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Article

Response of Native and Non-native to Subarctic Plant Species to Continuous Illumination by Natural and Artificial Light

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Abstract: The study addressed the following questions: How does continuous lighting (CL) impact plant physiology, photosynthetic and stress response? Does the impact of CL depend on the source of light and other environmental factors (natural vs artificial)? Do responses to CL differ for native and non-native plant species in the Subarctic and, if differences exist, what physiological reasons might they be associated with? Experiments were conducted with three native to Subarctic (*Geranium sylvaticum* L., *Geum rivale* L., *Potentilla erecta* (L.) Raeusch.) and three non-native plant species (*Geranium himalayense* Klotzsch, *Geum coccineum* Sibth. & Sm., *Potentilla atosanguinea* Loddiges ex D. Don) introduced in Polar-Alpine Botanic Garden (KPABG, 67°38'N). Obtained results have shown that plants lack specific mechanisms of tolerance to CL. Protective responses are non-specific and induced by developing photo-oxidative stress. In climate chambers under constant environmental conditions artificial CL causes leaf injuries due to oxidative stress, the main cause of which is circadian asynchrony. In nature plants are not photodamaged during the polar day as endogenous rhythms are maintained due to daily fluctuations of several environmental factors (light intensity, spectral distribution, temperature and air humidity). The obtained data show that among possible non-specific protective mechanisms, plants use flavonoids to neutralize excess ROS generated under CL. In local subarctic plants, their photoprotective role is significantly higher than in non-native introduced plant species.

Keywords: subarctic; native; non-native plants; photoperiod; continuous lighting; natural and artificial light

1. Introduction

All living organisms are constantly exposed to various environmental factors and their fluctuations. The most vulnerable to their effects are sessile organisms such as terrestrial plants that are not capable of moving in space. This means that their survival is directly dependent on the reliability and effectiveness of the protective and adaptive mechanisms that they have acquired in the course of long-term evolution. Some of them are general (non-specific), and some are specific. The first of them are the most universal and therefore are used more often by different plants in a wide variety of natural climatic conditions. It is not surprising that many plant species are characterized by significant numbers and large habitat areas, which include territories with significantly different environmental conditions. Since plants are constantly exposed to fluctuating environmental factors, this forces them to activate, depending on the situation, one or another part of their arsenal of adaptive responses. This is convincingly demonstrated by the results of numerous studies on the effects of such environmental factors as temperature, moisture supply and light, which

are considered to be the main ones for the plant life. The third of them (light) has been given, as it seems to us, somewhat less attention in recent decades than it was before, but with the advent of LED light sources and plant factories with artificial lighting (PFAL), the interest in this factor has increased significantly.

Three principal characteristics of light affect plant growth: quantity, quality and duration. Photoperiod quantifies the amount of time a plant is exposed to light, usually within a 24-hour circadian cycle. Photoperiod exerts profound effects on the growth and flowering of many plant species, and manipulation of daylength is essential for scheduling several greenhouse crops. Under natural conditions, the duration of daylight varies considerably over the Earth's surface, and is dependent on season and latitude. The duration of daylight for 67°44'N (the Kola Peninsula) (Figure 1) is presented in Table 1. At this latitude, the duration of daylength during the vegetation season (May-September) varies from about 18 hours the early May to 24 hours in June and early July and 12 hours in late September. The polar day, when the Sun remains visible at midnight, lasts at these latitudes about 50 days (from late May to mid-July) (Figure 2). This natural phenomenon occurs in the summer months in places north of the Arctic Circle or south of the Antarctic Circle. Moreover, at these latitudes the period when civil twilight lasts all night (also referred as white night) takes place almost a month before the beginning of the polar day and as many after. At this time the Sun does not sink low below the horizon and darkness does not occur. It turns out that plants in this region are exposed to continuous lighting (CL) for more than 3 months.

However, too much light can be harmful to plants as it significantly reduces the efficiency of photosynthesis which can lead to photoinhibition causing damage to photosystem II (PSII) [1]. Long photoperiods can also be harmful to plants. While the photoperiodic threshold is different for each plant species, generally, photoperiods longer than 17 hours can cause leaf damage observed as interveinal chlorosis and plants grown under CL tend to have higher carbohydrate and reactive oxygen species (ROS) levels which may lead to down-regulation of photosynthesis [2,3]. Theoretically, the implementation of CL strategy can increase yield if photoperiod-related injury is prevented [4,5]. Long photoperiods of lower light intensities could be used to achieve the desired daily light integral (DLI) with lower installed light capacity/capital costs and low electricity costs in regions with low night electricity prices [6]. Therefore, the application of CL in controlled environment agriculture (CEA) is a particular issue of interest to both plant scientists and commercial plant producers [7].

The underlying mechanism of CL-injury is unknown. Current hypotheses include (a) a mismatch between the endogenous circadian rhythm and exogenous environmental cues [8,9], (b) improper gene expression [10,11], and (c) over-accumulation of photosynthetic products leading to feedback inhibition [7,12,13]. Without question, growth under CL can cause plants to undergo a stress response. However, the extent to which the abiotic factor causing the stress response becomes harmful to the plant as opposed to beneficial is difficult to quantify [6].

