

Plant Physiological Acclimation to Irradiation by Light-Emitting Diodes (LEDs)

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Abstract

LEDs may be a suitable light source for future use as assimilation lighting in protected greenhouse cultivation. LEDs have properties which offer advantages compared to other light sources, but which also raise specific research questions. The narrow band spectrum of LEDs enables manufacturers to produce LED based light-sources specifically suitable for photosynthesis and other horticulturally relevant plant properties. The low radiated heat also makes LEDs suitable for interlighting (i.e. lighting from within the canopy), for which high pressure sodium lamps are not suitable. However, when using LEDs, crops must be able to acclimate their photosynthetic functioning to narrow band lighting (NBL) to efficiently use this light. Also, daylight-adapted leaves must be able to re-acclimate to NBL if LEDs would be used for interlighting in a high-wire grown crop. If low photosynthesis rates in older, lower leaves of the crop are also due to leaf age, besides low light, interlighting would be less effective. For investigating the intrinsic effect of NBL, we used 9 different arrays comprised of a single LED type (peak wavelengths in the range 460-668 nm) at light-limited irradiance ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$). *Spirodela polyrrhiza* (Lemnaceae) was cultivated as its leaves can not change in distance or orientation towards the light source. This enabled us to compare the effects of the different light sources on parameters such as growth rate and photosynthetic pigment composition. In order to separate the effect of light intensity and leaf age on photosynthesis, tomato plants were grown horizontally, so that older leaves were not shaded by younger leaves. Re-acclimation of leaves to NBL was investigated by illuminating older leaves (low in the canopy) using different LED arrays in a high-wire grown tomato crop. The light-harvesting apparatus of *Spirodela polyrrhiza* acclimated to the different NBL regimes within 6 days. Leaf age proved to be an irrelevant factor for photosynthetic capacity (P_{max}) of greenhouse grown tomato plants. P_{max} of leaves at a low position in a high-wire grown tomato crop, with a low P_{max} , did re-acclimate to the higher light intensities supplied by the supplemental NBL by progressively increasing their P_{max} . However, as it took 14 days for P_{max} to increase from 5.6 to $12.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, maintaining a continuously higher light level within the canopy would be more effective.

INTRODUCTION

Recently, light emitting diodes (LEDs) are subject of debate and research with respect to their possible future use for assimilation lighting in protected plant cultivation. At this moment LED lighting systems are too expensive and, more important, not efficient enough in terms of light output per Watt energy use, for large scale replacement of high pressure sodium (HPS) assimilation lamps for commercial greenhouse horticulture. However, the light-output efficiency of LEDs increased dramatically in recent years and production costs of LED systems aimed at the horticulture will decrease

if demand would be sufficient. So as well as yielding new, fundamental knowledge on plant functioning, research on plant responses to LED lighting is highly relevant for the horticultural industry.

Compared to other light sources used for plant production, LEDs have several properties which are potentially useful in relation to horticulture. LEDs emit light in a relatively narrow band spectrum, so they can be manufactured with a wavelength output tailored to the plant responses desired by growers. In order to enhance production, LED arrays with wavelengths favourable for photosynthesis would be desired. However, wavelengths influencing other plant properties such as morphology (blue light enhances compactness of plants) could be desirable for ornamental horticulture. Also, nutritional qualities of food crops may be influenced by the illumination spectrum used (e.g. antioxidant content; see e.g. Oelmuller and Mohr, 1985).

