University of São Paulo
“Luiz de Queiroz” College of Agriculture

Supplemental LED interlighting on the physiological response and yield of
mini-cucumber

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Thesis presented to obtain the degree of Doctor in
Science. Area: Crop Science

Piracicaba
2017

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Supplemental LED interlighting on the physiological response and yield of mini-cucumber
versão revisada de acordo com a resolução CoPGr 6018 de 2011

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Dedication

To my wife Carmen and my sons Felipe and Santiago, for having the courage to embark on this journey together.

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God, thank so much for guide my life with your love. For all the opportunities, protection and blessing in my life. Grateful forever! [Philippians Chapter 2,1-10].

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*Yo no quiero un cuchillo en manos de la patria.
Ni un cuchillo ni un rifle para nadie:
La tierra es para todos,
Como el aire.*

*Me gustaría tener manos enormes,
Violentas y salvajes,
Para arrancar fronteras una a una
Y dejar de frontera sólo el aire.*

*Que nadie tenga tierra
Como tiene traje:
Que todos tengan tierra
Como tienen el aire.*

*Cogería las guerras de la punta
Y no dejaría una en el paisaje
Y abriría la tierra para todos
Como si fuera el aire.*

*Que el aire no es de nadie, nadie, nadie,
Y todos tienen su parcela de aire.*

Jorge Debravo

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RESUMO

Suplementação luminosa com LED na resposta fisiológica e produtiva de plantas de minipepino

Na produção hortícola em ambiente protegido, o emprego de luz artificial é uma prática comum, quando condições de radiação natural reduzidas ou o autosombreamento das folhas nos estratos médio e inferior do dossel prejudicam a atividade fotossintética da planta, e consequentemente, limitam a produtividade. Na última década, em países de alta latitude, tem aumentado o uso de lâmpadas com tecnologia de luz LED para fazer essa suplementação luminosa. As luzes do tipo LED são mais eficientes energeticamente e apresentam baixo consumo de energia. Também nessas lâmpadas é possível regular a intensidade (quantidade) e a radiação (qualidade) na faixa ideal do espectro de luz visível para a conversão da energia luminosa em energia química. Em países de clima tropical, experiências dessa natureza não estão reportadas. Assim, foi realizado este trabalho com o objetivo de avaliar parâmetros fisiológicos relacionados com a atividade fotossintética e a produtividade de minipepino cultivado em um ambiente protegido com suplementação luminosa de tipo LED. Em Piracicaba (SP), foram conduzidos três experimentos em casa de vegetação, sendo realizados no inverno (anos 2015 e 2016) e no verão (ano 2017), usando plantas de minipepino híbrido Larino. Foi constatado nos três ciclos aumento na fotossíntese da planta e na produção de pepino comercial devido ao emprego da luz LED. Esse aumento no rendimento comercial variou entre 13 e 30%, sendo maior o efeito no ciclo do verão. No primeiro ciclo além do efeito da luz LED, plantas enxertadas foram testadas. Nesse caso, as plantas enxertadas mostraram também aumento na taxa fotossintética quando submetidas à suplementação luminosa, porém a produtividade não aumentou com a enxertia. Parâmetros de pós-colheita como acidez titulável, teor de sólidos solúveis e vida de prateleira não aumentaram pela suplementação com LED. Estudando as curvas de resposta à luz foi constatado que acima de $400 \mu\text{mol m}^{-2}\text{s}^{-1}$ na densidade do fluxo de fótons, as plantas com suplementação luminosa mostraram valores maiores na taxa de assimilação líquida de CO_2 . O ponto de compensação à luz também foi superior em plantas com LED. No caso da curva de resposta ao CO_2 , as plantas tratadas com luz LED mostraram maior incremento na taxa de assimilação de CO_2 quando a concentração interna de CO_2 na planta aumentou. Parâmetros como atividade da Rubisco carboxilase, taxa de transporte de elétrons e a respiração obscura foram também maiores em plantas sob luz LED. O menor consumo de eletricidade junto com o aumento na produtividade no ciclo de verão favoreceu maior eficiência no uso da energia e da luz nesse ciclo, quando comparado com os ciclos de inverno. Por esse mesmo motivo, a análise de custos revelou que a suplementação luminosa com luz LED somente foi benéfica no ciclo de verão. Nessa época, a renda líquida total foi positiva e a relação custo benefício favorável (1.15).

Palavras-chave: *Cucumis sativus*; Fotossíntese; Iluminação artificial; Produtividade; Pós-colheita; Curva de resposta; Custo

ABSTRACT

Supplemental LED interlighting on the physiological response and yield of mini-cucumber

On the protected horticultural industry, the artificial lighting is a common practice under reduced natural light radiation or self-shading of the leaves at lower canopies. Those conditions affect the photosynthetic activity and the yield is limited. In the last decade, the use of lamps with LED technology for supplemental lighting has increased on high-latitude countries. LED lights are more energy efficient and have lower power consumption. On these lamps is possible to regulate the intensity (quantity) and the radiation (quality) on the ideal range of light spectrum in order to convert the luminous energy into chemical energy. Not reports of use of this technology were found at the tropics. Thus, the objective of this work was to evaluate physiological parameters related with the photosynthetic activity and the yield on mini-cucumber plants grown on protected environment with supplemental LED interlighting. At Piracicaba (Sao Paulo), Brazil, three experiments were conducted on LED interlighting equipped greenhouse, two at winter seasons (2015 and 2016 years) and one at summer season (2017 year) using mini-cucumber hybrid Larino. Through the three stands, was verified an increase on the photosynthesis in the leaves and the commercial productivity of the mini-cucumber due to the LED lighting use. This increase on the commercial yield ranged between 13 and 30%, being higher the effect on the summer season. At the first stand, in addition to the light effect, grafted plants were tested. On this case, the photosynthetic rate increased with grafted plants when treated with LEDs. However, the yield did not increase with grafting. Postharvest parameters as titratable acidity, total soluble solids and long shelf life did not increase as consequence of supplemental LED lighting. Studying the light response curves, it was verified that above a photosynthetic photon flux density of $400 \mu\text{mol m}^{-2}\text{s}^{-1}$, plants treated with supplemental lighting showed higher values of net CO_2 assimilation. The light compensation point was also higher on plants with LEDs. In the case of the CO_2 response curve, plants treated with LED lighting verified higher increase on the CO_2 assimilation rate when the plant internal CO_2 concentration increased. Parameters like Rubisco carboxylase activity, rate of electron transport and leaf dark respiration were also higher on plants under supplemental LED lighting. The lower consumption of electricity and the yield increase on the summer stand allowed registering greater energy and light efficiency on this cycle when compared with the winter stands. For the same reason, the cost analysis revealed that only at the summer stand the LED interlighting was profitable. At that time, the total net income was positive and the benefit cost ratio favorable (1.15).

Keywords: *Cucumis sativus*; Artificial lighting; Photosynthesis; Performance; Postharvest; Response curve; Cost

1. INTRODUCTION

1.1. LED interlighting

Estimates indicate that human population will reach nearly 9.8 billion of people by the year 2050 (United Nations, 2017). Limited cultivated land, climate change and pressure for the water use are others factors that force for more efficient agricultural systems. Yielding increase in this context is necessary. Thus, have been appeared genotypes more productive and resistant to adverse biotic and abiotic conditions, new technologies and substantial improvements in production systems. In protected horticulture, air temperature, humidity, CO₂ concentration, photoperiod and light intensity are some factors that in the recent years have been manipulated to optimize plant production and quality (Dueck et al., 2016).

Artificial lighting is one of those technologies that contribute to this challenge of producing more and better. Lighting plants is not new. Some decades ago, in protected environments, artificial light was used to promote or control flowering on ornamental plants. With the popularization of greenhouses and the crop diversification in these environments, lighting techniques evolved. The first commercial plant production with LEDs started at Japan in 1998; since then, the more traditional High Pressure Sodium lamps (HPS) has been gradually changed by LED (Light-emitting diode) lamps systems (Morrow, 2008). Currently in the greenhouse horticultural industry, the global need to reduce CO₂ gas emissions to the environment, the highly energy dependent structure and the high cost of this energy are the major threats (Pinho et al., 2013), an LEDs can help reduce this impact.