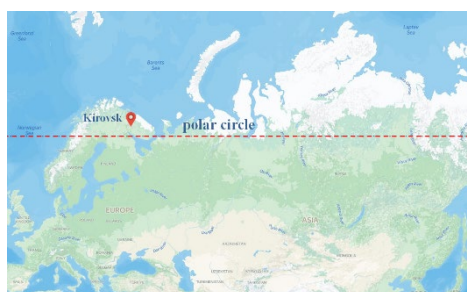


Figure 1. Location of the study site. Polar-Alpine Botanic Garden (KPABG), Kirovsk, Russia (67°38'N).

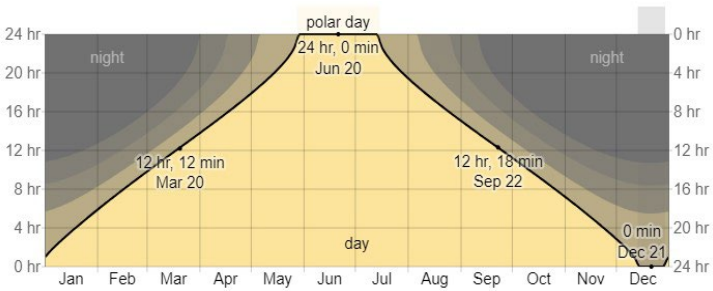


Figure 2. Hours of daylight and twilight in Kirovsk (67°38'N) © WeatherSpark.com.

The number of hours during which the Sun is visible (black line). From bottom (most yellow) to top (most gray) the color bands indicate: full daylight, twilight (civil, nautical, and astronomical) and full night.

Table 1. Day length in Khibiny Mountains (67°44'N), hour.

Days	May	June	July	August	September
1-5	17-42	23-07	24-00	18-41	14-38
6-10	18-24	24-00	23-59	17-57	14-00
11-15	19-08	24-00	22-44	17-18	13-22
16-20	19-53	24-00	21-27	16-40	12-42
21-25	20-46	24-00	20-29	16-02	12-04
26-30	21-52	24-00	19-35	15-09	11-28
Average by month	19-38	24-00	22-02	17-00	13-02

The use of artificial light generates light environments that are usually very different from the natural, sunlight-illuminated environments under which plants originally evolved [8]. Plants growing in natural environments experience diurnal fluctuations in irradiance and light quality, with gradual shifts between light and dark at dawn and dusk [14,15]. In contrast, experiments in controlled environment chambers typically expose plants to a constant irradiance during the day, and abrupt transitions between light and dark at dawn and dusk. Growth chambers do offer some advantages: the experiments are easily reproducible and allow responses to a single factor (e.g., day length, irradiance, or temperature) to be investigated [16,17]. In the strict sense, CL does not naturally occur [3], not even during the summer in arctic regions, because during the polar day considerable variations in light intensity and spectral distribution take place. However, for simplicity, we will refer to the lighting conditions during the polar day as CL.

In this study, we conducted an experiment to test the effect of CL on the responses of three native and three non-native to Subarctic plant species (Figure 3). We assumed that native plants have evolved over thousands of years to be adapted to specific lighting conditions of the northern polar region. Non-native plants were introduced to the Polar Alpine Botanic Garden (67°38'N), which is one of the three botanical gardens in the world located beyond the Arctic Circle. They originated from regions where CL conditions never exist and therefore should not have been adapted to CL. Thus, we observed plants that grew under natural CL during the polar day and plants that were placed in controlled environment chambers with 24 h photoperiod. We asked the following questions: (1) How does CL impact plant physiology, photosynthetic and stress response? (2) Does the impact of CL depend on the source of light and other environmental factors (natural vs artificial)? (3) Do responses to CL differ for native and non-native plant species in the Subarctic and, if differences exist, what physiological reasons might they be associated with?

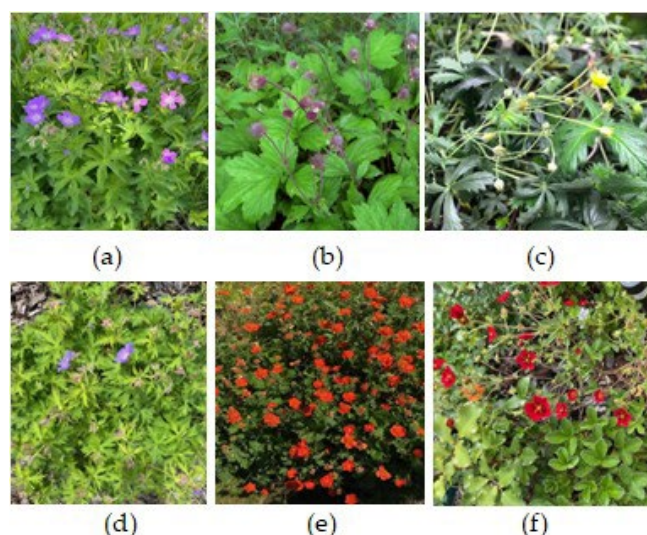


Figure 3. Plants under study. Native to Subarctic plant species: **(a)** *Geranium sylvaticum* L., **(b)** *Geum rivale* L., **(c)** *Potentilla erecta* (L.) Raeusch.; non-native plant species: **(d)** *Geranium himalayense* Klotzsch, **(e)** *Geum coccineum* Sibth. & Sm., and **(f)** *Potentilla atosanguinea* Loddiges ex D. Don.