At this moment, LEDs produce more heat than HPS lamps per Watt energy input, due to their lower energy/light conversion efficiency. However, the radiated heat of LEDs is very low compared to HPS lamps. This affords the opportunity to cool LED lighting systems and to reuse the (low caloric) heat for e.g. greenhouse heating. The low radiated heat output also makes LEDs suitable for interlighting, i.e. lighting inside the canopy, instead of or in combination with lighting above the canopy. Previous research on interlighting with HPS lamps (Hovi et al., 2004) indicated that interlighting may be more efficient than lighting from above. Interlighting could contribute to a more effective use of light by crops, as inside the canopy light levels are low, whereas above the canopy solar radiation already provides a considerable photosynthetic photon flux (PPF) during daytime. Especially in high-wire cultivated crops (e.g. tomato, cucumber and sweet pepper) with low photosynthesis rates lower in the canopy (see e.g. Xu et al., 1997), interlighting could offer a means to enhance production. The considerable percentage of radiated heat HPS lamps produce makes it impossible to situate HPS lamps close to the leaves of a crop. LEDs, on the other hand, with their low radiated heat output and overall lower temperature, can be in contact with the leaves. Though this may not be good for photosynthesis, the leaf will not be burned.

LED light output is proportional to electric current through the device, so light intensity can be controlled in a versatile way. Therefore, the light output of LED lighting systems can be easily, rapidly and efficiently adjusted to match environmental conditions or production requirements within the greenhouse, such as natural light or the stage of the crop in the production cycle. Also if lighting systems would be used comprised of different LED types, emitting light with different spectra, light quality could be adjusted to the needs of the crop. For example, the use of near far red light (690 nm) was found to increase the leaf area of developing leaves (Goins, 2002), which would result in a more effective use of the available light in early stages of the production cycle.

A socially relevant issue concerning assimilation lighting, called "light pollution" (public agitation and the disturbance of the natural rhythm of birds etc. due to lighted greenhouses at night) may be reduced by the replacement of HPS lamps by LEDs for assimilation lighting. The human eye is much more sensitive to yellow/orange light (the dominant colour of HPS lamps) than it is to red light (a potentially useful colour for LED lighting systems), so an equal amount of μmol PPF can give a very different perception of light intensity for the (human) eye (Fig. 1). Mammals and birds are also more sensitive to yellow/orange light than to red (Goldsmith, 2006). Furthermore, interlighting would reduce radiation loss from the greenhouse considerably due to increased light interception within the canopy.

Nonetheless, several questions need to be addressed, concerning the possible use of LEDs for assimilation lighting. In order to grow crops efficiently using narrow band lighting (NBL), plants have to be able to acclimate their photosynthetic apparatus to NBL. Any imbalance in excitation of the photosystems I and II would lead to a loss of quantum yield for CO_2 fixation in a leaf. Also, the dynamics of re-acclimation to NBL of daylight-adapted leaves in a crop are essential for the efficiency of interlighting in a canopy. As the production time of a leaf in a high-wire grown crop is relatively short (approximately 8-

10 weeks for tomato), a daylight-adapted leaf which is then exposed to NBL, must be able to acclimate to NBL in a short time and to a sufficiently large extent to benefit from the extra light. Note also that lower photosynthetic capacity in the lower part of the canopy may not only be due to lower light, but also due to leaf age effects, which would not be reversed by supplemental lighting. If leaves lower in the canopy, where light levels are low, are able to produce more assimilates due to interlighting at all, then the question remains if, and to what extent these assimilates would be partitioned to harvestable parts of the plant.

We investigated the acclimation abilities of *Spirodela polyrrhiza* (Lemnaceae, also known as duckweed) to NBL using different arrays comprised of single LED type. This aquatic floating angiosperm grows quickly, and as a floating monolayer of leaves, the distance and orientation of the leaves to the light-source does not change during growth, and there are no complications due to changes in leaf properties due to self-shading of the leaves. This enabled us to compare the intrinsic effects of the different light sources on parameters such as growth rate and photosynthetic pigment composition in a reliable way. We separated the effect of light intensity and leaf age on photosynthesis by growing tomato plants horizontally, so that older leaves were not shaded by younger leaves. Also, we assessed re-acclimation dynamics of daylight-adapted leaves to NBL by illuminating older leaves (low in the canopy) in a high-wire grown tomato crop using LED arrays.