LEDs are solid state light emitting device and a unique type of semiconductor diode. This diode is designed to allow electrons and holes to recombine to generate photons (Singh et al., 2015). Their structure consists of a chip, which is a light-emitting semiconductor material, a lead frame to place the die and the encapsulation which protects the die (Yeh and Chung, 2009).

LEDs have many characteristics important to horticultural purposes. The ability to control the spectral composition of visible light is one of the most important. Thereby, is possible to control plant growth and morphology, or modifies metabolic process and internal content of plant compounds. LEDs also have high energy efficiency. Lighting energy use in greenhouse modules can decrease up to 70% due to LED devices when compared with traditional lamps with similar effects on the plants pattern. LEDs have a very long operation life, with about an estimated duration of 50.000 hours. They have very little radiant heat output, which means reduction of heat stress on plants; thus, lamps can be operated close to plant tissue. Their reliability, compact size and the reduction in maintenance are other advantages of LED lamps (Morrow, 2008; Singh et al., 2015).

The light quantity and quality have direct influence on the growth and other plant responses. According to Fan et al. (2013), an increase on net photosynthesis and plant growth correlates with high light intensity, while changes in leaf anatomy, physiology and morphology appear with alterations on the light spectrum. For example, far-red light stimulates flowering of long-day plants and promotes internode elongation, while blue light regulates stomatal opening and is important for phototropism (Massa et al., 2008). Thus, the LED lamps use is useful to stimulate the photosynthetic activity on the specific waveband light spectrum for many economic important crops, including vegetables. Benefits on yield and the quality of the products due to the LED and interlighting use have been reported in the literature for crops like tomato (Deram et al., 2014), cucumber (Hovi et al., 2004), sweet pepper (Hovi-Pekkanen et al., 2006), lettuce (Son and Oh, 2015) and strawberry (Choi et al., 2015).

Traditionally, supplemental lighting has been common in northern latitude countries, especially at winter season, where the photoperiod is short and the light intensity decays significantly (Gunnlaugsson and Adalsteinsson, 2006). However other circumstances can also make necessary the artificial light use. The mutual shading of lower leaves of the canopy can limit the productivity of high stand crops. High plant density is a common practice in greenhouses in order to maximize the productivity of the stands on crops like tomato or cucumber. This situation promotes an intense competition for light (Pettersen et al., 2010). With LED interlighting is possible to avoid this problem in the lower parts of the canopy. Operating close to the canopy allows a better interception of the light by the plant. Consequently improves the use efficiency of the lighting system (Morrow, 2008).

1.2. Mini-cucumber

Cucumber is one of the most expressive crops for the greenhouse industry. Represent one of the three most important crops in protected agriculture in Europe and in the United States of America (Heuvelink et al., 2006; Shaw et al., 2007). According to the FAO (2017), almost 75 millions of tons of cucumber were produced in the world in 2014, considered both open field and protected agriculture. China, Iran and Russia are the main producers worldwide. Cucumber represents for Brazil one of the ten vegetables crops of greater economic importance, with more of 215 000 annual tons produced (Carvalho et al., 2013).

Mini-cucumber is a Beit Alpha type cucumber first originated at Israel. This genotype is a hybrid that is gynoeocious. Differing from traditional cucumber, the fruit production for Beit Alpha cucumber is prolific and up to six fruits are set at each plant node. The plant is parthenocarpic and the fruits have thin skin (Shaw et al., 2000). The fruit length usually does not exceed 18 cm, but it is ideal to harvest with a size of 8 to 11 cm for marketing reasons. Mini-cucumber fruits are normally packed in small plastic boxes or bags of 200 g (6 to 8 fruits approximately) appropriate for the target market (Hochmuth, 2012). A more sweet taste of this cucumber compared to the traditional one, is another differential of the Beit Alpha genotype fruits.

The above described characteristics for the mini-cucumber fruit represent an opportunity to reach more select markets and a stimulus for snack type consumption. Snack products have high added-value, which would benefit a better economic return on investment. There are currently more than 50 species of mini-vegetables grown and marketed worldwide. For their convenience and great flavor, these innovative products are ideal for use as snack and their consumption increase every year following market trends (García et al., 2012.). The snack or cocktail name is used for a new concept of healthy food, where fresh and natural products are preferred between meals. The target markets for these products are students and office workers, for the ease of being consumed during recess. Furthermore, are very practical for people who have a healthier lifestyle and want a nutritious and equilibrated diet, where the flavor, the visual stimulus and the vegetables convenience are important. Ready-to-eat products are also consumed by small families, single people and time-pressed consumers, which contribute to a demand increase for this kind of natural products (Olsen et al., 2012; Schreiner et al., 2013).

No reports were found for LED interlighting experience on tropical latitudes. Similarly no scientific information is available for mini-cucumber crop at Brazil. Due to the increase in the LEDs use in commercial greenhouses and the introduction of innovative vegetables genotypes to respond to market demands, there is a need to generate local knowledge to make the necessary adjustments and take advantage of the new technologies. Therefore, this study was undertaken in order to evaluate physiological parameters related with the photosynthetic activity and the yield on mini-cucumber plants growing on protected environment with supplemental LED

interlighting on tropical conditions. Chapter 1 emphasizes on the LED interlighting effect on the photosynthetic response, the yield and quality parameters of grafted mini-cucumber plants in one winter stand. Chapter 2 compares both winter and summer stands in the photosynthesis and the yield and shows the light and CO₂ response curves of plants treated with LED interlighting. Chapter 3 details and compares the yield distribution of the three mini-cucumber crop cycles carried out and presents a cost analysis of the investment required by the use of LEDs in the study conditions.

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2. THE RESPONSES OF PHOTOSYNTHESIS, FRUIT YIELD AND QUALITY OF GRAFTED MINI-CUCUMBER TO LED-INTERLIGHTING

Abstract

Supplemental lighting is becoming a common practice for horticultural greenhouse industries, especially at high-latitude countries. This increase is because artificial light stimulates the photosynthetic process and enhances the total yield. However no scientific reports were found on this topic in tropical climate countries. This study investigated the effects of LED interlighting and grafted seedlings on photosynthetic response and the yield and quality of mini cucumber in Brazil. In the light treatment, at 15 cm of distance from the bar, the LED devices emitted a photon flux of $220 \mu\text{mol m}^{-2} \text{ s}^{-1}$ by red light (80%) with a peak wavelength of 662 nm and blue light (20%) with a peak wavelength of 452 nm. Lighting was used for 12 h d⁻¹ from 30 days after seedling transplanting until the end growth period. Mini cucumber hybrid ‘Larino’ was grafted on rootstock cultivar ‘Keeper’ (pumpkin hybrid) and rootstock cultivar ‘Shelper’ (*C. moschata*, pumpkin hybrid). The air temperature and relative humidity (RH) were maintained at $23.5 \pm 4 \text{ }^{\circ}\text{C}$ and $72 \pm 10 \%$ during the light period of day, respectively. At night, average temperature was $18.6 \text{ }^{\circ}\text{C} \pm 5 \text{ }^{\circ}\text{C}$ and the RH was $90 \pm 5 \%$. The results showed increased CO₂ net assimilation rate of plants under LED interlighting than plants grown under natural light in the greenhouse. Plants grafted onto both rootstocks had higher CO₂ net assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), apparent carboxylation efficiency ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$) and apparent electron transport rate ($\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$). The early yield increased 11.6 % with LED interlighting and 24 % with grafted plants. The commercial yield also increased with LED light at rate of 13 %, but did not enhance with grafted plants. Postharvest quality parameters as titratable acidity, total soluble solids and long shelf life did not increased with the LED light supplementation.

Keywords: *Cucumis sativus*; LED lamp; Supplemental lighting; CO₂ rate; Grafting; Postharvest

2.1. Introduction

Cucumber is one of the main crops cultivated in protected environment. Particularly specialty cultivars with high market value as snack cucumber, with great flavor and reduced size fruits, favor the fresh consumption by an increasing consumer sector more exigent on food quality and healthy products (García et al., 2012).