2. Results

2.1. Plants under Artificial Lighting

Plants grown in climate chambers and treated by A-CL had significantly lower values of potential quantum yield of photochemical activity of PSII (F_v/F_m) compared to A-16 h plants (Table 2). Values of F_v/F_m varied between 0.691 and 0.806 for A-CL plants.

Leaf mass per area (LMA) values were much greater in A-CL plants than in their A-16 h counterparts. LMA values of A-CL-treated plants were 7-53% higher for native species and 52-66% for non-native species (Table 2).

Total chlorophyll (Chl) content was decreased in A-CL-treated plants by 9-38% in native and 19-53% in non-native plant species. Carotenoids were little affected by A-CL in native plant species, but their content decreased in non-native *G. coccineum* and *P. atosanguinea*. A-CL increased Chl *a/b* ratio and decreased Chl/carotenoids ratio in plants, although not all the differences were significant. Share of Chl in light-harvesting complex II (LHCII) was decreased by A-CL treatment in *G. sylvaticum*, *G. rivale* and *P. atosanguinea*.

All plants grown under A-CL had higher H_2O_2 content compared to A-16 h plants. Thus, it increased by 39-138% in native and by 37-68% in non-native plants (Table 2). The intensity of lipid peroxidation was also higher in these plants. The MDA content increased by 27-93% in native and by 45-88% in non-native plant species. Besides, they had higher flavonoid and anthocyanin contents. Native and non-native plants had 27-83% and 5-56% higher content of flavonoids, correspondingly. Anthocyanin content showed a broad range of species-specific variation. The A-CL-induced increase in anthocyanins content varied between 18% in *P. erecta* to 5.3- and 8-fold increase in *G. sylvaticum* and *P. atosanguinea*.

Analysis of the daily course of stomatal conductance (g_s) of plants showed that after two weeks of exposure to A-CL under constant temperature and air humidity the variation range of g_s significantly decreased compared to that at A-16 h treatment by 2.6-6 times. The maximum and minimum g_s values in the daily cycle of A-16 h and A-CL plants are presented in the Table 3. As an example, the daily course of g_s in *P. atosanguinea* is shown on Figure 4. The other species demonstrated similar trends.

Table 2. Physiological and biochemical parameters of native and non-native plant species exposed for 2 weeks to artificial lighting with 16 h (A-16 h) or 24 h (A-CL) photoperiod.

Parameter	Photoperiod	<i>Geranium sylvaticum</i>	<i>Geranium himalayense</i>	<i>Geum rivale</i>	<i>Geum coccineum</i>	<i>Potentilla erecta</i>	<i>Potentilla atosanguinea</i>
LMA, mg/cm ²	A-16 h	3.52b	3.00b	4.78b	4.63b	4.94b	5.25b
	A-CL	5.37a	4.98a	6.87a	7.3a	5.29a	7.96a
F_v/F_m	A-16 h	0.766a	0.778a	0.783a	0.782a	0.823a	0.804a
	A-CL	0.691b	0.755b	0.773a	0.753b	0.806b	0.741b
Chl, SPAD	A-16 h	28.3a	37.9a	44.6a	40.2a	50.4a	49.6a
	A-CL	26.0a	33.8a	43.9a	22.5b	49.8a	37.2b
Chl <i>a+b</i> , mg/g DW	A-16 h	3.60a	3.87a	8.21a	7.96a	8.98a	7.11a
	A-CL	2.64b	3.14a	5.12b	2.39b	8.13a	3.32b
Chl <i>a/b</i>	A-16 h	2.3b	2.9a	2.5a	2.4a	2.0a	3.15b
	A-CL	2.6a	3.0a	2.6a	2.6a	2.2a	3.34a
Carotenoids, mg/g DW	A-16 h	0.51a	0.68a	1.0a	0.98a	0.72a	1.02a
	A-CL	0.49a	0.69a	0.7a	0.35b	0.56a	0.61b
Chl/Carotenoids	A-16 h	7.1a	5.7a	8.6a	8.1a	12.5a	7.0a
	A-CL	5.4b	4.6a	6.8b	6.8b	14.5a	5.0b
LHCII, %	A-16 h	69.9a	56.4a	64.0a	67.9a	79.3a	54.4a
	A-CL	60.4b	54.8a	51.6b	64.7a	81.7a	48.4b
Anthocyanins, mg/mg FW	A-16 h	0.80b	1.98b	0.98b	1.06b	0.59b	0.78b
	A-CL	4.23a	3.94a	1.95a	3.73a	0.70a	6.23a
Flavonoids, mg/mg FW	A-16 h	271.60b	404.3b	191.2b	189.9b	85.0b	205.1b
	A-CL	497.40a	632.5a	243.1a	233.7a	140.5a	215.9a
MDA, μ mol/g FW	A-16 h	53.9b	51.4b	45.6b	93.1b	52.9b	45.4b
	A-CL	79.0a	76.4a	88.0a	175.3a	67.3a	65.9a
H_2O_2 , μ mol/g FW	A-16 h	1.36b	1.10b	0.78b	1.47b	1.06b	0.89b
	A-CL	2.58a	1.85a	1.86a	2.02a	1.47a	1.44a

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

Table 3. Daily variation (maximum/minimum, mmol/m² s) of stomatal conductance (g_s) in native and non-native plant species exposed for 2 weeks to artificial lighting with 16 h (A-16 h) or 24 h (A-CL) photoperiod and exposed to natural 16 h photoperiod (N-16 h) in the second decade of August or natural continuous lighting (N-CL) in the end of June in Polar-Alpine Botanic Garden (KPABG, 67°38'N).

