

THE EFFECTS OF RED LIGHT ON BLUE LIGHT-BASED PHOTOTROPISM IN
ARABIDOPSIS THALIANA

by
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ABSTRACT

Red and blue light pathways interact with one another to guide the phototropic growth and development of seedlings. Both roots and hypocotyls experience phototropism. This study aims to describe the effects of red light on blue-light phototropism and to examine the relationship between red and blue light pathways. Two wild-type ecotypes of *Arabidopsis thaliana*, germinated either under white light or in darkness, were exposed to different red and blue light conditions and used to examine these relationships.

Red light exposure preceding growth under solely blue light was shown to have an inhibitory effect on root lengths, regardless of ecotype or etiolation. Surprisingly, red light did not produce significant phototropistic curvature in roots of either ecotype. The remainder of the results illustrated the fact that different ecotypes exhibit different phototropistic responses to light treatments and etiolation. Seedlings did not uniformly respond to constant red and blue light in the same manner as they responded to pretreatment by red light. In different circumstances, simultaneous red and blue light led to results statistically identical to blue light's results, red pretreatment's results, or an intermediate between the two.

Our results both highlight the importance of testing multiple ecotypes within a species and reveal interesting trends in the relationship between red and blue light pathways. Red and blue light pathways interact, but different *Arabidopsis* ecotypes respond to each light treatment and germination condition differently, and have differing capacities to prioritize one pathway over the other when both are available.

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LIST OF ABBREVIATIONS AND SYMBOLS

Col	Columbia
ecotype	genetically distinct population
Ler	Landsberg erecta
v/v	volume/volume
WT	wild-type
w/v	weight/volume

INTRODUCTION

While plants cannot move from one location to another, they do have the ability to bend towards or away from environmental stimuli, such as sunlight or gravity, in order to better accommodate their needs. This response is called a tropism, defined as growth-mediated directional response to a stimulus vector (Kaufman and Song 1987; Whippon and Hangarter 2004). Multiple environmental stimuli are sensed by plants, and they respond accordingly. Though the phototropic response is usually attributed to blue light, red light has been found to influence it as well (Correll and Kiss 2002; Kiss et al. 2003).

When exposed to unilateral white light, phototropism occurs when the growth hormone auxin accumulates on the dark side of plant stems and induces growth on that side of the stem. This induction of growth results in curvature of the stem towards the source of light. Plants sense light through multiple photoreceptor proteins. Of these receptors, cryptochromes and phototropins sense blue light, while phytochromes sense red and far-red light. These photoreceptors mediate the plant's responses to their environment by regulating the production and transportation of auxin molecules (Halliday et al. 2009). Though phototropism is typically associated with movement of the above-ground portion of a plant, it occurs in roots as well (Kiss et al. 2002).

Under unilateral blue light irradiation, plant shoots tend to curve towards the light source, exhibiting positive phototropism. Roots, alternatively, generally exhibit negative phototropism, turning away from the light (Briggs and Christie 2002; Kutschera and Briggs 2012). Red-light exposure, however, leads to positive phototropism in roots, while not significantly affecting shoot curvature on its own (Kiss et al. 2002). *Arabidopsis thaliana* exposed to red light, before growing under blue light, experiences an enhanced phototropistic response to the blue light by shoots, and in certain ecotypes, a diminished response in roots (Millar et al. 2010; Sindelar et al. 2014).

Many phototropism experiments focus on plant shoots, even though roots exhibit their own important phototropistic response. In nature, light pierces several centimeters deep into the soil, with red and far-red wavelengths traveling deeper than blue wavelength light. Root phototropism in *Arabidopsis* seedlings has been found to affect the architecture and growth of the roots as well as the entire plants' metabolic rates and physiologies (Mo et al. 2015). For this reason, this study examined both root and shoots responses.

These experiments attempt to further elucidate the interactive relationship between red and blue-light pathways. The findings of Sindelar et al. (2014) suggest different phototropic response among two different ecotypes of *Arabidopsis*. Here, phototropism and growth rates of two ecotypes (Landsberg and Columbia) are examined using both light and dark-germinated seedlings. In order to look for interactions between red and blue-light pathways, seedling growth was compared under three different unilateral light treatments: continuous blue light, a ten-minute red light pretreatment

followed by blue light, and continuous growth under constant combined red and blue-light treatment. It was hypothesized that the blue-light treatment and the red-light pretreatment would produce results consistent with previous studies, while the combined red and blue-light treatment would result in a more amplified version of red-light pretreatment's results.

MATERIALS & METHODS

Growth Conditions for Tropism Studies

This study used wild-type (WT) *Arabidopsis thaliana* seeds of ecotypes Landsberg (Ler) and Columbia (Col). Before surface sterilizing the seeds, 100 x 100 x 15-mm gridded square petri dishes was prepared. Sterilized agar consisting of 1.2% (w/v) *Arabidopsis* growth medium bacto-agar, as described by Kiss et al. (1997), half-strength Murashige and Skoog salts medium, and 1% (w/v) sucrose at pH 5.5 was poured into the plates. Once the agar solidified, a sterilized nitrocellulose film (Promega, V7131) was laid on top of the agar as a membrane. Prepared plates were double-wrapped in Parafilm and chilled for 24 hours at 4°C.

