*, 478 186.
21. Zhang, Y.; Ntagkas, N.; Fanourakis, D.; Tsaniklidis, G.; Zhao, J.; Cheng, R.; Li T. The role of light intensity in modulating ascorbic acid content during postharvrst tomato ripening: A transcriptomic analysis. *Postharvest Biol. Tec.* 180.
22. Shibaeva, T.G.; Sherudilo, E.G.; Rubaeva, A.A.; Titov, A.F. Continuous LED lighting enhances yield and nutritional value of four genotypes of *Brassicaceae* microgreens. *Plants* **2022**, *11*, 176.
23. Velez-Ramirez, A.I.; van Ieperen, W.; Vreugdenhil, D.; van Poppel, P.M.J.A.; Heuvelink, E.; Millenaar, F.F. A single locus confers tolerance to continuous light and allows substantial yield increase in tomato. *Nat. Commun.* **2014,** *5,* 4549.
24. Haque, M. S.; Kjaer, K. H.; Rosenqvist, E.; Ottosen, C. O. Continuous light increases growth, daily carbon gain, antioxidants, and alters carbohydrate metabolism in a cultivated and a wild tomato species. *Front. Plant Sci.* **2015**, *6*, 522.
25. Haque, M.; de Sousa, A.; Soares, C.; Kjaer, K.H.; Fidalgo, F.; Rosenqvist, E.; Ottosen, C.-O. Temperature variation under continuous light restores tomato leaf photosynthesis and maintains the diurnal pattern in stomatal conductance. *Front. Plant Sci.* **2017**, *8*, 1602.
26. Velez-Ramirez, A.I.; Dunner-Planella, G.; Vreugdenhil, D.; Millenaar, F.F.; van Ieperen, W. On the induction of injury in tomato under continuous light: circadian asynchrony as the main triggering factor. *Funct.Plant Biol*. **2017**, *6*, 597-611.
27. Shibaeva, T.G.; Mamaev, A.V.; Titov A.F. Possible physiological mechanisms of leaf photodamage in plants grown under continuous lighting. *J. Plant Physiol.* **2023,** *70:15.*
28. Murage, E. N.; Masuda, M. Response of pepper and eggplant to continuous light in relation to leaf chlorosis and activities of antioxidative enzymes. *Sci. Hortic.***1997**, *70*, 269–279.
29. Highkin, H.R.; Hanson, J.B. Possible interaction between light-dark cycles and endogenous daily rhythms on the growth of tomato plants. *Plant Physiol.* **1954**, *29*, 301–302.
30. Chen, X.l.; Li, Y.l.; Wang, L.C.; Yang, Q.C.; Guo W.Z. Responses of butter leaf lettuce to mixed red and blue light with extended light/dark cycle period. *Sci Rep* **2022**, *12*, 6924.
31. Ho, L.C. The relationship between rates of carbon transport and of photosynthesis in tomato leaves. *J.Exp.Bot.* **1976,** *27*, 87-97.
32. Bradley, F.M.; Janes, H.W. Carbon partitioning in tomato leaves exposed to continuous light. *Acta Hortic.* **1985,** *174,* 293-302.

33. Logendra, S.; Putman, J.D.; Janes, H.W. The influence of light period on carbon partitioning, translocation and growth in tomato. *Sci. Hort.* **1990,** *42,* 75.
34. Vézina, F.; Trudel, M.J.; Gosselin, A. Influence du mode d'utilisation de l'éclairaged'appoint sur la productivité et la physiologie de la tomate se serre. *Can. J. Plant Sci.* **1991,** *7,* 923-932.
35. Dorais, M.; Yelle, S.; Gosselin, A. Influence of extended photoperiod on photosynthate particioning and export in tomato and pepper plants. *N. Z. J. Crop Hortic. Sci.* **1996**, *24*, 29-37.
36. Globig, S.; Rosen, I.; Janes, H.W. Continuous light effects on photosynthesis and carbon metabolism in tomato. *Acta Hort.* **1997**, *418,* 141-152.
37. Cushman, K.E.; Tibbitts, T.W. The role of ethylene in the development of constant-light injury of potato and tomato. *J. Am. Soc. Hort. Sci.* **1998,** *123,* 239-245.
38. Demers, D.A.; Dorais, M.; Wien, H.C.; Gosselin, A. Effects of supplemental light duration on greenhouse tomato (*Lycopersicon exculentum* Mill.) plants and fruit yields. *Sci. Hort.* **1998**, *74,* 295-306.
39. Demers, D.A.; Gosselin, A. Growing greenhouse tomato and sweet pepper under supplemental lighting: optimal photoperiod, negative effects of long photoperiod and their causes. *Acta Hortic.* **2002**, *580*, 83-88.
40. Haque, M.S.; Kjaer, K.H.; Rosenqvist, E.; Ottosen, C.-O. Recovery of tomato (Solanum lycopersicum L.) leaves from continuous light induced injury. *J. Plant Physiol.* **2015**, *185*, 24-30.