Plant name	Maximum/minimum daily g_s , mmol/(m ² s)			
	A-16 h	A-CL	N-16 h	N-CL
<i>Geranium sylvaticum</i>	229/142	312/275	996/330	686/244
<i>Geranium himalayense</i>	363/90	297/188	711/267	645/248
<i>Geum rivale</i>	440/250	395/320	845/304	776/286
<i>Geum coccineum</i>	215/98	123/101	477/183	450/197
<i>Potentilla erecta</i>	507/160	521/223	812/195	790/217
<i>Potentilla atosanguinea</i>	342/114	297/260	995/302	806/124

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

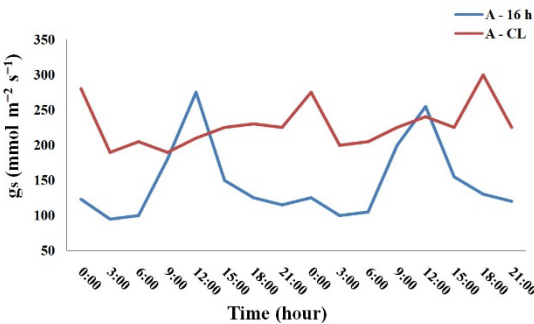


Figure 4. The daily course of stomatal conductance of A-16 and A-CL leaves of *Potentilla atrosanguinea*.

Exposure of plants to A-CL resulted in leaf injuries of varying degrees. In some plants, such as *P. erecta* and *G. rivale* the leaves demonstrated just slight degreening. Injuries of leaves of *G. sylvaticum*, *G. himalayense*, *G. coccineum* and *P. atrosanguinea* were seen as yellow discoloration with tissue necrosis along the margins. Some of the leaves of *G. sylvaticum* and *G. coccineum* have turned rusty red (Figure 5).

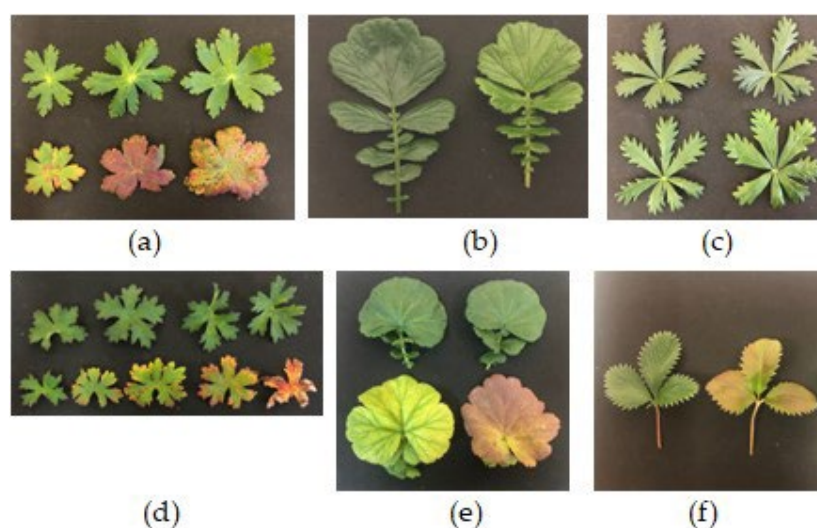


Figure 5. Leaves of plants exposed to A-16 (top on (a), (c), (d), (e) and left on (b), (f)) and A-CL (bottom on (a), (c), (d), (e) and right on (b), (f)) for 2 weeks. (a) *Geranium sylvaticum*, (b) *Geum rivale*, (c) *Potentilla erecta*, (d) *Geranium himalayense*, (e) *Geum coccineum* and (f) *Potentilla atrosanguinea*.

2.1. Plants under Natural Lighting

Plants exposed to N-CL during the polar day did not have any visible leaf injuries. Much later, in August, when days were shorter, we observed age-dependent injuries that were similar to those seen on A-CL-treated leaves (Figure 6).

During the polar day N-CL plants had significantly similar or slightly lower values of F_v/F_m compared to N-16 h plants (Table 4). However, the lowest recorded value of F_v/F_m was 0.794, which means that the PSII reaction centers were not damaged and N-CL-induced photoinhibition did not occur either in native or non-native plants.

Leaf mass per area (LMA) values were higher by 7-52% and 12-56% in N-CL-treated native and non-native plants, correspondingly, than in their N-16 h counterparts (Table 4).