The seeds were surface sterilized under a laminar flow hood via rinsing with multiple ethanol and Triton X-100 concentrations. The first rinse was 70% (v/v) ethanol and 0.002% (v/v) Triton X-100 solution for 5 minutes. Next, seeds were given 2 rounds of rinsing by 95% (v/v) ethanol, each rinse lasting for 1 minute. Seeds were then rinsed given a 1 minute rinse with 0.01% (v/v) Triton X-100 solution before undergoing 4 consecutive sterilized water rinses. Seeds were sown onto the plate membranes with 2 rows of 6 seeds per plate. Plates were once again wrapped in two layers of Parafilm and subjected to a 24-hour cold treatment at 4°C. Eight plates were made for each ecotype.

Half of the plates for each ecotype were placed upright under continuous white light (Gro-Lux white light fluorescent tubes ($70\text{-}80 \mu\text{mol m}^{-2}\text{s}^{-1}$) for 96 hours at 23°C . The other half were placed upright in the same room, but germinated instead in complete darkness. Darkness was accomplished by placing a styrofoam container inside a cardboard box which was placed inside a metal cabinet.

After the 4-day germination period, seedlings were given one of three different 24-hour light treatments in a temperature and light-controlled room: continuous blue light, ten minutes of red light followed by blue light, or continuous red and blue light. Blue ($60\text{-}75 \mu\text{mol m}^{-2}\text{s}^{-1}$), red ($20\text{-}30 \mu\text{mol m}^{-2}\text{s}^{-1}$), and red+blue (red:blue ratio 165:60) light was produced by 110-V LED panels. Light produced by the blue panels was 465 nm and light from red panels was 650 nm. Panels were placed perpendicular to plants, so that reactive growth by seedling ends could be clearly seen as towards or away from the light.

Data Collection and Analysis

Seedling length and curvature measurements were taken using ImageJ64, a public domain Java image processing program (Schneider et al. 2012). Seedlings that failed to germinate by time 0, had detached from the membrane surface, had made contact with the sides of the plate or with another seedling, or whose images were determined to be unreadable were omitted from the data. Designated time points (t) for data collection were 0, 0.5, 1, 2, 4, 8, 12, and 24 hours from initial light treatment exposure. Sample size ranged from 21 to 43 plants, with 4 plates per each experimental category. Curvature

measurements considered any growth towards the light to be a positive angle, and growth away from light to be negative; a purely vertical angle was 0.

Analysis of Variance (ANOVA) was used to determine if any significant differences occurred between treatments, followed by Tukey's HSD (Honestly Significant Difference) to adjust for multiple comparisons. Tests compared the data from each light treatment, ecotype, and germination condition. Categories tested were mean phototropic curvature of stems and of roots, and average overall changes in length from 0 to 24 hours for stems and roots.

RESULTS

Curvature

There was no significant difference in shoot curvature between Landsberg and Columbia ecotypes (fig. 3, 4). Light-grown Ler shoots exhibited significantly more curvature when subjected to the red light pretreatment than under continuous blue light alone; under the continuous red and blue-light treatment, curvature was not significantly different from either of the other treatments (fig. 3A-3C). Dark-grown Ler seedling shoots under the red and blue light treatment exhibited enhanced positive curvature compared to light-grown Ler seedlings (fig. 3C). The continuous blue treatment and the continuous red and blue treatment elicited a stronger shoot curvature response from dark-grown Ler plants than the red light pretreatment did (fig. 3A-3C). Dark-grown Col seedlings exhibit significantly more positive shoot curvature under the blue light treatment than under the red light pretreatment, while the red and blue treatment did not produce significantly different results (fig. 4A-C). There were no significant differences found in root curvature for any light treatment, ecotype, or etiolation comparison.

Shoot Length

Under red or blue light, dark-grown Landsberg seedling shoots experienced higher growth rates than light-grown seedlings, though this was not seen in Col plants (Fig 6). No significant differences were found between light and dark-grown Ler shoots grown under the red light pretreatment (Fig. 5). Under continuous blue-light, dark-grown Ler seedlings outgrew dark-grown Col seedlings (Fig. 6). Regardless of whether Ler seedlings were germinated in darkness or with light, plants grown under the blue light treatment experienced higher growth rates than those grown under the red and blue light treatment (Fig. 5). There were no differences between Col seedlings between these two treatments.

Root Length

Light-grown roots in both ecotypes tended to have higher growth rates than in dark grown plants, except with red light pretreatment. In seedlings germinated in the light and grown under continuous blue conditions, Ler roots experienced higher growth rates than Col seedlings' roots. In both ecotypes, red light pretreatment resulted in plants with significantly shorter root lengths than in those grown under the blue treatment or the red and blue treatment (Fig 7).