For cucumber plants, fruit yield and quality have been influenced by the light quantity and quality (Dorais, 2003) as well as the localization of the light source in relation to the position of the photosynthetic plant surface. The lamp design defines the light’s orientation angle and height at canopy. LED lamps and interlighting have been used successfully to grow tomatoes, bell peppers and cucumbers (Fan et al., 2013; Massa et al., 2008). Cucumber plants are grown vertically and intra-canopy artificial radiation assists in reducing the self-shading of the plant’s lower canopy. Therefore, particularly in temperate climates, the use of top and interlighting lamps with light emitting diodes, known as LED technology, has increased in protected cultivation. LED technology has advantages as possibility to calibrate the spectrum radiation for different species, low heat, low power consumption, easy handling and thus to adapt to different canopy structures and prolonged equipment life (Mitchell et al., 2012). Artificial light with LED lamps may increase the carbon assimilation that it is directly related with the photosynthetically active radiation intercepted by the leaves, which converts light energy into chemical energy by photosynthesis (Radin et al., 2003). Positive results

have been reported for several protected crops growing under different lighting arrangements of blue, red and far red LED's (Fan et al., 2013; Olle and Viršillė, 2013; Park and Runkle, 2017). Such arrangements allow a good correspondence between the blue and red light spectra provided by LED with the action spectra of chlorophyll and carotenoids (Yeh and Chung, 2009). Terashima et al. (2009) reported that leaves might absorb 90% of the available blue and red light. The ratio of blue to red light provided by LED to cucumber showed that leaves illuminated only with red wavelength are photosynthetically dysfunctional. This problem was prevented with only 7% blue light, which photosynthetic capacity increased up to 50%. Blue light also increases plant biomass and fruit yield in cucumber plants (Menard et al., 2006). Hernández and Kubota (2014) used the total light energy during the day (day light integral - DLI) to calibrate the proportion of blue and red lights provided by LED. Under high DLI conditions ($16.2 \pm 5.3 \text{ mol m}^{-2} \text{ d}^{-1}$) no differences were observed to dry weight, leaf number and leaf area of cucumber plants, but at low DLI ($5.2 \pm 1.2 \text{ mol m}^{-2} \text{ d}^{-1}$), these characteristics improved with red:blue ratio of 80%:20% supplied by LED light.

The response of cucumber plant to supplemental LED light also may vary as function as rootstock-scion combination. Grafting is a useful practice to increase the vigor and yield of plants as compared to those cultivated from conventional seedlings in greenhouses (Hernández et al., 2014; Liu et al., 2015). Grafted plants have vigorous roots, whose plant root system is more able to improve the water and nutrient uptake. Appearance and postharvest quality of the fruits also can be strongly affected by grafting (Fallik and Ilic, 2014; Flores et al., 2010).

No reports of LED interlighting use were found on scientific literature for tropical climate countries. The objective of this study was to evaluate the photosynthetic parameters, yield and quality of snack cucumber plants grafted on two rootstock cultivars under supplemental LED light in greenhouse.

2.2. Materials and methods

2.2.1. Experimental site

The experiment was carried out from April to August 2015 in the experimental site of Crop Science Department of University of São Paulo, Piracicaba (SP), Brazil ($22^{\circ}42'$ South Lat.; 546 m of altitude; altitude tropical climate, type Cwa according with Köppen). The cucumber plants were grown in a greenhouse with 345 m² floor area and 3.4 m gutter height. The greenhouse structure had a climate control evaporative cooling system (pad and fan) activated when temperature raised higher than 25 °C. Temperature, humidity and PAR radiation inside the greenhouse were registered continuously by meteorological station (WatchDog 2400 Mini Station External Sensor, Spectrum® Technologies, Inc.; Aurora, Illinois, USA) placed at 1.5 m of height. Temperature and air relative humidity (RH) were maintained at 23.5 ± 4 °C and 72 ± 10 % during the daytime. At night, the temperature and RH ranged from $18.6 \text{ °C} \pm 5 \text{ °C}$ and from $90\% \pm 5 \%$ respectively.

2.2.2. Treatments and experimental design

The experiment was established as a randomized block design with three replicates and six treatments at factorial scheme. Treatments included two light conditions (LED supplemental light and natural light as control) and three types of seedlings (ungrafted hybrid, hybrid grafted on rootstock cultivar Keeper (pumpkin hybrid) and hybrid

grafted on rootstock cultivar Shelper (*C. moschata*, pumpkin hybrid)). Each plot had 12 plants grown in pots. The pots were distributed in double lines, with 0.4 m of spacing between plants, 0.8 m between lines and 2.0 m between the double lines, resulting in a density of 2.5 plants m⁻².

2.2.3. Lighting

Light supplementation was provided by LED interlighting lamps (Philips GreenPower LED, Philips Lighting Holding B.V., Amsterdam, The Netherlands). The LED bar lamp dimensions were 250 cm length, 4.2 cm width and 7 cm height, with light linear arrangement. They were placed horizontally at 1.5 m height in the middle of a double row of cucumber plants. Light colors emitted were red and blue in a ratio of 80% red light (662 nm) and 20% blue light (452 nm). The light intensity emitted was in a range near to 220 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Light photoperiod was 12 hours, from 9:00 AM to 9:00 PM.

2.2.4. Cultivar

The cultivar used at the experiment was the mini-cucumber hybrid ‘Larino’ (Rijk Zwaan®). Fruits of this hybrid are parthenocarpic and have 9 to 11 cm long. They may be consumed as a fresh-tasty snack. This cultivar needs vertical conduction system and can produce three to five fruits per bud in a period of two to four months.

2.2.5. Growth conditions

The seedlings of grafted and non-grafted cucumber were produced in a nursery company (Hidroceres®) and transplanted (one plant per pot) into 8 L capacity plastic containers filled with substrate based on coconut fibers (medium texture). This substrate had a cation exchange capacity (CEC) of 220 mmol kg⁻¹, pH of 5.7 and an electrical conductivity of 2.07 dS m⁻¹.

The nutrient solution was applied by drip irrigation controlled by moisture-sensors (Irrigation controller-MRI, Hidrosense, Jundiai, Brazil) to maintain the substrate moisture at field capacity. The nutrient solutions for two development stages were composed by (mg L⁻¹): Vegetative period: 120 N, 42 P, 120 K, 108 Ca, 32 Mg and 60 S; Reproductive period: 150 N, 105 P, 200 K, 133 Ca, 50 Mg and 84 S. In both mineral solutions, micronutrients were supplied as a cocktail fertilizer (1.82% B, 1.82% Cu, 7.26% Fe, 1.82% Mn, 0.73% Zn and 0.36% Mo) at rate of 25 mg L⁻¹. The average electrical conductivity (EC) and pH of nutrient solutions were 2.2 dS m⁻¹ and 6.5, respectively.

Plants were grown vertically by plastic strips and supported by horizontal wires, which were positioned at 3 m height. Plants had only one main stem with pruning made primarily at the beginning of the plant growth period.

2.2.6. Photosynthetic parameters

Photosynthesis was evaluated at 55 DAT using a portable infrared gas analyzer (IRGA, LI 6400XT, LI-COR; Lincoln, Nebraska, USA). The photosynthesis measurements were made in the third fully expanded leaf between 8:30 am to 11 am in a completely sunny day. The photosynthetic parameters recorded were net assimilation rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2}\text{s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), internal CO_2 leaf concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$), apparent electron transport rate (ETR , $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) as a parameter of chlorophyll a fluorescence, A/E and A/C_i . A/E determines the water use efficiency (WUE , $\mu\text{mol CO}_2 (\text{mol H}_2\text{O})^{-1}$) and A/C_i the apparent carboxylation efficiency ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$), determined by the relationship between the CO_2 assimilation rate and the intercellular CO_2 concentration. Photosynthesis parameters were obtained under a constant light of $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in the chamber and using the ambient value (approximately $380 \mu\text{mol mol}^{-1}$ of air), as a reference for the CO_2 concentration.

2.2.7. Agronomic parameters

The same leaves used to analyze photosynthesis were collected, in a total of five leaves per plot, sent to laboratory, rinsed with tap water, dipped in a phosphate free detergent solution (0.1% w/v), and rinsed three times with deionized water. Leaves were dried at 68°C until they reached a constant weight and analyzed for N, P, K, Ca, Mg and S contents. At the end of the growth period (124 DAT), two plants of each plot were collected to measure the internodes length (IL). Later, the plants were dried at 68°C to determine the dry weight.

Fruits from each plot were harvest three or four times per week, counted, weighted and classified to determine the commercial yield and curved fruit yield. The fruits well formed, without injury and with size between 9 cm and 11 cm of length were classified as commercial yield.