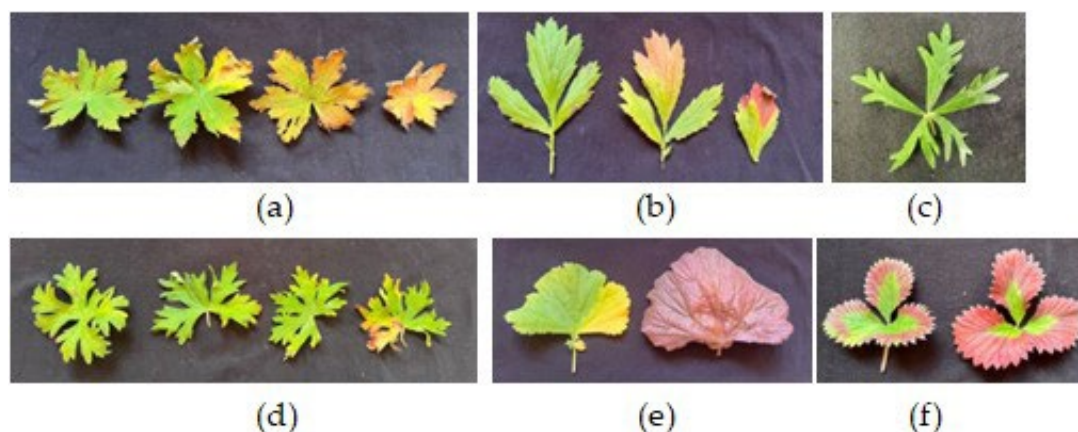


Figure 6. Leaf color pattern in aging N-16 h leaves. **(a)** *Geranium sylvaticum*, **(b)** *Geum rivale*, **(c)** *Potentilla erecta*, **(d)** *Geranium himalayense*, **(e)** *Geum coccineum* and **(f)** *Potentilla atrosanguinea*.

All N-CL plants showed a decreased in Chl content to varying degrees, although the most pronounced decrease was observed in non-native plant species (Table 4). N-CL-induced increase in Chl *a/b* and decrease in the share of Chl in LHCII were recorded for all plant species. Carotenoid content was decreased by N-CL by 15-26% in native plant species and by 40-86% in non-native plants. The ratio Chl/carotenoids was little affected by N-CL in native plants, but significantly decreased in non-native plants.

All plants grown under N-CL had higher H₂O₂ content compared to A-16 h plants. It increased by 22-47% in native plants and by 3-90% in non-native plants (Table 4). The MDA content increased by 7-22% in native and by 6-22% in non-native plant species.

Flavonoid content changes in response to N-CL significantly varied between plants in a species-specific manner. For instance, N-CL reduced their content by 20% in *G. himalayense*, but increased by 70% in *P. erecta* (Table 4).

Under natural conditions, the daily course of stomatal activity did not differ significantly between N-16 h and N-CL plants and the difference between maximum and minimum *g_s* values in the daily cycle was only 2-12% smaller in N-CL plants (Table 3). It means that under polar day conditions natural CL did not make the sinusoidal pattern of the daily course of *g_s* to be flatter as the artificial CL in climate chambers did. The differences between aboriginal plant species and introduced plants were species-specific in nature and did not allow to identify any pattern.

Table 4. Physiological and biochemical parameters of native and non-native plant species exposed to natural 16 h photoperiod (N-16 h) in the second decade of August or natural continuous lighting (N-CL) in the end of June in Polar-Alpine Botanic Garden (KPABG, 67°38'N).

Parameter	Photoperiod	<i>Geranium sylvaticum</i>	<i>Geranium himalayense</i>	<i>Geum rivale</i>	<i>Geum coccineum</i>	<i>Potentilla erecta</i>	<i>Potentilla atrosanguinea</i>
LMA, mg/cm ²	N-16 h	3.83b	3.77b	3.23a	4.82b	3.95b	5.57b
	N-CL	4.74a	4.23a	3.47a	7.50a	5.99a	6.81a
<i>F_v/F_m</i>	N-16 h	0.805a	0.821a	0.820a	0.813a	0.820a	0.808a
	N-CL	0.795a	0.802b	0.818a	0.794b	0.821a	0.800a
Chl, SPAD	N-16 h	22.8a	41.3a	43.3a	40.2a	40.8a	45.1a
	N-CL	20.6a	33.4b	38.3a	26.5b	45.1a	38.9b
Chl <i>a+b</i> , mg/g DW	N-16 h	3.21a	5.24a	3.03a	6.43a	7.28a	4.51a
	N-CL	2.71b	2.26b	1.98b	2.27b	6.47b	1.24b
Chl <i>a/b</i>	N-16 h	2.6b	3.0b	3.0a	1.9b	1.8b	2.9b
	N-CL	3.4a	3.6a	3.4a	2.5a	2.5a	3.7a
Carotenoids, mg/g DW	N-16 h	0.52a	0.68a	0.50a	0.57a	0.82a	0.77b
	N-CL	0.44b	0.41b	0.37a	0.28b	0.62b	0.66a
Chl/Carotenoids	N-16 h	6.2a	7.8a	6.1a	11.3a	8.9a	5.9a
	N-CL	6.3a	5.5b	5.4a	8.1b	10.4a	1.9 b
LHCII, %	N-16 h	61.2a	6.1a	55.0a	77.9a	78.5a	56.8a
	N-CL	50.5b	47.8b	49.7a	51.2b	61.0b	42.6b
Anthocyanins, mg/mg FW	N-16 h	1.18a	0.84a	0.87a	0.82b	1.17a	0.98a
	N-CL	1.04a	0.95a	1.02a	1.17a	1.07a	0.76b
Flavonoids, mg/mg FW	N-16 h	254.7a	354.1a	279.8b	334.8a	82.1b	131.4b
	N-CL	277.5a	283.6b	343.1a	351.3a	139.0a	215.7a
MDA, μmol/g FW	N-16 h	57.1b	37.0b	101.2b	61.3a	52.0a	38.1a
	N-CL	69.7a	45.0a	120.9a	72.8b	55.7a	40.3a
H ₂ O ₂ , μmol/g FW	N-16 h	4.48b	2.10b	1.9b	1.62b	1.82b	1.76a
	N-CL	6.42a	4.01a	2.8a	2.54a	2.22a	1.82a