MATERIALS AND METHODS

Acclimation of *Spirodela polyrrhiza* (Duckweed) to NBL

Wild-type *Spirodela polyrrhiza* plants were collected and cultivated under white fluorescent tubes ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$). One healthy plant was selected for further cultivation, to exclude genetic variation between the plants. The growth medium was half-strength Hoagland's E-Medium (<http://www.mobot.org/jwcross/duckweed/media.htm>).

Plants were transferred to a trough above which 9 different arrays comprised of single LED type were suspended. Each LED array had its own floating cultivation grid, consisting of 6 cells and each cell of the grid was seeded with *Spirodela* plants (2 to 3 cm^2 of leaf area per cell), which were allowed to grow to a maximum total area of 11 cm^2 . Each grid was assured of radiation from only one LED array by means of opaque white screens. Nutrient solution (as during cultivation) was pumped through the trough to ensure that all plants received the same nutrient solution. The light intensity at the leaf surface was set to $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ for all LED arrays by adjusting the current to each of the LED arrays. This light intensity was in the light-limited range for growth of these plants, determined by growth-analysis of plants under white fluorescent light (data not shown). Light spectrum and intensity were measured using an Ocean Optics USB2000 spectroradiometer. The peak wavelengths of the spectra of the different LED arrays used were 460 (blue), 470 (blue), 517 (cyan), 537 (green), 595 (amber), 630 (orange/red), 642 (red), and 668 nm (red); the white LEDs had a broad spectrum.

The plants were grown under the different LED arrays for a period of 6 days and digital photos of the plants were taken after 0, 1, 2, 3 and 6 days of growth. The leaf area was determined by analysing the images using custom-written software (software developed by Joost Ruijsch, Wageningen University). The relative growth rate per day was determined for growth between the third and the sixth day, as growth was not stable for the first two days of the treatment. The relative growth rate per day, k , was calculated using the expression $k = (\ln(A_{\text{day6}}/A_{\text{day3}}))/3$, in which A = leaf area. The data are based on 4 repetitions.

After 6 days of growth under the different light sources, dry weight (DW) and chlorophyll a (chl a) and b (chl b) content of the plants were determined (3 compartments for DW and 3 compartments for chlorophyll content per light treatment; 4 repetitions for DW and 2 repetitions for chlorophyll). Chlorophyll was extracted in DMF in darkness at -22°C . The chlorophyll content was analysed spectrophotometrically and calculated as described by Wellburn (1994).

Leaf Age Effects on Tomato

Tomato plants (*Lycopersicon esculentum* 'Pronto') were grown during autumn and winter 2005/2006 in a greenhouse in Wageningen, the Netherlands. The plants were forced to grow horizontally by binding the growing top shoot to a wooden frame three times a week (Fig. 5A). The plants were grown in 10 L pots filled with perlite. A constant water content of 70% (v/v) was maintained in the pots. Water level was continuously controlled by using an ECH₂O dielectric aquameter (Decagon devices, Pullman, WA, USA). A standard nutrient solution for a tomato crop on substrate was supplied with an EC of 2.7 and a pH of 5 (Sonneveld and Bloemhard, 1994). Carbon dioxide fixation under saturating light conditions (i.e. P_{\max}) of 5-6 leaves per plant (3 plants in total), varying in leaf age from 20-70 days (leaf age defined as 0 when the leaves were 1-2 cm long), was monitored per plant within a two day period using a Licor 6400 portable photosynthesis system (Lincoln, Nebraska, USA). Results were analysed with regression analysis using the statistical package Genstat (release 8.11, Rothamsted experimental station, Harpenden, UK).