DISCUSSION

Curvature

Light-grown WT Landsberg seedlings exhibited greater shoot curvature after the red light pretreatment compared to the blue treatment, an effect seen in previous studies (Hangarter 1997, Sindelar et al. 2014, Whippo and Hangarter 2004). Interestingly, when Landsberg plants were germinated in darkness, less curvature was seen after the red light pretreatment compared to after the blue treatment. Etiolated Ler seedlings under continuous red and blue light, however, exhibited shoot curvature that was statistically identical to the blue light treatment, bending more dramatically towards the light than the plants that received the red pretreatment.

The red pretreatment appeared to inhibit phototropism in dark-grown Col WT stems versus continuous blue light, while the seedlings exposed to simultaneous red and blue light did not significantly differ from either of the other two treatments. Light-grown Columbia ecotype shoots did not significantly differ from one another, which contrasts with previous results in which the red pretreatment should have increased curvature in comparison to the blue treatment.

There were no significant differences among root curvatures in any condition. This contrasts results reached by other studies, in which exposure to a red light pretreatment significantly reduced root curvature in wild-type Landsberg plants compared to exposure only to unilateral blue light irradiation, and had the opposite significant effect

on wild-type Columbia plants (Sindelar et al. 2014). It is possible that sample size played a role in this result. Only 21 to 43 plants per category were used in these experiments, compared to the 52 to 78 wild-type plants used in the previously published study.

That constant red and blue light does not tend to produce an exaggerated version of the red pretreatment's effects, as compared to solely blue light, is interesting. Dark-grown Ler seedlings tended to react to the simultaneous red and blue light exposure as if it were only blue light, while Col and light-grown Ler under the red and blue treatment failed to significantly differ from either other treatment. These results suggest that red and blue light pathways interact with one another and generally operate at the same time, though Ler plants may favor the blue pathway in the presence of blue light. This effect is perhaps only seen in the dark-grown plants because etiolation often induces an exaggerated phototropic response (Kang and Burg 1974).

Lengths

Red-light pretreatment of seedlings resulted in a reduction in root growth rate for all seedlings. In both of the other light treatments, roots in light-grown seedlings significantly outgrow etiolated roots. The red light pretreatment stunted shoot growth in dark-grown Ler seedlings, but did not cause differences in Col shoot growth, likely due to genetic differences between ecotypes.

Under continuous blue-light treatment, dark-grown Ler shoots experienced increased growth rates, but this trend did not extend to light-grown seedlings. Col shoots, however, were unaffected. Blue light-treated Ler seedlings significantly outgrew their

blue-and-red treatment, though the red light pretreated cells (with the exception of etiolated Ler under blue light, which experienced enhanced growth as mentioned above) fell in the middle, failing to significantly differ from either other treatment.

Landsberg seedlings experienced greater growth compared Columbia seedlings under blue light, but germination conditions determined whether the difference occurred in the roots or the shoots. Light-grown Ler roots outgrew light-grown Col roots, with no significant shoot length differences. Among dark-grown seedlings, Ler shoots outgrew Col shoots. Red light pretreatment prevented this effect; there were no significant differences among seedlings that received the pretreatment.

Conclusions

It is important to note that results of this study cannot necessarily be extended to other *Arabidopsis* strains or to other plants, as evidenced by various differences in shoot curvature and seedling lengths. Although *Arabidopsis thaliana* is a common model organism, this study, as well as previous ones, has demonstrated that different genotypes, even non-mutants, within the same species respond differently in experiments (Hangarter 1997, Kumar et al. 2008, Sindelar 2014).

The attenuation by red light of root lengths in all conditions is intriguing considering red light's ability to penetrate soil (Mo et al. 2015). Also, even though the results of these experiments did not reveal a significant connection between phototropic root curvature and red light pretreatment as previous experiments have revealed, there was an inhibitory effect regardless of etiolation or ecotype (Sindelar 2014).

The inclusion of continuous red and blue light in this study served to further elucidate the relationship between red and blue light pathways. Plants exhibited varied responses to this light treatment according to ecotype and germination differences, rather than exaggerated versions of the same trends that were induced by red light pretreatment. In some circumstances, simultaneous red and blue light resulted in growth responses statistically identical to blue light's results; in others, it resulted in growth more similar to the effects of red pretreatment. Other times, it caused an intermediate reaction, creating growth in between, but not significantly different than, the other two treatments. While red light does affect blue light phototropism and growth in *Arabidopsis* roots and shoots, different ecotypes respond to it differently and possess differing capacities to prioritize the two pathways when both are present. Further research will continue to elucidate the interactions among light qualities, growth, and the development of plants.