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

3. Discussion

In this study we investigated plant responses to CL provided by artificial light sources and under natural conditions. Plants under study exposed to A-CL in the climate chamber where other parameters were held constant exhibited an entire spectrum of responses aimed at protection and adaptation to excessive illumination as were observed in many crop species exposed to artificial CL in growth chambers and PFALs [2,3,18–36]. These included degreening, higher Chl *a/b* ratio, lower Chl/carotenoid ratio and lower relative chlorophyll content in the LHCII compared to plants grown under 16 h photoperiod. Elevated H₂O₂ and MDA levels indicated the development of oxidative stress in these plants, and the increased antioxidant capacity was insufficient to cope with the oxidative stress induced by A-CL. The contents of flavonoids and anthocyanins which protect plant tissues from excess radiation were increased. In our experiments A-CL-treated plants had greater DLI compared to plants grown under A-16 h conditions (21.6 and 14.4 mol/(m² day), correspondingly) and therefore the enhancement of photoprotective mechanisms was rather expected. Some decrease in *F_v/F_m* values was also observed, suggesting a possible decrease in photosynthetic activity, although most species showed an increase in LMA values, indicating an accumulation of assimilates in leaves, presumably as a result of their hindered export under CL conditions due to an imbalance between source and sink strengths [3,10,18,35]. Exposure to CL means plants are under constant photon pressure which continuously drives photosynthesis. Constant photosynthesis means continuous production of photosynthetic products such as soluble sugars and starch [13,36].

Excessive accumulation of carbohydrates can cause damage to chloroplast membranes, which inevitably leads to a downregulation of photosynthesis through a feedback mechanism caused by over-reduction of the electron transport chain components [37,38]. Many authors also believe that the accumulation of photosynthetic products during CL affects the expression of genes involved in the control of photosynthesis, and ultimately this leads to complete or partial inhibition of this process [39–42].

Under natural conditions during the polar day leaves of N-CL plants looked healthy, but later in August we observed changes in leaves associated with senescence (Figure 6). The symptoms were similar to those observed on A-CL leaves (Figure 5). Color changes of aging leaved were due to progressive loss of Chl coinciding with the partial retention of carotenoids, new synthesis of anthocyanins and the formation of dark oxidation products of phenolics. Thus, leaf photodamages induced by A-CL were actually symptoms of accelerated leaf senescence. Although the regulation of leaf senescence is age dependent it is heavily influenced by internal and environmental stimuli. It is thought that one of the processes that is involved in the control and response to senescence regulatory networks is that of sugar status. A high level of sugar in plants has been shown to both decrease the levels of photosynthetic activity and to initiate the senescence process. Our results are consistent with other research results [10,43–45] suggesting that a higher nighttime light intensity without a change in spectrum can raise the carbohydrate levels in plants and accelerate leaf senescence that are associated with CL-injury.

Why were the leaves not injured by N-CL during the polar day? Velez-Ramirez et al. [3] rightly noted that in the strict sense, CL does not naturally occur. In natural conditions even during the polar day in the absence of complete darkness light intensity and spectral distribution considerably vary. Irradiance from sunlight changes in a sinusoidal manner during the day (Figure 7). As for the spectral fluctuations, it was shown long ago that they set the circadian clock of arctic birds [46]. Great difference between spectral quality of artificial and natural light may be one of the reasons why plants did not become injured under N-CL. Artificial lighting produce light spectra with very uneven wavelength distributions, with sharp peaks around the main emission wavelengths and little or no light in between. In contrast, the visible light spectrum of sunlight is much more homogeneous [47]. Earlier experiments have shown that if CL was partially or totally provided by solar light, the injury was reduced or even absent [2,3,18,48,49]. Thus, the question was risen if the CL-induced injury is caused by the continuity of light itself or by an interaction between photoperiod and light spectral distribution?