2.2.8. Postharvest quality

Fruit quality was evaluated by titratable acidity (TA) and total soluble solids (TSS) at harvest (H1) and 20 days postharvest (H2). TA was determined through 10 g of aliquot pulp diluted in 100 ml distilled water with 0.1N NaOH until a solution reach a pH of 8.1, according to the method of AOAC (2010). TSS was measured in a digital refractometer (Atago Co., Tokyo, Japan) using an aliquot of cucumber pulp. In addition, mass loss of cucumber fruit was evaluated every two days during 20 days after harvest. During this period, the fruits were kept in a chamber at 10°C and 90% relative humidity.

2.2.9. Statistical Analysis

For the statistical analysis of the results obtained in the different evaluations, variance analyses were made by the F test. The treatment means were compared by Tukey test ($P < 0.05$) using SAS/STAT® program (SAS Institute, Cary, NC).

2.3. Results and Discussion

2.3.1. Photosynthetic parameters

The effects of LED supplemental light and grafting on the photosynthetic parameters of mini-cucumber plants are shown on Table 1.

Table 1. Effects of LED interlighting and grafting on CO₂ net assimilation rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), stomatal conductance (G_s , $\text{mol m}^{-2}\text{s}^{-1}$), internal CO₂ leaf concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), water use efficiency (WUE , $(A/E) \mu\text{mol CO}_2 (\text{mol H}_2\text{O})^{-1}$), apparent carboxylation efficiency (A/C_i) and electron transport rate (ETR , $\mu\text{mol electrons m}^{-2}\text{s}^{-1}$) in mini-cucumber plants.

Main Effects	A	G_s	C_i	E	WUE	A/C_i	ETR
Interlighting (I)							
Control	9.22 b ^z	0.395 a	352.51 a	8.703 a	1.018 b	0.027 a	59.04 a
LED	12.93 a	0.423 a	342.96 a	6.412 b	2.059 a	0.038 a	65.84 a
Grafting (G)							
Ungrafted	5.36 b	0.405 a	372.12 a	7.045 a	0.820 b	0.015 b	51.85 b
Keeper	13.37 a	0.428 a	330.86 b	8.190 a	1.747 a	0.041 a	64.56 a
Shelper	14.48 a	0.395 a	340.23 ab	7.438 a	2.048 a	0.043 a	70.92 a
Interaction Effects							
I*G	ns	ns	ns	ns	ns	ns	ns
C.V.%	27.4	15.35	6.36	13.16	35.92	35.17	12.16

^zMeans followed by the same letter in the column did not differ from each other by Tukey range test at $P \leq 0.05$

Plants under LED interlighting showed higher CO₂ net assimilation rate (A) at 55 DAT than plants grown under natural light in the greenhouse (Table 1). The increase of A may be explained first by the direct effect of the increase in the photosynthetic photon flux by the LEDs. Plants treated with supplemental lighting received higher light intensity than control plants only affected by natural sunlight. Also the ratio between red and blue LED of 80%/20%, that emitted wavelength peaks of 662 nm and 452nm respectively, gave quality light in the spectral ratio favoring the CO₂ assimilation. The red light is the main wavelength for chlorophyll excitation (Kriedemann, 2010) and has a direct impact on cucumber plant growth (Su et al., 2014). Additionally, red light also stimulates chlorophyll synthesis and chloroplast development (Olle and Viršillè, 2013; Su et al., 2014). Blue light is involved in many process as stomatal opening, leaf photosynthetic functioning (Whitelam and Halliday, 2007), biomass production and photosynthetic capacity (Hogewoning et al., 2010). According to the last authors an adequate red:blue light combination is required for enhance the photosynthetic machinery. The absence of blue light results on dysfunctional photosynthetic operation. Leaves grown at an irradiance containing less 15% blue light may lead to reductions in A .

It was also expected an increase of stomatal opening, electron transfer in the photosystem II and Rubisco carboxylation in plants due to interlighting (Pettersen et al., 2010). However, these parameters were not influenced by LEDs as shown by the stomatal conductance (G_s), electron transport rate (ETR) and apparent carboxylation efficiency (A/C_i), respectively (Table 1), although there was a trend of increase the G_s , ETR and A/C_i in plants illuminated by LEDs. Close to Capricorn tropic, southeast Brazil is considered a tropical climate, with about 11 to 12 light hours photoperiod in the location of the study (Piracicaba, SP) in the winter season, when our experiment took place. With more restricted light circumstances, as example of high latitude countries, LED effects on all photosynthetic parameters could be more evident than in our conditions.

The water use efficiency (WUE) also increased in plants under LED interlighting. Since WUE is the ratio between A and E , plants illuminated with LEDs used water more efficiently than the control (without LED) because photosynthesis was 1.4 times higher and the transpiration rate (E) was 26.3% lower with LEDs as compared to non LED interlighting. 20% of B LED did not increase enough the G_s which may justify why transpiration did not increase significantly with supplemental LEDs.

Plants grafted onto both rootstocks showed higher A , A/C_i and ETR than ungrafted plants of mini-cucumber. It is known that grafting can improve the net photosynthesis of cucumber plants (Rouphael et al., 2012) and photosynthetic parameters related to this metabolic change as apparent carboxylation efficiency (A/C_i) and electron transport rate (ETR). ETR is an indicator of the photosystem II (PSII) operating efficiency and electron flux through PSII reaction centers. Thus, it has direct connection with the CO_2 assimilation process. As long carbon is assimilated, electrons flowed through the photosystems and ETR also increased in grafted cucumber as reported by Pettersen et al. (2010) and Zhou et al. (2004).

2.3.2. Yield and plant dry weight

The early yield of cucumber (27 to 55 DAT) increased 16.4% with interlighting LED compared to non-interlighting LED (Table 2). These results are very similar to those obtained by Hao et al. (2012), who observed that LED interlighting increased cucumber fruit yield in early production period. For Marcelis (1993), the early yield of cucumber under intracanopy light is resulted of higher rate of individual fruit growth associated with a greater number of fruits growing at the same time on the plants.

Table 2. Effects of interlighting LED and grafting on commercial and curved fruit (not marketable) yield, in three different periods of days after transplant (DAT), in mini-cucumber plants cultivated on greenhouse.

Main effects	Commercial yield (kg plant ⁻¹)			Curved fruit yield (kg plant ⁻¹)		
	DAT			DAT		
	27-55	56-107	27-107	27-55	56-107	27-107
Interlighting (I)						
Control	0.67 b ^z	3.05 b	3.72 b	0.11 a	1.08 a	1.20 a
LED	0.78 a	3.43 a	4.21 a	0.11 a	0.84 b	0.95 b
Grafting (G)						
Ungrafted	0.63 b	3.41 a	4.04 a	0.11 a	0.96 a	1.07 a
Keeper	0.77 a	3.13 a	3.90 a	0.11 a	0.96 a	1.07 a
Shelper	0.78 a	3.17 a	3.95 a	0.11 a	0.96 a	1.08 a
Interaction Effects						
I*G	ns	ns	ns	ns	ns	ns
CV%	9.49	7.34	7.18	25.72	6.04	4.16

^zMeans followed by the same letter in the column did not differ from each other by Tukey range test at $P \leq 0.05$.

The yield in the period between 56 and 107 DAT and total yield (27 to 107 DAT) also were 12.4% and 13.2% higher with intracanopy light than control, respectively (Table 2). The results indicate that yield increase with intracanopy lighting LED was due the increased absorbed radiation in the lower part of the canopy. According to Pettersen et al. (2010), intracanopy light could increase photosynthetic rates and consequentially the assimilate supply to the fruits because the interlighting LED alters the spectral distribution in the canopy by enhancing the red and blue intensity. LED interlighting also improved cucumber fruit's quality by reduction of curved fruits (Table 2). Fruits need to be straight and uniform to increase profits of growers.