Re-acclimation of Tomato Leaves

Tomato plants (*Lycopersicon esculentum* 'Pronto') were grown during autumn and winter 2005/2006 in a greenhouse in Wageningen, the Netherlands using the high-wire cultivation system. The plants were grown under the same conditions as described above. Leaves at a low position along the vertical axis of the crop (the 22-25th fully grown leaf and approximately 70 days old), thus in a low-light position and with a low P_{\max} , were subjected to supplemental narrow band irradiance by arrays comprised of single LED type. The leaves received a supplemental irradiance of 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from LED arrays with peak wavelengths of 470 nm, 537 nm and 642 nm (18 hours a day, between 5:00 and 23:00). P_{\max} of these leaves was monitored every 3-4 days for a period of 14 days. Data are based on 2 repetitions. Analysis of variance was used to test differences in response between the different light treatments using Genstat.

RESULTS

Growth Response *Spirodela polyrrhiza* (Duckweed) to NBL

Compared to the other NBL treatments used and also to white fluorescent light, the greatest growth rates (leaf-area production per day) were achieved for plants illuminated with LEDs emitting in the red and the blue parts of the spectrum (Fig. 2). The pattern of growth response to the different NBL treatments approximately resembled the quantum yield for CO₂ fixation along the PAR spectrum found in previous research (e.g. McCree, 1972; Balegh and Biddulph, 1970). The spectrum used had no significant effect on specific leaf weight (SLW, g/cm^2 ; Fig. 3A). Only for the treatment with white LEDs SLW was slightly, but still significantly higher than for the blue light (460 nm) treatment ($P < 0.01$). As expected, increasing light intensity strongly increased SLW (Fig. 3B) of *Spirodela polyrrhiza*, at least for white fluorescent light.

Chl a/b ratios were notably altered by the different spectra used (Fig. 4). The chl a/b ratio was markedly higher for the plants grown under blue light (460 and 470 nm peak wavelength) compared to the ratio for plants from the other light treatments. Differences in total chlorophyll content were minor, except for the plants grown under the 668 nm peak wavelength light, which had a lower chlorophyll content (data not shown).

Leaf Age Effects and Re-acclimation

Tomato leaves of various ages up to 70 days of plants grown horizontally in day-light did not show a decrease in photosynthetic capacity (i.e. P_{\max} ; see Fig. 5B, no significant slope was found ($P = 0.26$)).

P_{\max} of the lowest leaves of a high-wire grown tomato crop increased dramatically after adding 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ supplemental NBL light (Fig. 6). The leaves subjected to blue NBL had a significantly higher P_{\max} on the 14th day than the leaves subjected to red and

green NBL had ($P < 0.001$; $LSD = 1,019$). However, the different response of P_{max} to the different LED types used was small and the experiment was repeated only once. The slight increase in P_{max} of the control may be due to acclimation to higher light intensities used during the P_{max} measurement itself.

DISCUSSION

For *Spirodela polyrrhiza*, some LED types clearly enhanced growth better than others (Fig. 2). However, it would be wrong to draw the conclusion that those LEDs producing the most efficient wavelengths for this experiment would thus be the best option for assimilation lighting in a crop. For growers, not only the growth rate per photon produced is relevant, but also the photon output of a (LED) light source per Watt energy use. In this experiment only arrays comprised of single LED type were used, whereas a combination of LED types may be more efficient for growth per $\mu\text{mol PPF}$ (see e.g. Kim et al., 2003). Furthermore, duckweed forms a single leaf layer, making direct absorbance of the incident light a decisive factor for growth. In a crop with multiple leaf layers, photons transmitted by the upper leaf layer may be absorbed by a lower leaf layer. Under high irradiance, where growth is no longer light-limited, photons of the relatively poorly absorbed part of the PPF spectrum (e.g. green) may be more effective than photons that are relatively well absorbed (e.g. red), as irradiance beyond the light-limited range results in a progressive loss of quantum yield for photosynthesis.