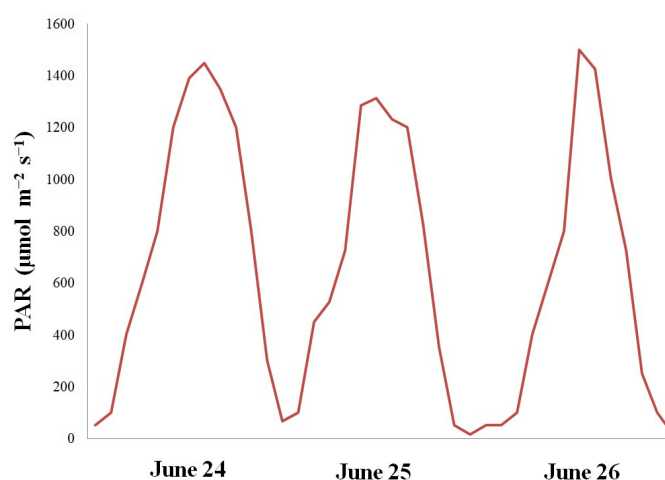


Figure 7. Photosynthesis active radiation (PAR) measured during the polar day on June 24-26, 2024 at Kirovsk, Russia (67°38'N).

We add to this that temperature fluctuations under CL can also set the circadian clock in plants under artificial CL [2,3,18,24,25,36,44,53]. As is known, in natural conditions the temperature fluctuates during the day/night cycle, whereas in most experiments in climate chambers the temperature is maintained at a constant level. Thus, results of CL treatments when other environmental parameters are held constant should be interpreted taking proper care.

It was shown by many authors that plants grown in controlled environments can show significantly different phenotypes from plants of the same genotype grown in more natural conditions or in the field [14,15,54–56]. However, the lack of information does not allow us to clearly judge which of the parameters that distinguish the natural and artificial environments have the greatest impact on plant metabolism and growth [47]. A unique series of experiments was carried out in the 1930s-1960s by the founders and first researchers worked for the Polar-Alpine Botanical Garden in (KPABG, Kirovsk, Russia, 67°38' N). They studied responses to CL during the long polar day in indigenous plants and plants that were introduced from other parts of the world. Non-native plants were brought over for agricultural, medicinal and ornamental purposes. In order to exclude the influence of other environmental factors, plants were tested under either natural day length (24 hours) or shortened day length (8, 10, 12 or 14 hours). Daylight hours were shortened by shading using specially designed photoperiodic cabins that moved along rails. The cabin area was 6 m², the height was 1.2 m. As many as 37 species of trees and shrubs and 21 species of herbaceous plants were tested. The effects of the long summer daylight on transpiration, photosynthesis, chemical composition, mineral uptake as well as rhythms of growth and development were investigated and reported. The reports on the experiments conducted have been preserved and are currently available in the archives of the Kola Research Center of the Russian Academy of Sciences. We did not find any mention of leaf injuries due to CL in them, although a slight change in color was sometimes noted. It is what we also observed in our research.

It is known that exposure of plants to CL can significantly increase the photo-oxidative stress [2,3]. ROS are a normal by-product of photosynthesis, but when generated in large quantities during periods of high irradiance or prolonged light exposure (e.g., CL), they can be harmful to plants. In particular, excessive ROS accumulation can cause severe and irreversible DNA damage leading to cell death [57]. ROS can also be used as a signaling molecule to alert the plant to stressful conditions such as high or prolonged light. Thus, a normal balance between ROS production and scavenging can maintain homeostasis since ROS accumulation can initiate expression of genes whose products are involved in cellular detoxification [57]. Plants with naturally higher levels of antioxidants and enzymes that scavenge ROS have been shown to suffer less damage when exposed to long photoperiods, even CL [29]. Therefore, it is suggested that the ability to eliminate excess ROS may also play an important role in preventing CL damage based on their role in photooxidative stress [58]. In our study, native plants exposed to CL had higher (by 30-80%) content of flavonoids

compared to their non-native counterparts. Under N-CL plants generally had more flavonoids than under N-16 h conditions. Flavonoids are a numerous and widespread group of phenolic compounds in the plant kingdom [59]. Due to their pronounced antioxidant properties, they participate in the protection of plants from oxidative stress caused by adverse environmental influences. By absorbing ultraviolet radiation (330–350 nm) and some visible rays (520–560 nm), flavonoids protect plant tissues from excess radiation. Thus, the obtained experimental data indicate the participation of flavonoids in plant adaptation to CL. Apparently, the role of flavonoids in the mechanism of the photosynthetic apparatus protection from photodamage under CL conditions is greater in native to Subarctic plants compared to introduced plants. This supports the hypothesis that the qualitative and quantitative composition of flavonoids accumulated by plants is closely related to the origin and evolution of a particular species. By conducting this comparative analysis, our research offers valuable insights into the optimal lighting conditions necessary for the successful cultivation of plants in CEA. Sinusoidal LED light recipes instead of square wave light regimes may be of interest for optimizing indoor lighting regimes to more closely match the requirements of the crop in order to maximize photosynthetic efficiency and consequently marketable yield.