As result of this increase on photosynthetic metabolism, grafted plants had enhanced 24% the early yield (27 to 55 DAT) as observed by Cansev and Ozgur (2010) and Farhadi and Malek (2015) on grafted plants of cucumber. Grafting may affect uptake, synthesis and translocation of water, nutrients and plant hormones. For Hu et al. (2006) and Zhu et al. (2006), increased nutrient uptake in grafted plants increases photosynthesis, yield and sometimes fruit quality of cucumber. Zhou et al. (2009) reported in grafted cucumber plants that cytokinin was directly related to synthesis of chlorophyll resulting in an increase of ribulose-1,5-bisphosphate (Rubisco) enzyme and photosynthetic performance. It is known that cytokinin controls chlorophyll biosynthesis (Yaronskaya et al., 2006; Cortleven et al., 2016). After grafting, plant vasculature needs to be connected and the healing process seems to be strongly affected by cytokinin and auxin, as they promote cell proliferation (Melnyk et al., 2015).

Cucurbita species has been used as rootstocks for cucumber. However, in the end of the experiment grafted plants did not show increased commercial yield and biomass (Table 3) as compared to ungrafted plants. Several studies report variable effects on yield of grafted vegetables with different rootstocks genotypes (Edelstein et al., 2004; Davis et al., 2008a). Specific interaction and compatibility between the scion and the rootstocks and the

environmental conditions may be responsible for this growth response, since there is not a trend that all grafted plants are necessarily more vigorous and accumulate higher biomass (Huang et al., 2013; Lee et al., 2010).

Table 3. Internode length (IL) and biomass dry weight of cucumber plants cultivated on greenhouse with LED interlighting and grafted on pumpkin rootstocks.

Main Effects	IL (cm)	Biomass (g)
Interlighting (I)		
Control	8.92 a ^z	64.84 a
LED	8.60 a	65.76 a
Grafting (G)		
Ungrafted	10.55 a	67.53 a
Keeper	7.92 b	62.57 a
Shelper	7.82 b	65.80 a
Interaction Effects		
I*G	ns	ns
C.V.%	10.3	13.4

^zMeans followed by the same letter in the column did not differ from each other by Tukey range test at $P \leq 0.05$.

Grafted plants had shorter internode length (IL) compared to non-grafted, while lighting did not affect the IL and the biomass produced. Plant growth could be influenced by hormonal scion from the rootstock that could alter shoot physiology, indicating that roots can be involved in control of stem elongation (Schwarz et al., 2010; Pérez-Alfocea et al., 2010). Internode length may be reduced with interlighting (Hao and Papadopoulos, 2005), but the particular climatic conditions on this study contributed to reduce the effect of LEDs on the observed growth plant pattern.

2.3.3. Postharvest quality

The results of the titratable acidity (TA), total soluble solids (TSS) and loss of mass (LM) are shown in Table 4.

Table 4. Titratable acidity (TA), total soluble solids (TSS) and loss of mass (LM) of cucumber fruits from plants cultivated on greenhouse with LED interlighting and grafted on pumpkin rootstocks during the storage at 0 and 20 days after harvest (DAT).

Main effects	TA (%)		TSS (°Brix)		LM (%)
	0 DAT	20 DAT	0 DAT	20 DAT	20 DAT
Interlighting (I)					
Control	1.40 a ^z	2.18	2.42 a	2.76 a	23.18 a
LED	1.43 a	2.36	2.54 a	2.78 a	23.49 a
Grafting (G)					
Ungrafted	1.42 ab	2.30	2.56 a	2.73 a	25.82 a
Keeper	1.50 a	2.31	2.47 a	2.72 a	22.95 a
Shelper	1.32 b	2.21	2.39 a	2.84 a	21.24 a
Interaction Effects					
I*G	ns	*	ns	ns	ns
C.V.%	6.78	7.62	12.42	11.40	14.74

^zMeans followed by the same letter in the column did not differ from each other by Tukey range test at $P \leq 0.05$.

*Significant at $P \leq 0.05$.

Cucumbers fruits are very sensitive to water loss, main factor for deterioration, dehydration and poor visual quality (Hochmuth, 2012). However, interlighting LED used in cucumber plants was not able to decrease the mass loss of fruits (LM) at harvest (0) and in the storage 20 days after harvest (20). Hovi and Tahvonen (2008) also reported that cucumber fruits grown with interlighting LED did not extend long shelf life either. TA at harvest and TSS at 0 and 20 days after harvest also were not affected by LED interlighting in our study.

More than 21% of mass was lost at 20 days after harvest, which means around 1% of daily weight loss. These results in cucumber fruits are similar with those found by dos Reis et al. (2006) who reported 9% of mass loss after 8 days of storage also on cold chamber.

A right selection of a rootstock is fundamental to get improvement on fruit quality of cucumber (Fallik and Ilic, 2014). TA was affected by grafting in the storage at harvest (0), which rootstock 'Keeper' was better than 'Shelper', while grafting didn't affect the TSS. Huang et al. (2009) found that grafted cucumber plants in Figleaf Gourd and Chaofeng Kangshengwang had fruits with higher titratable acidity than other rootstocks. Liu et al. (2015) comparing cucumber rootstocks did not find benefits of grafting on TSS. Discrepancies between beneficial and detrimental effects on the scion fruit vegetables quality attributes are common, but it is agreed that the rootstock/scion combination and the environment conditions interfere strongly the quality of fruit flavor (Davis et al., 2008b).

In the storage at 20 days after harvest (20), there was interaction ($p=0.0021$) between interlighting LED and grafting to TA in the fruits. Mini-cucumber fruits from plants grafted in Keeper rootstock showed higher TA than control and Shelper rootstock with LED supplemental light. Without LED interlighting, there were not statistical differences between values of TA to grafted and ungrafted plants.

2.4. Conclusions

LED interlighting of the lower canopy leaves had a positive effect on photosynthesis, early yield and total yield of mini-cucumber. Postharvest benefits due to LED were not verified. Grafted plants of mini-cucumber cultivar ‘Larino’ on rootstock cultivar Keeper (pumpkin hybrid) and on rootstock cultivar Shelper were able to increase photosynthetic parameters, water-use-efficiency (WUE) and early yield, but not the total yield. In summary, supplemental LED light may be used to enhance the yield of mini-cucumber in the winter at tropical conditions. However, to recommend the adoption of the supplemental LED interlighting technology for mini-cucumber crop in the study conditions, is necessary at least a cost analysis to define the economic feasibility.

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3. LED INTERLIGHTING: PHYSIOLOGICAL RESPONSES TO INCREASE CUCUMBER YIELD PRODUCTION

Abstract

LED interlighting supplies artificial light for the greenhouse horticulture industry mainly at high latitude countries. In this study, the objectives were to determine photosynthetic parameters including the light and CO₂ response curves and to evaluate the LED interlighting effect on the yield of mini-cucumber plants on winter (WP) and summer (SP) periods at tropical weather conditions in southeastern Brazil. The study was conducted at Piracicaba, Sao Paulo, Brazil, in a greenhouse equipped with LED interlighting bars. Those LED bars emit an intensity of 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with peak wavelengths of 662 nm and 452 nm at the red (R) and blue (B) light spectrum respectively. The R:B proportion of the lamp was 4:1. The seedlings of mini-cucumber hybrid Larino were planted 9 days after sowing. LED lamp bars were used for 12 hours a day at winter and 10 hours for summer from 30 days after seedling transplanting. The average temperatures inside the greenhouse were 25,9 °C (maximum) and 14,3 °C (minimum) at WP; 26,9 °C (maximum) and 20,4 °C (minimum) at SP. Total plant period was 120 days for the WP and 90 days for the SP. The results showed that the net CO₂ assimilation rate increased with LED interlighting in both seasons, which increase was more expressive at winter. Stomatal conductance and the transpiration rate were higher at WP, while at SP the water use efficiency was higher, both with LED treatment. Apparent carboxylation efficiency increased with LEDs in both periods. According to the light response curves, it was verified that above 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux density (PPFD), plants treated with supplemental lighting had higher values of net CO₂ assimilation. The light compensation point was also higher on plants with LEDs. For the CO₂ response curve, plants treated with supplemental LED lighting showed higher increase on the CO₂ assimilation rate with increase of the intercellular CO₂ concentration. The rate of electron transport and leaf dark respiration were also higher on plants under LED lighting. The commercial yield increased 19% at WP and 30% at SP due to the LED interlighting use.