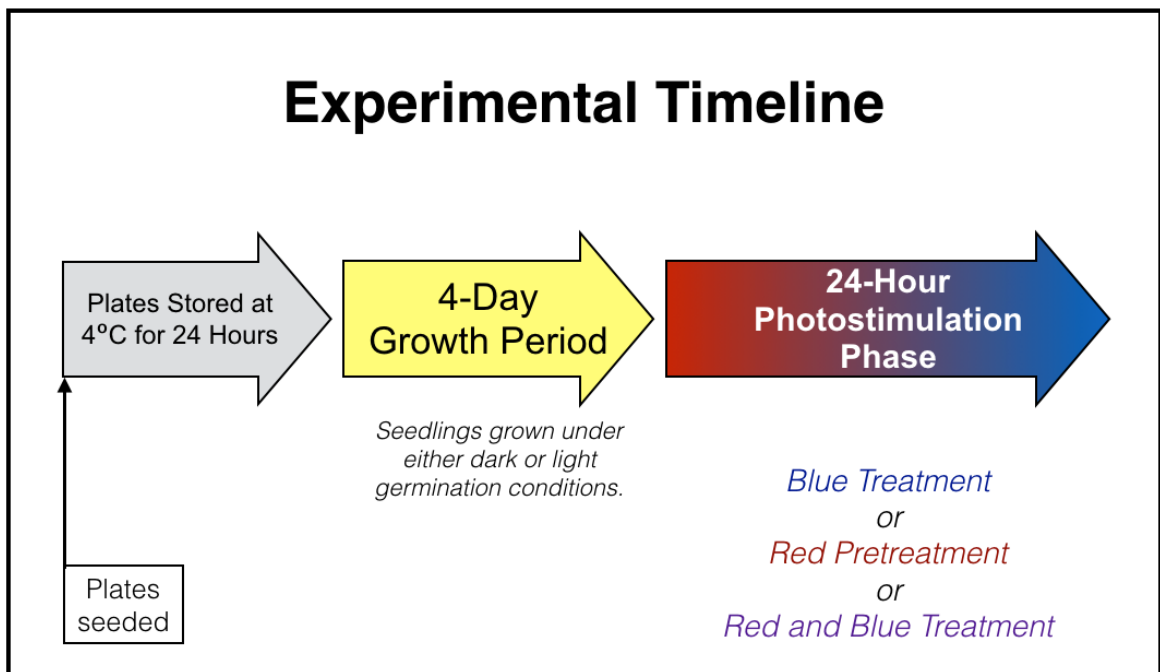


Figure 1: Timeline of events for phototropism study

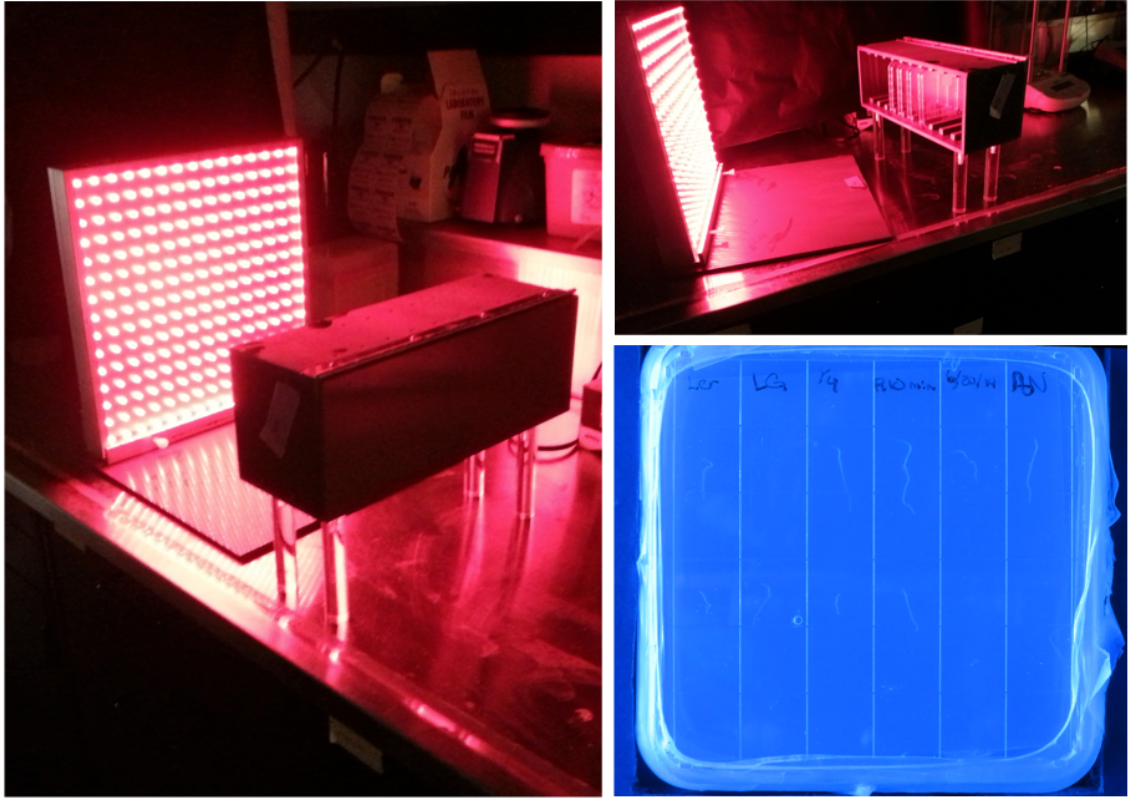
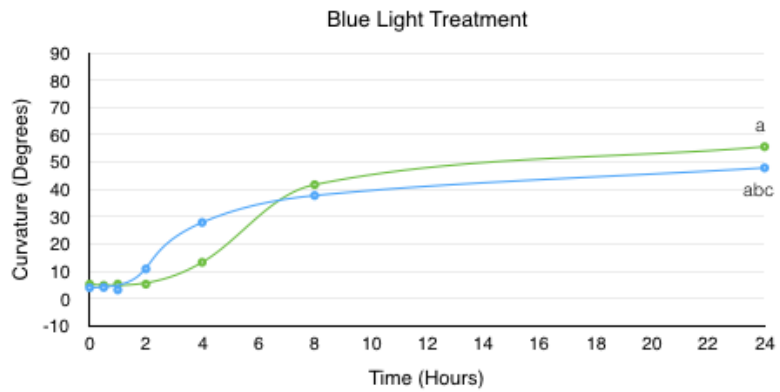
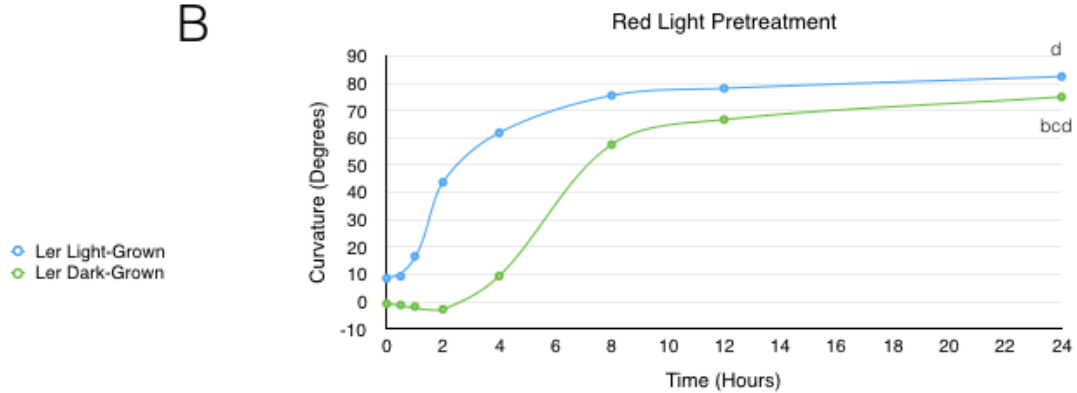


Figure 2: Photostimulation phase experimental setup. *Arabidopsis* seedlings were sown onto plates, twelve seeds per plate (bottom right). After chilling and germination periods, plates were oriented perpendicular to the light source for photostimulation period (left, top right) under one of three light treatment experiments.