41. Ikkonen, E.N.; Shibaeva, T.G.; Rosenqvist, E.; Ottosen, C.O. Daily temperature drop prevents inhibition of photosynthesis in tomato plants under continuous light. *Photosynthetica,* **2015**, *53*, 389-394.
42. Shibaeva, T.G.; Sherudilo, E.G. Immediate and delayed effects of diurnal temperature drops on growth and reproductive development of tomato plants grown under continuous lighting. *Russ. J. Plant Physiol.* **2015**, *62*, 328-333.
43. Murage, E.; Watashiro, N.; Masuda, M. Leaf chlorosis and carbon metabolism of eggplant in response to continuous light and carbon dioxide. *Sci. Hort*. **1996,** *67,* 27-37.
44. Ikkonen, E.N.; Shibaeva, T.G.; Shedrudilo, E.G.; Titov, A.F. Daily short-term temperature drops can alliviate the negative effects of continuous lighting of the photosynthetic apparatus in plants. *Russ. J. Plant Physiol.* **2023**, *70:79*.
45. Nilwik, H.J.M. Growth analysis of sweet pepper (*Capsicum annuum* L.). Interacting effects of irradiance, temperature and plant age in controlled conditions. *Ann. Bot*. **1981**, *48*, 137-146.
46. Wheeler, R.M.; Tibbitts, T.W. Utilization of potatoes for life support systems in space. I. Cultivar-photoperiod interaction. *Am. Potato J.* **1986**, *63*, 315-324.
47. Cao, W.; Tibbitts, T.W. Physiological responses in potato plants under continuous irradiation. *J. Am. Soc. Hort. Sci.* **1991**, *116*, 525-527.
48. Cushman, K.E.; Tibbitts, T.W.; Sharkey, T.D.; Wise, R.R. Constant-light injury of potato: temporal and spatial patterns of carbon dioxide assimilation, starch content, chloroplast Integrity, and necrotic lesions. *J. Amer. Soc. Hort. Sci.* **1995**, *120,* 1032-1040.
49. Wolff, S.A.; Langerud, A. Fruit yield, starch content and leaf chlorosis in cucumber exposed to continuous lighting. *Eur. J. Hortic. Sci.* **2006,** *71,* 259-261.
50. Hata, N.; Masuda, M.; Kobayashi, A.; Muranaka, T.; Okazawa, A.; Murakami, K. Application of continuous light in a plant factory system 3. Moderation of injuries induced by continuous light and relative tolerance to continuous light. *J. Shita* **2011**, *23*, 137-143.
51. Zha, L.; Liu, W.; Zhang, Y.; Zhou, C.; Shao, M. Morphological and physiological stress responses of lettuce to different intensities of continuous light. *Front. Plant Sci.* **2019**, *10,* 1440.
52. Highkin, H.R.; Hanson, J.B. Possible interaction between light-dark cycles and endogenous daily rhythms on the growth of tomato plants. Plant Physiol. **1954**, *29*, 301-302.
53. Shibaeva, T.G.; Markovskaya, E.F. Growth and development of cucumber *Cucumis sativus* L. in the prereproductive period under long photoperiods. *Russ. J. Dev. Biol.* **2013**, *44*, 78-85.
54. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.***1983**, *603*, 591–592.
55. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol*. **1987**, *148*, 350–382.
56. Shibaeva, T.G.; Mamaev, A.V.; Sherudilo, E.G. Evaluation of a SPAD-502 PLUS chlorophyll meter to estimate chlorophyll content in leaves with interveinal chlorosis. *Russ. J. Plant Physiol.* **2020**, 67, 690-696.

57. Kang, J.H.; Sugumaran, K.; Atulba, S.L.S.; Jeong, B.R.; Hwang, S.J. Light intensity and photoperiod influence the growth and development of hydroponically grown leaf lettuce in a closed-type plant factory system. *Hort. Environ. Biotechnol.* **2013**, *54*, 501–509.
58. Meng, X.; Xing, T.; Wang, X. The role of light in the regulation of anthocyanin accumulation in Gerbera hybrid. *Plant Growth Regul*. **2004**, *44*, 243–250.
59. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198.
60. Velikova, V.; Yordanov, I.; Edreva, A. Oxidative stress and some antioxidant system in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* **2000**, *151*, 59–66.
61. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207.
62. Aebi, H. Catalase in vitro. *MerhodsEnzymol*. **1984**, *105*, 121-126.
63. Giannopolitis, C.N.; Ries, S.K. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol*. **1977**, *59*, 309–314.
64. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. **1981**, *22*, 867–880.
65. Maehly, A.C.; Chance, B. The assay catalases and peroxidases. In *Methods of Biochemical Analysis*; Glick, D., Ed.; Interscience Pub., New York, **1954;** Volume 1, pp. 357-424.
66. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976,** *72*, 248–254.