The pigment data (Fig. 4) show that *Spirodela polyrrhiza* is able to adapt its light absorbing system to different narrow band light spectra in only a few days. The high chl a/b ratio for the blue light treatments may be due to preferential excitement of photosystem II (PSII). As photosystem I (PSI) has a higher chl a/b ratio than PSII, the leaves may have responded to preferential excitement of PSII by increasing the PSI/PSII light harvesting complex ratio of their photosynthetic apparatus. The opposite reaction (a low chl a/b ratio) would then be expected to light in the near far red region (>680 nm; see e.g. Kim et al., 1993). However, as *Spirodela polyrrhiza* developed new leaves during cultivation under the NBL, these data can not be extrapolated to a situation in which a day light adapted leaf is subjected to NBL.

Our data clarify that the loss of photosynthetic capacity in lower, older leaves in a tomato crop can not be attributed to leaf age, at least not during the life cycle of a leaf as grown in a commercial greenhouse (Fig. 5) and can most probably be attributed to the light environment (intensity and spectrum). As P_{max} of the lowest, oldest leaves in a high-wire tomato crop increased dramatically after the addition of supplemental narrow band lighting (Fig. 6), the oldest leaves are able to re-acclimate again to higher irradiance. Therefore supplemental lighting lower in the canopy could make leaves with low productivity productive again and thus increase plant assimilate production. However, it must be taken into account that the re-acclimation was progressive over a period of 14 days (P_{max} was not measured after 14 days), whereas in a commercial greenhouse the three oldest leaves are picked weekly. This does not leave sufficient time to fully re-acclimate to supplemental lighting for lower leaves. Therefore, in order to keep low leaves in a shaded position productive and benefit optimally from interlighting, the decrease in photosynthetic capacity from top to bottom has to be prevented.

The optimum wavelength, or combination of wavelengths, for interlighting and for assimilation lighting in general (with or without natural daylight) is not clear from our research yet. The partitioning of the additional assimilates produced due to interlighting (to harvestable parts of the crop or to other plant organs) also needs to be clarified to get a complete image of the possible benefits of interlighting.

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Figures

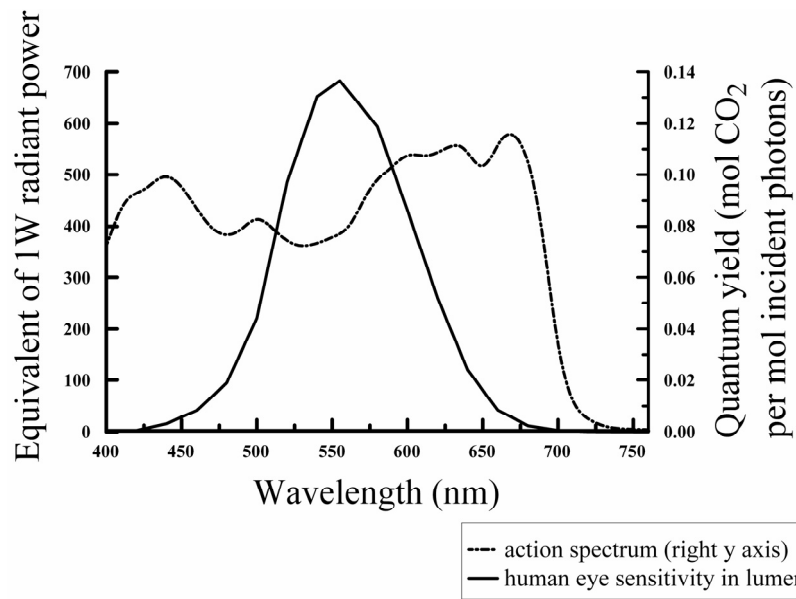


Fig. 1. Light sensitivity of the human eye along the PAR spectrum (data from <http://www.giangrandi.ch/optics/eye/eye.shtml>) and quantum yield for CO₂ fixation of a bean leaf (data from Balegh and Biddulph, 1970).