4. Materials and Methods

4.1. Plant Material

We selected three native plant species (*Geranium sylvaticum* L., *Geum rivale* L., *Potentilla erecta* (L.) Raeusch.), which are common in Subarctic woods, and three non-native plant species (*Geranium himalayense* Klotzsch, *Geum coccineum* Sibth. & Sm., *Potentilla atosanguinea* Loddiges ex D. Don) that were introduced in Polar-Alpine Botanic Garden (KPABG), the Kola Scientific Center, Russian Academy of Sciences, Kirovsk, Russia (67°38'N) (Figure 2) and commonly used in city landscaping in Subarctic. *Geranium himalayense* and *Potentilla atosanguinea* are native to Himalayas. *Geum coccineum* is native to the mountains of the Balkans. They are all herbaceous perennials that belong to Geraniaceae and Rosaceae families. Species selection was done to match congeneric pairs of native and non-native plant species.

4.2. Experimental Design

In the natural environment, measurements of physiological and biochemical parameters were carried out at the peak of the polar day (in the end of June), when the plants were illuminated continuously, and in the second decade of August, when the day length was about 16 hours.

Five plants of each of the three species pairs were placed into containers with soil and grown for a month in the climate chamber (Vötsch, Germany) at 16 h photoperiod, light intensity of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 23°C, air humidity of 70±5%. Then, some of the plants were grown for two weeks under the same conditions, but 24 h photoperiod. Light was provided by cool-white fluorescent tubes supplemented with red-enhanced fluorescent tubes. The PPFD value was measured using LI-250A Light Meter (Li-COR Biosciences, Lincoln, NE, USA).

Thus, experimental groups included 3 species pairs exposed to 1) natural 16 h photoperiod (N-16 h), 2) natural CL (N-CL), 3) artificial 16 h photoperiod (A-16 h) and 4) artificial CL (A-CL).

4.3. Leaf Mass per Area

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

4.4. Chlorophyll Fluorescence Measurements

Chlorophyll 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.

4.5. Stomatal Conductance Measurements

Stomatal conductance (g_s) was measured using a steady state leaf porometer (SC1, Decagon Devices Inc., Pullman, WA, USA). The diurnal course of g_s was measured every 3 hour during 3 days, from one of the topmost fully expanded leaf. Leaves used for measurements were tagged for repeated measurements. All the measurements were done on the abaxial side of the leaves.

4.6. Photosynthetic Pigment Analysis

The content of chlorophyll a and b and carotenoids was measured in 96% ethanol extracts with a SF2000 spectrophotometer (Spectrum, St. Petersburg, Russia) and calculated according to the known formulas [60]. The percentage of Chl in light-harvesting complex II (LHCII) was calculated by accepting that almost all chlorophyll b is in LHCII and that the ratio of chlorophyll a/b in LHCII is 1.2 [61].

A SPAD-502 Plus chlorophyll meter (Konica Minolta Optics, Japan) was used to determine a chlorophyll content index (CCI).

4.7. Anthocyanins and Flavonoids Content

Anthocyanins were extracted from leaves, according to Kang et al. [62]. Fresh samples (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 530 nm (peak of absorption of anthocyanin) and 657 nm (peak of absorption of chlorophyll 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 $\Delta A = A_{530} - 1/4A_{657}$ was used to deduct the absorbance contributed by chlorophyll and its degradation products in the extract [63].

The relative amounts of flavonoids were measured spectrophotometrically [64–66]. The supernatant for anthocyanins was diluted 10 times and the absorbance was measured at 300 nm. Flavonoids content in the sample was expressed as absorbance at 300 nm g^{-1} FW.

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 [67]. The value for nonspecific absorption of each sample at 600 nm was also recorded and subtracted from the absorbance recorded at 532 nm. The concentration of MDA was calculated using an extinction 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. [68]. Leaf tissues (0.1 g) were homogenized in an 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 the supernatant was measured at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve and expressed in $\mu mol g^{-1}$ FW.

4.10. Data Analysis

Five plants per treatment were employed in the experiment. The tables and figures show mean values and standard errors. Significant differences between the means were revealed at $p < 0.05$ using the least significant difference test.

5. Conclusions

This research presents a novel approach by examining the specific effects of different lighting environments on plants of different origin. While previous studies have addressed aspects of responses of important crops to long photoperiods including CL under controlled climate conditions with artificial lighting, there was a lack of comprehensive research comparing natural and artificial lighting. A comprehensive comparison between natural light and artificial lighting sources have shown that plants lack specific mechanisms of tolerance to CL. Protective responses in this case are non-specific and induced by developing photo-oxidative stress. Under constant environmental conditions artificial CL causes photodamage to leaves due to oxidative stress, the main cause of which is circadian asynchrony, i.e., a mismatch between the internal (endogenous) rhythms of the organism and external light-dark cycles [8].

In nature, during the polar day, plants avoid photodamage because, unlike under artificial conditions, endogenous rhythms are maintained by daily fluctuations in other environmental factors (light intensity, spectral distribution, and temperature), which can probably obviously also act as pacemakers. Among various non-specific defense mechanisms, plants actively use flavonoids to neutralize the increased amount of ROS generated by light. At the same time, the role of flavonoids in the photoprotection mechanism is obviously higher in local subarctic plants than in non-native ones. This not only indicates their special role under CL conditions, but also confirms the hypothesis that the qualitative and quantitative composition of flavonoids accumulated by plants is closely related to both the origin of a particular species and its evolution, during which those adaptive mechanisms that were distinguished by their simplicity, reliability, and efficiency were selected and consolidated.

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