A



B



C

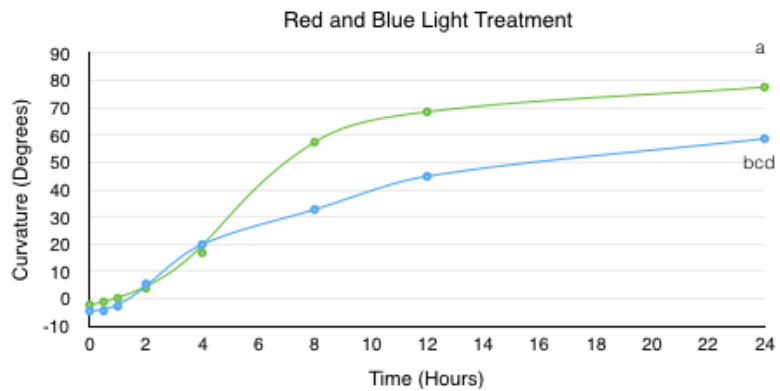


Figure 3: Shoot Curvature in Landsberg Ecotype. Curvature of stems over twenty-four hour photostimulation phase under each light treatment. Letter-labels for each set are taken directly from Tukey’s HSD test results. Sets sharing any similar letter (a, abc) are not statistically significant, and sets that do not share letters (a, bcd) are significantly different.