Keywords: *Cucumis sativus*; Light and CO₂ curve; Photosynthesis; Supplemental light

3.1. Introduction

The greenhouse industry uses high technology to cultivate value-added vegetable crops. Cucumber is one of those crops considered important in protected environment and it has an expressive consumption demand and high market value (Shaw et al., 2007). New genotypes classified as specialties varieties appear to add value to the production, which is the case of the mini-cucumber. This kind of product consumed as natural snack have an increasing demand in defined target market as children, students and office's workers and in general people with healthier eating habits (García et al., 2012; Olsen et al., 2012).

Mini-cucumber plants are cultivated in greenhouses with high technologies as light supplementation with LED (Light-emitting diode) to reach high yield and quality of fruits. The use of LED lamps has become popular in the recent years in the technical horticultural industry, due to its proven efficiency, increasing lighting configuration alternatives and reduction on its costs. LED interlighting is specially designed for high wire crops, helping to reduce the shade at the plants lowest canopies (Mitchell et al., 2012) and improving the physiological and metabolic activities of the plants (Olle and Viršile, 2013). The main differential effect of the LED's light supply when compared with

other light sources is the option to use specific wavelengths to stimulate the photosynthetic activity of plants (Morrow, 2008). This light quality manipulation is a valuable tool for growers for enhances production especially under greenhouse conditions and high latitudes with poor natural light supply (Demotes-Mainard et al., 2016; Moe et al., 2006).

Light quantity and quality are two of the main factors, which determine plant growth responses to environment (Dorais, 2003). Net photosynthesis, growth of plants, anatomy and morphology of the leaves are correlated with light intensity (Trouwborst et al., 2011a) and spectral light quality (Fan et al., 2013). Plants have many photoreceptors with specific function and organization that respond over a broad range of wavelength from UV-B (280 nm) to far red (750 nm) and to variations in light direction and photoperiod (Galvão and Fankhauser, 2015). Absorbance spectra of molecules show higher efficiency at blue and red wavelengths, corresponding to 80 to 95% of the total absorbed light (Terashima et al., 2009).

For Trouwborst et al. (2011b), a higher yield with LED interlighting is expected due an increase in light absorption and homogeneously light distribution over the vertical axis plant. Production increases with supplemental interlighting were reached for many crops like tomato (Tewolde et al., 2016), cucumber (Hovi-Pekkanen and Tahvonen, 2008), sweet pepper (Hovi-Pekkanen et al., 2006), cut flowers and foliage plants (Runkle and Heins, 2006), strawberry (Choi et al., 2015), lettuce (Lin et al., 2013) and lamb's lettuce cultivated in greenhouses (Wojciechowska et al., 2015).

According to Hernández and Kubota (2014), cucumber plants are more sensible to light quality irradiance than other traditional greenhouse vegetables such as peppers and tomatoes. A production of photoassimilates due to the photosynthetic process is strongly affected by light quality and diurnal temperature alternations that are the main responsible for the plant dry mass development (Xiong et al., 2011). Therefore, the supplemental lighting may promote an increase in daily net photosynthetic CO₂ assimilation, which can lead to increase productivity. Measuring CO₂ assimilation rates and the light and CO₂ response curves is possible to characterize the photosynthetic activity of the plant, which defines the biomass production influencing crop productivity and quality (Amaro et al., 2014). Those metabolic rates are influenced by environmental conditions, been a light one of the strongest factors that defines proportions of photosynthesis, respiration and eventually yield (Melis, 2009; van Dongen et al., 2011).

The supplemental lighting studies were always been conducted on high latitudes regions with particular climate conditions characterized by limited natural radiation. No scientific information on this topic was found on tropical or subtropical regions, where naturally light incidence is higher than temperate climate, especially at summer season. The objectives of this study were to determine photosynthetic parameters including the light and CO₂ response curves and to evaluate the LED interlighting effect on the yield of mini-cucumber plants in greenhouse under winter and summer season at tropical weather conditions in southeastern Brazil.

3.2. Materials and methods

3.2.1. Growth conditions and plant material

Two experiments were developed in greenhouse located at experimental site of Crop Science Department, University of São Paulo (USP), Piracicaba, São Paulo, Brazil (22°42' South Lat.; 546 m. altitude; altitude tropical climate, type Cwa according with Köppen). The first one was conducted from April to August 2016 corresponding to a winter season growth period (WP) and the second one from October 2016 to January 2017 in the

summer season growth period (SP). The greenhouse had 345 m² floor area and 3.4 m height. The greenhouse structure was equipped with a climate control evaporative cooling system (pad and fan) automatically activated when temperature raised 25 °C.

The genotype employed at the experiment was the F1 hybrid Larino, a mini-cucumber cultivar for greenhouse conditions. It is a high wire crop that requires vertical conduction system and it can produce 3 to 5 fruits per bud in a period of two or four months. The fruits have a size between 9 and 11 cm.

Seedlings of cucumber cultivar Larino were planted into 8 L capacity plastic containers filled with coconut fibers. In a drip irrigation system, a daily fertigation program was applied considering two different growth stages (mg L⁻¹): vegetative: 120 N, 42 P, 120 K, 108 Ca, 32 Mg and 60 S; reproductive: 150 N, 105 P, 200 K, 133 Ca, 50 Mg and 84 S. In both growth periods, the micronutrients were supplied by a commercial cocktail (7.26% Fe, 1.82% Cu, 0.73% Zn, 1.82% Mn, 0.36% Mo and 0.36% Ni). The average of electrical conductivity (EC) and pH of nutrient solutions were 2.2 dS m⁻¹ and 6.5, respectively. The plants were grown with the main stem and supported by plastic wires at height of 3 m.

3.2.2. Climate conditions

A meteorological station (WatchDog 2400 Mini Station External Sensor, Spectrum® Technologies, Inc.; Aurora, Illinois, USA) was placed at 1.5 m to register climate conditions at greenhouse. The mean, maximum and minimum temperatures and the accumulated PAR radiation for the winter (WP) and summer (SP) growth periods are shown in Fig. 1. Inside the greenhouse, the average temperatures were 25.9 °C (maximum) and 14.3 °C (minimum) at WP and 26.9 °C (maximum) and 20.4 °C (minimum) at SP.

Fig. 1. Mean, maximum and minimum temperatures registered and accumulated PAR radiation by day in the greenhouse for the winter (WP) and summer (SP) growth periods.

3.2.3. LED devices

The light supplementation was performed by LED interlighting lamp bars (Philips GreenPower LED, Philips Lighting Holding B.V., Netherlands) that were placed horizontally at 1.5 m distant from the floor in the middle of a double row cucumber plantation. Lamp light intensity emitted 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$ corresponding to 80% red light with a peak wavelength of 662 nm and 20% blue light with a peak wavelength of 452 nm (Fig. 2). The relative intensity was measured with a portable spectroradiometer (LI-1800, LI-COR; Lincoln, Nebraska, USA) at 10 cm distant of the lamp. Supplemental lighting was provided for 12 hours day⁻¹ at winter and 10 hours day⁻¹ at summer, from 28 days after transplanting (DAT) to the end of the growth periods.

Fig. 2. Relative intensity of spectral photon flux density for LED interlighting lamp device.

3.2.4. Treatments and experimental design

Two environments (LED interlighting and non-LED interlighting) were arranged at the greenhouse. Three replications were considered in each area, containing 12 plants per plot. The plants were grown in double lines, with 0.4 m of spacing between plants, 0.8 m between lines and 2.0 m between the double lines, resulting in a density of 2.5 plants m^{-2} .

3.2.5. Photosynthetic measurements

All the CO_2 assimilation rates (including those for light and CO_2 response curves) were measured with a portable infrared gas analyzer (IRGA, LCpro+, ADC BioScientific; Hoddesdon, Herts, England). The photosynthetic measurements were taken 67 DAT in the winter growth period and 52 DAT in the summer growth period. Differences between evaluation dates were due to cold environmental conditions that delayed plant development at winter season. Artificial lighting at the IRGA was provided by a mixed red/blue LED array coupled to the chamber and with an intensity of $696 \mu\text{mol m}^{-2}\text{s}^{-1}$. It was defined the ambient value as a reference for the CO_2 concentration. In both experimental environments (LED interlighting and non-LED interlighting), readings were taken at the third fully expanded leaf and were made from 10 to 11 am in a completely sunny day for four plants per plot. The photosynthetic parameters considered were CO_2 net assimilation rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (G_s , $\text{mol m}^{-2}\text{s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) and internal CO_2 leaf concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$). The ratios of A/E and A/C_i were calculated, which determines the water use efficiency (WUE , $\mu\text{mol CO}_2 (\text{mol H}_2\text{O})^{-1}$) and the apparent carboxylation efficiency ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$), respectively.