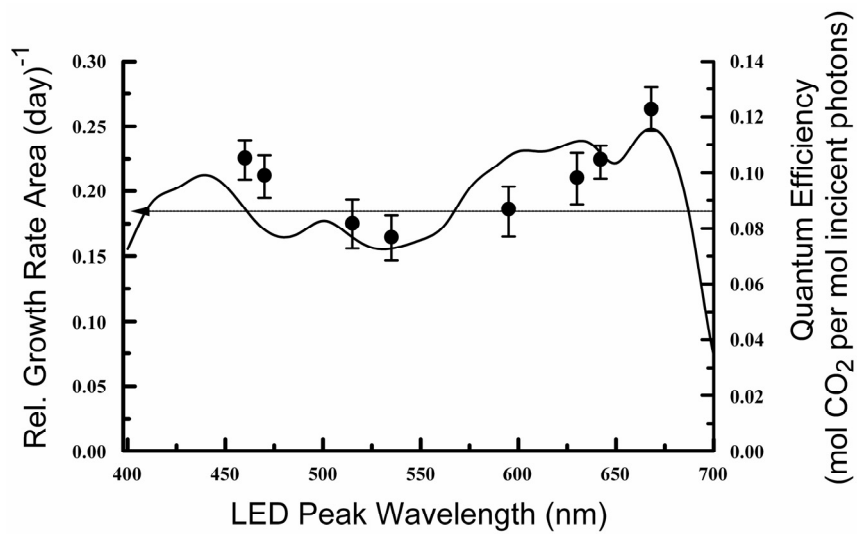


Fig. 2. Data points indicate the relative growth rate per day of *Spirodela polyrrhiza* between day 3 and day 6. The solid line indicates the quantum yield for CO₂ fixation of a bean leaf (data from Balegh and Biddulph, 1970). The dashed line indicates the relative growth rate per day at 50 μmol m⁻² s⁻¹ white fluorescent light.

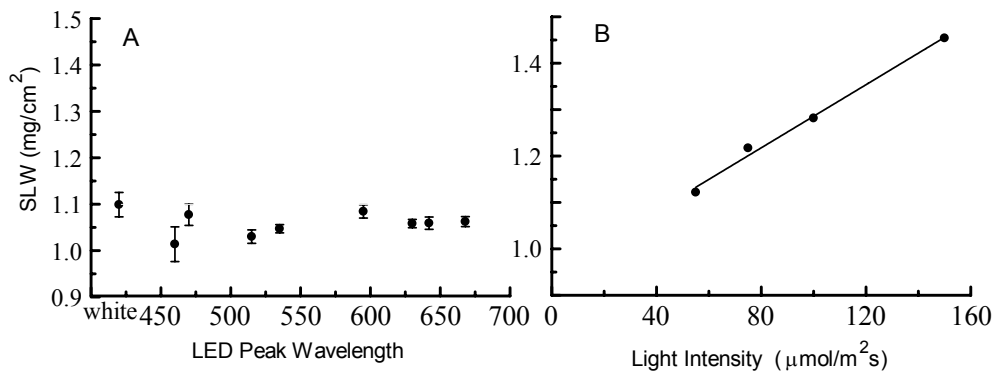


Fig. 3. A: Specific Leaf Weight (SLW) of *Spirodela polyrrhiza* after 6 days of cultivation under different LED arrays (“white” indicates white LED light treatment). B: SLW versus light intensity of white fluorescent tubes (apparent linearity can not be extrapolated beyond the data points).