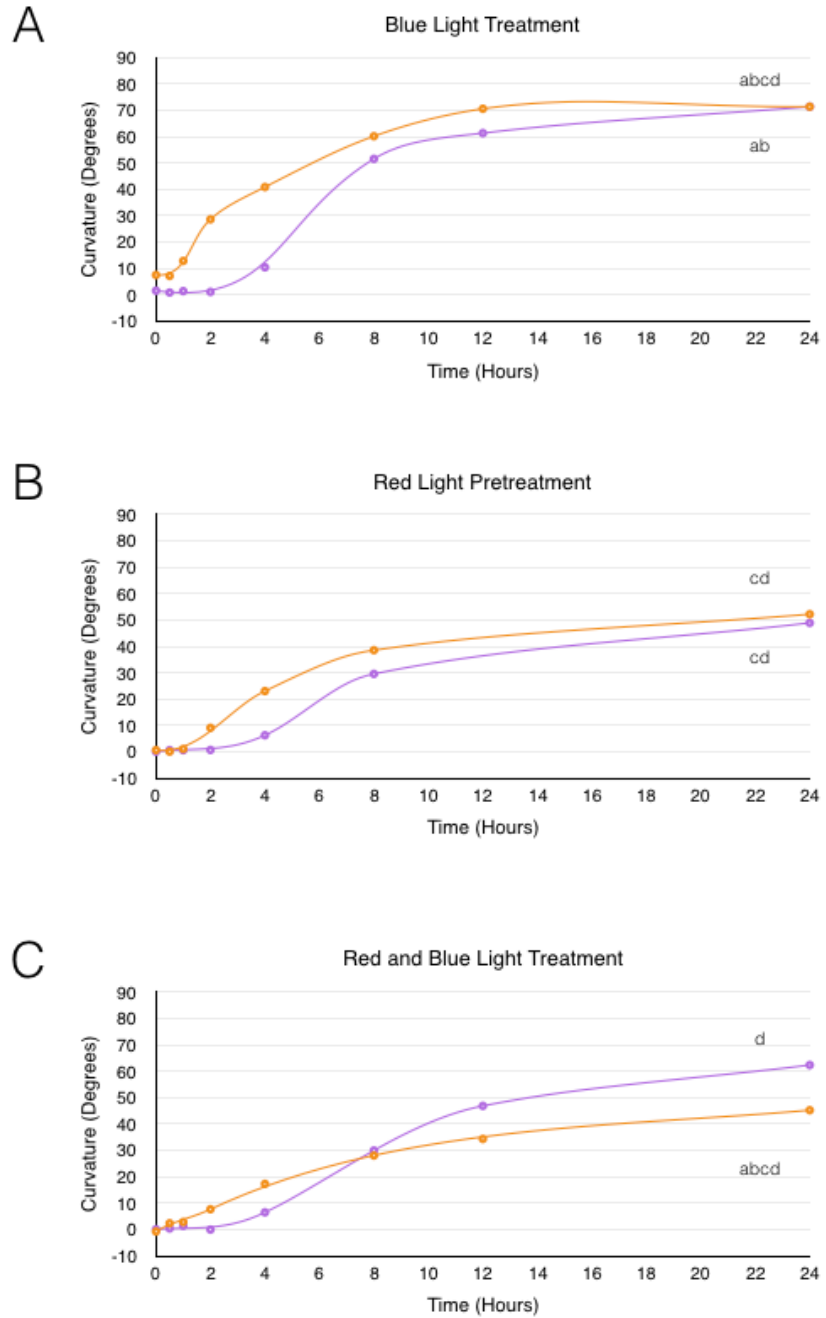


Figure 4: Shoot Curvature in Columbia Ecotype. Curvature of stems over twenty-four hour photostimulation phase under each light treatment. Letter-labels for each set are taken directly from Tukey’s HSD test results. Sets sharing any similar letter (a, abc) are not statistically significant, and sets that do not share letters (a, bcd) are significantly different.