3.2.5.1. Light response curves

The response curves of the net photosynthesis rate as a response to photons flux density in cucumber plants with and without LED interlighting supplementation were obtained by 15 steps of increments from 0 to 1740 photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2}\text{s}^{-1}$). It was used a LED light coupled chamber at the leaf cuvette incorporated into the IRGA and were considered steady-state conditions before each measurement. The measures started with lower interval values at the more sensitive curve slope areas (until $348 \mu\text{mol m}^{-2}\text{s}^{-1}$). Measurements were made 48 DAT, considering the fourth fully expanded leaf. Temperature registered inside the cuvette was 28°C and $[\text{CO}_2]$ was the environmental. The photosynthetic light response curves were fitted using the nonrectangular hyperbola based model according to Lobo et al. (2013):

$$P_N = ((f_{(j_0)} \times I + P_{\text{gmax}} - ((f_{(j_0)} \times I + P_{\text{gmax}})^2 - 4\theta \times f_{(j_0)} \times I \times P_{\text{gmax}})^{0.5}) / 2\theta) - R_D$$

where P_N = net photosynthesis rate [$\text{mmol (CO}_2\text{) m}^{-2} \text{s}^{-1}$]; I = photosynthetic photon flux density [$\text{mmol (photons) m}^{-2} \text{s}^{-1}$]; $f_{(j_0)}$ = quantum yield at $I = 0$ [$\text{mmol (CO}_2\text{) mmol}^{-1} \text{(photons)}$]; P_{gmax} = maximum gross photosynthesis rate [$\text{mmol (CO}_2\text{) m}^{-2} \text{s}^{-1}$]; θ = convexity (dimensionless) and R_D = dark respiration rate [$\text{mmol (CO}_2\text{) m}^{-2} \text{s}^{-1}$].

3.2.5.2. A/Ci response curves

Similarly, the CO_2 response curves in cucumber plants in LED lighting and non-lighting environments were obtained using the IRGA and an incorporated controlled microenvironment leaf cuvette. Light was fixed with a coupled LED chamber at PPFD of $783 \mu\text{mol m}^{-2}\text{s}^{-1}$, temperature inside the cuvette were 28°C and measurements were registered only after steady-state conditions. Fifteen records were considered to register the A/C_i curve by increasing the CO_2 concentration level starting from 0 ppm until 2000 ppm with intervals of 50 ppm at the more sensitive points of the curve (until 300 ppm). At 400 ppm, increasing intervals were from 200 ppm until reached 1600 ppm and thereafter a reading was made at 2000 ppm of CO_2 to close the construction curve. Those changes in the increasing intervals were made for more accuracy of the curve slope. Three replications were considered for more reliability, using the fourth fully expanded leaf at 50 DAT. To build the CO_2 curve, the response of A to $[\text{CO}_2]$, it was used the biochemical model proposed by Farquhar et al. (1980), using values of C_c instead C_i through the program developed by Sharkey et al. (2007).

At low intercellular partial pressures (CO_2 assimilation rate limited by Rubisco) to fit the A it was used the following linear regression approach equation, used to estimate the V_{cmax} as the slope and the $-R_d$ as the intercept:

$$A = \frac{V_{\text{cmax}} (C_c - \Gamma^*)}{C_c + K_c (1 + O/K_o)} - R_d$$

where V_{cmax} = the maximum carboxylation velocity of Rubisco; C_c = CO_2 partial pressure at Rubisco; K_c = Michaelis constant of Rubisco for CO_2 ; O = O_2 partial pressure at Rubisco; K_o = Michaelis constant of Rubisco for O_2 ; Γ^* = photorespiratory compensation point and R_d = day mitochondrial respiration

At higher intercellular CO₂ partial pressures (*A* limited by RuBP regeneration) *J* was obtained fitting the following equation:

$$A = \frac{J(C_c - \Gamma^*)}{4C_c + 8\Gamma^*} - R_d$$

where *J* is the rate of electron transport, assuming on this equation four electrons per carboxylation and oxygenation (Sharkey et al., 2007).

3.2.6. Yield

Three times per week, mini-cucumber fruits from each plot were harvest, counted, weighted and classified to determine the total, commercial and non-commercial yield. The fruits with sizes between 9 and 11 cm of length, non-curved and without injury were classified as commercial yield; fruits non-curved and without injuries, but size outside the standard were classified as non-commercial yield. Fruits with excessive curvature and other malformation were considered as curved fruit. The total yield was calculated by sum of all fruits categories.

3.2.7. Statistical Analysis

Variance analyses were made for the statistical analysis of the results obtained. Treatment means were compared by Tukey test at 5% probability, using SAS/STAT release 9.3 ® program (SAS Institute Inc., Cary, NC, USA).

3.3. Results

LED interlighting affected all photosynthetic parameters for both growth seasons except to internal CO₂ leaf concentration in the WP (Table 1). The increase of net assimilation rate of CO₂ (*A*) with LED interlighting supplementation was more evident in WP because in this season the natural light intensity was lower than SP. It was verified 22% more CO₂ assimilation in the leaves of plants in WP. In the summer, LED interlighting besides benefiting *A*, also showed significantly higher *WUE* and *A/Ci* than control plants. Plants not treated with LED showed higher *Gs*, *Ci* and *E* than plants treated with LED in SP. Only the variables *Ci* and *WUE* did not have statistical differences in winter season. A higher apparent carboxylation efficiency (*A/Ci*) always corresponded with an increases in *A* for both seasons directly related with the supplemental LED light.

Table 1. Effects of LED interlighting on net assimilation rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), stomatal conductance (G_s , $\text{mol m}^{-2}\text{s}^{-1}$), internal CO_2 leaf concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), water use efficiency (WUE , $(A/E) \mu\text{mol CO}_2 (\text{mol H}_2\text{O})^{-1}$) and apparent carboxylation efficiency (A/C_i) in mini-cucumber plants cultivated on greenhouse at winter (WP) and summer (SP) seasons on tropical conditions.

WP						
Treatment	A	Gs	Ci	E	WUE	A/Ci
Control	12.46 b*	0.143 b	219.33 a	3.422 b	3.666 a	0.057 b
LED Interlighting	15.16 a	0.187 a	238.89 a	4.139 a	3.702 a	0.064 a
C.V.	6.95	21.11	10.54	13.51	11.30	10.85
SP						
Treatment	A	Gs	Ci	E	WUE	A/Ci
Control	10.88 b	0.178 a	237.00 a	4.602 a	2.36 b	0.046 b
LED Interlighting	12.56 a	0.128 b	182.25 b	3.312 b	3.38 a	0.070 a
C.V.	6.19	3.78	4.24	2.17	6.39	10.83

*Means followed by the same letter within the column did not significantly differ from each other (by Tukey range test at $P \leq 0.05$).

The Fig. 3 presents the light response curve for mini-cucumber plants submitted to LED. Plants grown with artificial light showed higher values of net assimilation CO_2 rate as compared to control when PPFD was above nearly $400 \mu\text{mol m}^{-2}\text{s}^{-1}$. Light compensation point (LCP) was also significantly greater for plants with supplemental LED light than control (Table 2). Even with higher LCP registered to plants with LED interlighting, the light saturation point was not affected for plants treated with LED light. The maximum quantum yield of photosynthesis (Φ) and the quantum yield at net assimilation rate equally to zero (ϕ_{I_0}) were significantly lower in plants exposed to LED light. No statistical difference was observed for the convexity (θ) of the light response curves between treatments.

Fig. 3. Light fitted response curve in LED interlighting and non-interlighting (control) mini-cucumber plants in response to increasing photosynthetic photon flux density. Plots represent an average of three measurements.

Table 2. Parameters estimated for light compensation point (LCP, $\mu\text{mol m}^{-2}\text{s}^{-1}$), light saturation point (LSP, $\mu\text{mol m}^{-2}\text{s}^{-1}$), maximum quantum yield of photosynthesis (Φ , $\text{mol CO}_2 \text{ mol photon}^{-1}$), convexity (Θ , dimensionless) and quantum yield at net assimilation rate = 0 (ϕ_{Io} , $\mu\text{mol CO}_2 \mu\text{mol photon}^{-1}$) from light response curves in LED interlighting and non-interlighting (control) mini-cucumber plants.