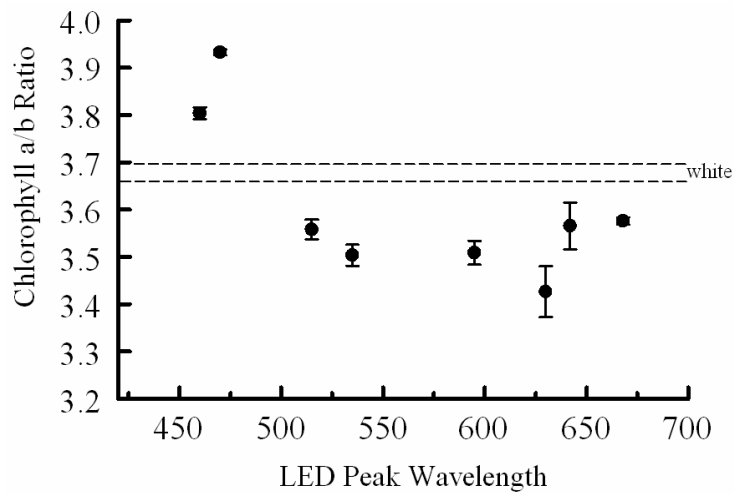


Fig. 4. *Spirodela polyrrhiza* chl a/b ratios for the different light treatments after 6 days cultivation. The dashed lines indicate chl a/b ratio level for the white LED treatment and for white fluorescent tubes ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$).

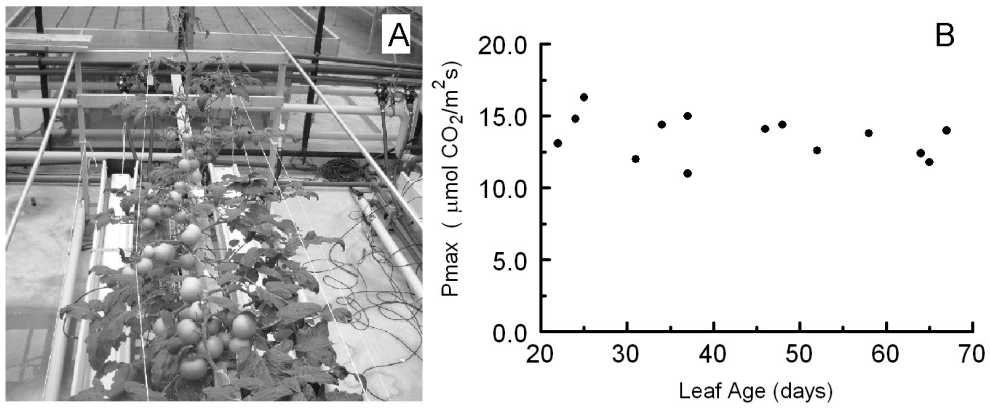


Fig. 5. A: Tomato plant grown horizontally in greenhouse. B: P_{max} of leaves differing in age, from horizontally grown tomato plant.

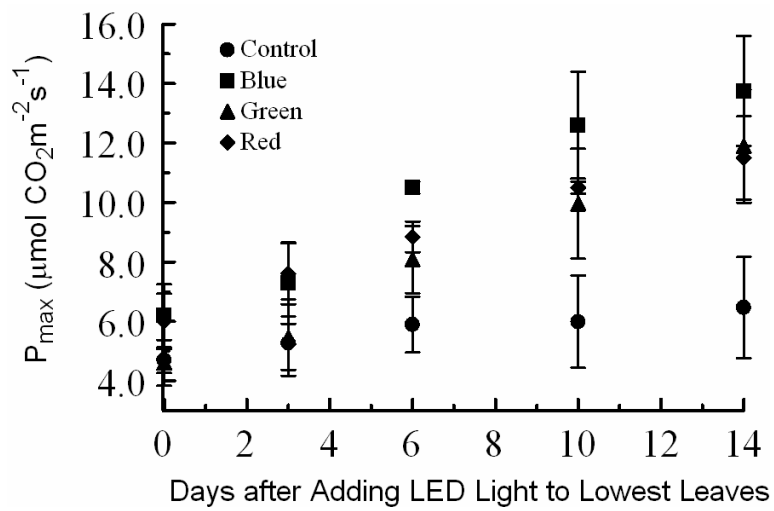


Fig. 6. P_{max} measured after adding supplemental narrow band light to the lowest leaves in a high-wire grown tomato crop, using blue, green and red LED light.