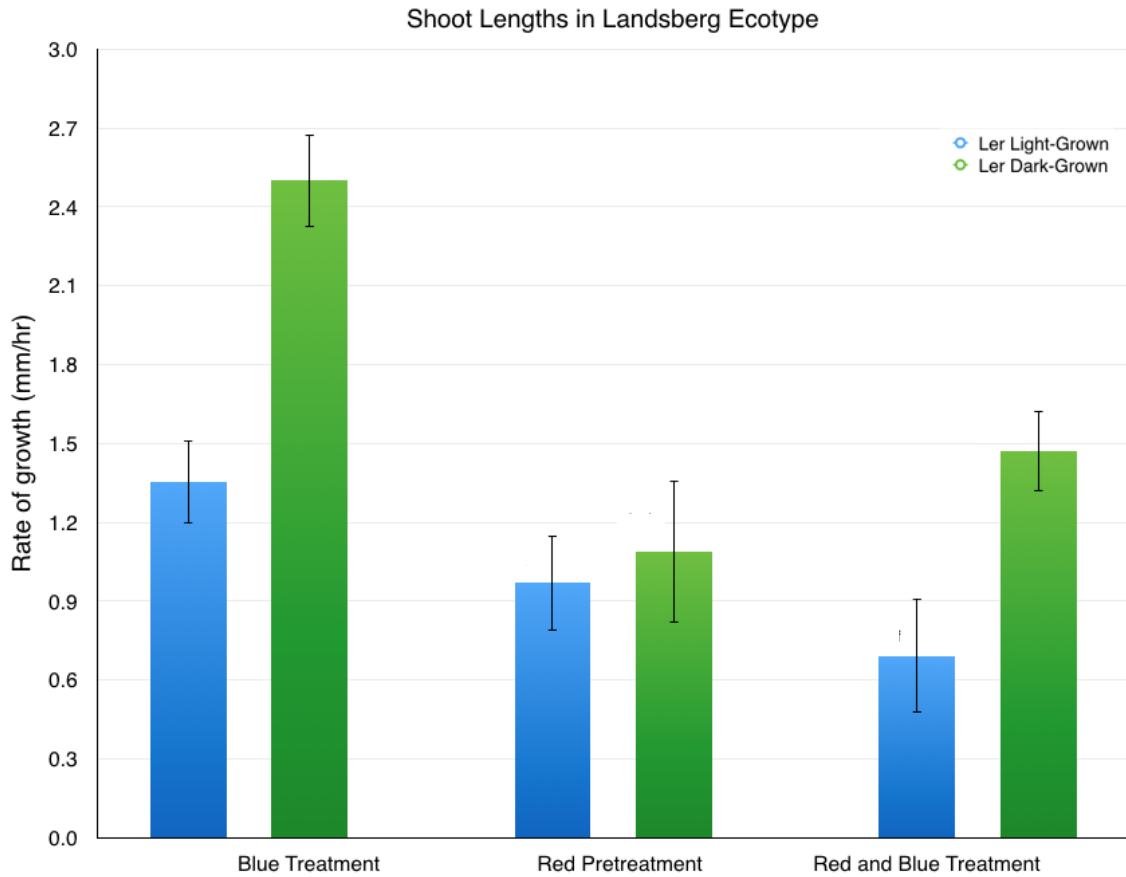


Figure 5. Growth rates of Ler WT shoots over 24 hour photostimulation phase. Letter-labels for each set are taken directly from Tukey’s HSD test results. Sets sharing any similar letter (a, abc) are not statistically significant, and sets that do not share letters (a, bcd) are significantly different.

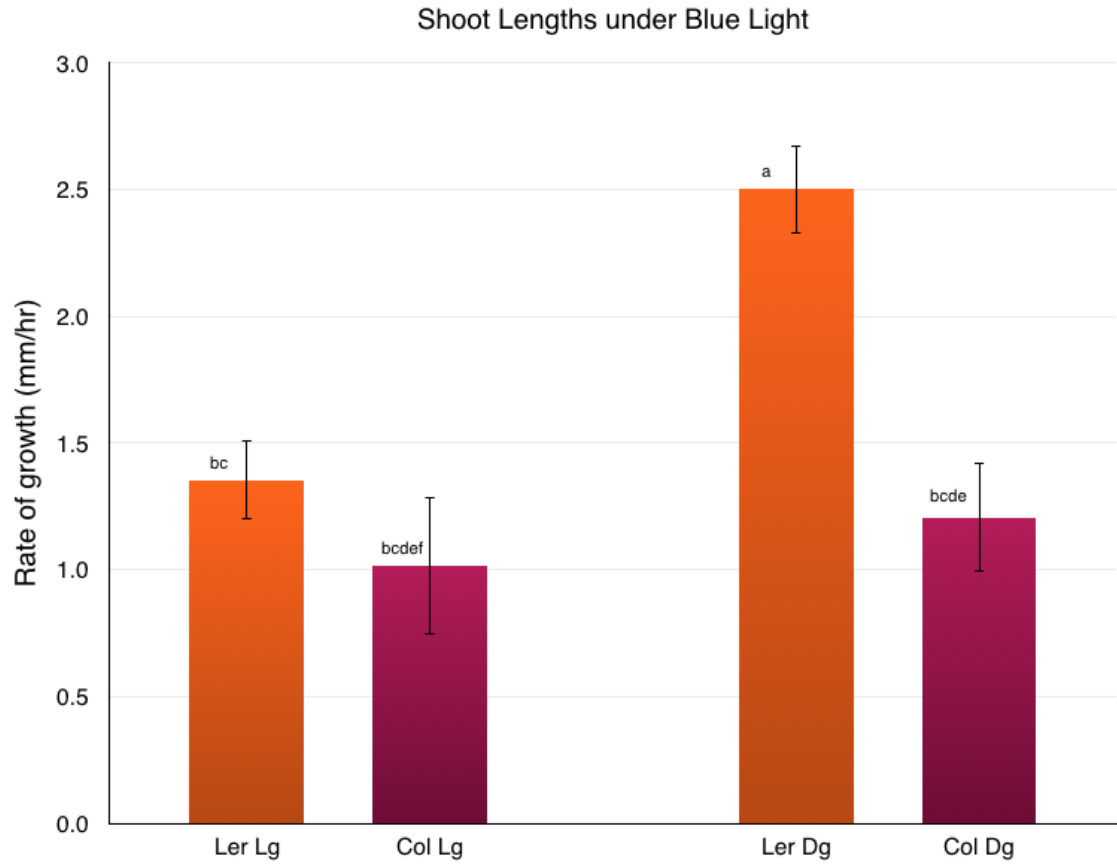


Figure 6. Growth rates of all shoots receiving the blue treatment over 24 hour photostimulation phase. Letter-labels for each set are taken directly from Tukey's HSD test results. Sets sharing any similar letter (a, abc) are not statistically significant, and sets that do not share letters (a, bcd) are significantly different.

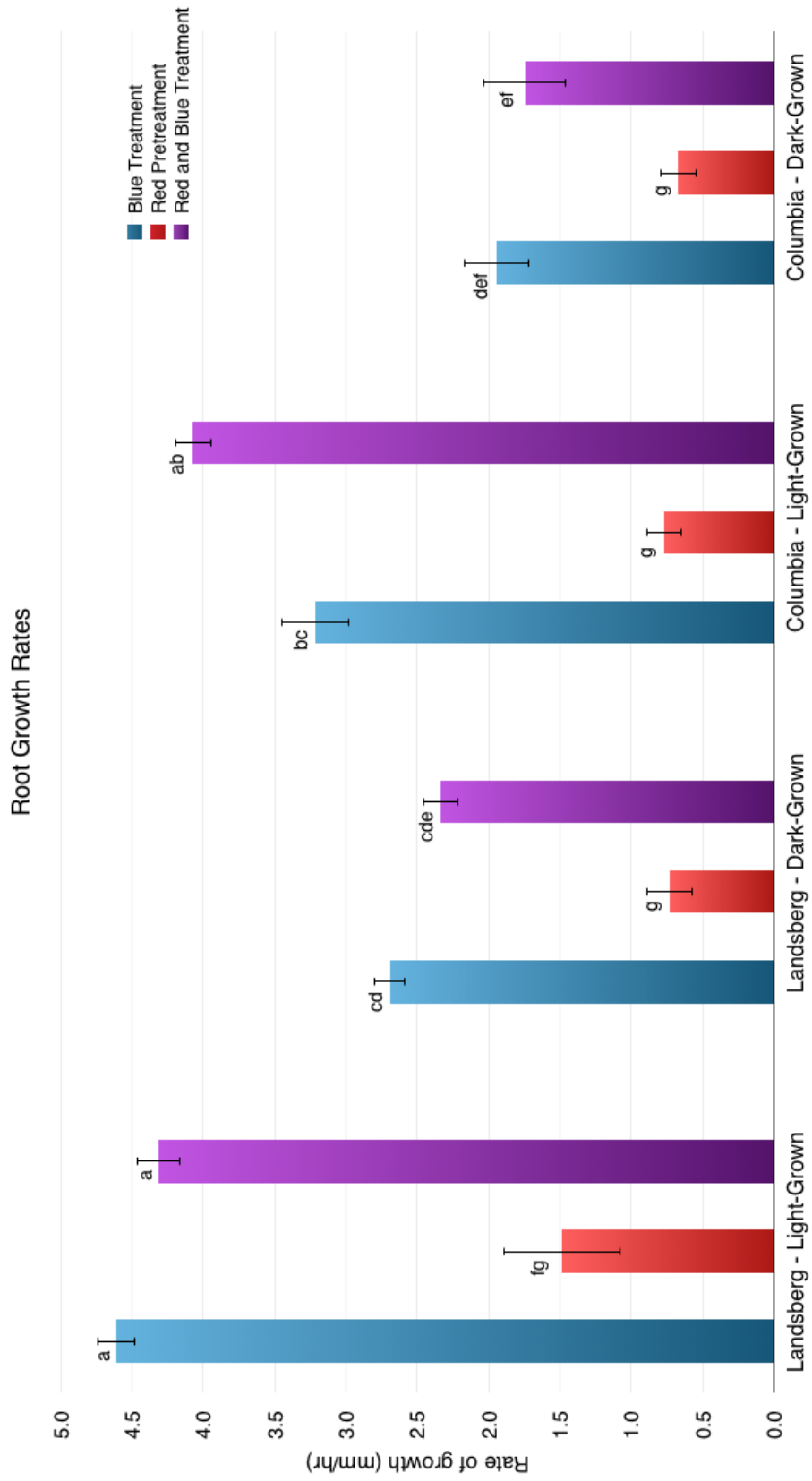


Figure 7. Comparison of growth rates of all roots under all different light treatments over 24 hour photostimulation phase. Letter-labels for each set are taken directly from Tukey's HSD test results. Sets sharing any similar letter (a, abc) are not statistically significant, and sets that do not share letters (a, bcd) are significantly different.

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