Treatment	LCP	LSP	Φ	Θ	ϕ_{Io}
Control	12.5 b*	740.0 a	0.0619 a	0.8088 a	0.0634 a
LED Interlighting	24.9 a	910.0 a	0.0444 b	0.9133 a	0.0450 b
C.V.	25,41	22,22	12,27	22,36	13,24

*Means followed by the same letter within the column did not significantly differ from each other (by Tukey range test at $P \leq 0.05$).

Additional CO_2 in the cell increases the A until a saturation point, normally near 100 Pa for C3 plants (Larcher, 2000), similar trend observed in this study. In the case of the parameters calculated from the CO_2 response curve (Fig. 4), no significant differences were observed on the maximum rate of Rubisco carboxylation ($V_{c_{\max}}$) and triose-phosphate utilization (TPU) due to LED interlighting supply (Table 3). The rates of electron transport (J) and the dark respiration (R_d) were higher in plant grown with LED when compared to control.

Fig. 4. CO_2 assimilation (A/C_i) fitted curve in LED interlighting and non-interlighting (control) mini-cucumber plants in response to increasing intercellular CO_2 chloroplast concentration. Plots represent an average of three measurements.

Table 3. Characterization of maximum Rubisco carboxylase activity ($V_{c_{max}}$), rate of electron transport (J_{max}), leaf dark respiration (R_d) and triose-phosphate utilization (TPU), in LED interlighting and non-interlighting (control) mini-cucumber plants.

Treatment	$V_{c_{max}}$	J_{max}	TPU	R_d
Control	101 a*	86 b	6.9 a	0.39 b
LED Interlighting	127 a	112 a	11.4 a	2.06 a
C.V.	10,32	3,43	24,69	10,18

*Means followed by the same letter within the column did not significantly differ from each other (by Tukey range test at $P \leq 0.05$).

The number of fruits and the total yield was unaffected by the LED interlighting in WP (Table 4). However, the commercial yield (Kg pl^{-1}) increased approximately 19% with the LED supplementation. In SP, all the yield parameters evaluated increased significantly with LED interlighting. The total and commercial yield of plant with LEDs increased 14% and 30%, respectively. The yield at the winter season (WP) was almost the half of yield at the summer season (SP) due to the colder temperatures in WP.

Table 4. Number of fruits per plant (Fr. pl^{-1}) and commercial and total mini-cucumber yield in plants with LED interlighting and non-interlighting (control) at different year seasons: winter (WP) and summer (SP) growth periods.

WP			
Treatment	Fr. pl^{-1}	Commercial (Kg plant^{-1})	Total (Kg plant^{-1})
Control	65.43 a*	1.87 b	2.60 a
LED Interlighting	67.44 a	2.23 a	2.81 a
C.V.	9.21	9.80	9.39
SP			
Treatment	Fr. pl^{-1}	Commercial (Kg plant^{-1})	Total (Kg plant^{-1})
Control	87.00 b	3.50 b	4.84 b
LED Interlighting	97.00 a	4.54 a	5.50 a
C.V.	2.26	1.58	2.37

*Means followed by the same letter within the column did not significantly differ from each other (by Tukey range test at $P \leq 0.05$).

3.4. Discussion

Red and blue light combination (4R:1B) and the additional photosynthetic photon flux density emitted inside the mini-cucumber plant canopy activated the metabolic pathways that enhanced the net photosynthesis (A) independently of the growth period in this study. As expected, a higher A/C_i ratio was consistent with the bigger A value in LED supplemented plants in both periods. According to Darko et al. (2014), photosynthetic process can be

modified in plants grown under LED light because lamps cover intensity and wavelength required for biomass and metabolic products of plants. Intracanalopy lighting for high-wire grown vegetables preserve CO₂ assimilation capacity deeper in the canopy, contributing to higher photosynthetic rate (Trouwborst et al., 2011b). Higher CO₂ assimilation values are achieved in cucumber plants under red:blue LED interlighting (Wang et al., 2009; Trouwborst et al., 2016) compared with monochromatic blue or red (Savvides et al., 2012).

Commonly higher A values are associated with higher stomatal conductance (G_s) due to increase of intercellular CO₂ concentration (C_i) (Shibuya et al., 2015). However, LED light use leaded to increase of A with decrease of stomatal conductance (G_s) and consequently of CO₂ leaf concentration (C_i) and transpiration rate (E), under higher temperatures and solar radiation registered in SP. It is known that net photosynthesis is associated not only with G_s and C_i , but with non-stomatal contributions as stomatal density and/or size (Shibuya et al., 2015). Therefore, LED interlighting can have been responsible to increase A through the stomatal density and/or size increase at higher solar radiation available in the SP.

Net photosynthesis and stomatal behavior that regulated the transpiration in leaves of mini-cucumber plants may explain the results of water use efficiency (WUE) in both periods (WP and SP). Climatic conditions associated with LED light use conducted to changes in stomatal conductance and consequently variations of transpiration. The regulation of stomata opening determines the amount of water loss through transpiration (Lawson et al., 2014). The stomatal response to light stimuli by LED lamps resulted in higher transpiration and net photosynthesis in WP. However, the conversion of CO₂ into photoassimilates was not enough to increase the WUE limited by light intensity and lower temperatures at winter period of growth mini-cucumber.

Light fitted response curve for mini-cucumber plants followed the usual pattern (Cocetta et al., 2017). Thus, there was a quickly increases on CO₂ assimilation rate at low density of photons and then the photosynthetic rate of the plants increased with higher light intensities until a plateau was reached.

In this study, plants growing in a richer supplemental light environment had a higher A and presented a light saturation point (LSP) at higher irradiance values, agreeing with Pettersen et al. (2010a). These authors obtained similar results with interlighting on different canopy parts of cucumber plants. They revealed that also the light compensation point (LCP) showed reduced values in darker than lighter canopy levels. Our findings demonstrated that LCP was significantly lower without LED interlighting (control plants). A trend of leaves growing on more dark conditions is to register lower LCP (Larcher, 2000), common fact in self-shading leaves in the lower canopy levels of high-wire conducted crops as the mini-cucumber plants.

Quantum yield (Φ) was lower in plants with LED interlighting and the convexity (Θ) did not differ significantly between plants with and without supplemental lighting. These results were also obtained by Pettersen et al. (2010b) who studied those parameters in different canopy levels of lighting cucumber. They suggested that supplemental light may cause some stress level that may lead a reduction in the Calvin cycle efficiency in terms of the utilization of ATP and NADPH.

Environments with low irradiance besides directly affects the A , also limits the $V_{c_{max}}$, the J_{max} and the R_d (Pettersen et al., 2010a; Trouwborst et al., 2016). These is frequent in the middle or the bottom part of canopies with the leaves characteristics and growth habits of the cucumber, especially at high plant density levels used in greenhouse production systems. In the present study, this fact was proved statistically for the J_{max} and R_d parameters but not for the $V_{c_{max}}$ variable in plants without LED interlighting. Trowburst et al. (2011b) also reports J_{max} lower in cucumber plants without LED interlighting. It means that the electron transport is less active in not LED light stimulated leaves corresponding to a lower photochemical reactions and CO₂ assimilation rate than plants treated

with supplemental LED lighting. In the opposite way, an increase of A accompanied an enhance in the maximum Rubisco carboxylase activity ($V_{c_{max}}$), rate of electron transport (J) and quantum efficiency of photosystem II as showed by Zhou et al. (2004) in chilled cucumber leaves.

A lower stomatal conductance, characteristic of low photosynthetic rates, may also partly explain lower J_{max} (Pettersen et al., 2010a). The same was expected for the maximum rate of carboxylation ($V_{c_{max}}$) and triose-phosphate utilization (TPU), because light quality influences the expression of photosynthesis-related genes as verified by Su et al. (2014) in cucumber seedlings. However, in our study, TPU was not affected by LED interlighting. Also specific photosynthetic pigments (Wang et al., 2009) and the quantities of PSI and PSII structural proteins (Viazau et al., 2015) in cucumber leaves are affected by light quality spectrum, playing an important role regulating the CO_2 assimilation rate and their related parameters. However Agüera et al. (2006) didn't found significant changes in the stomatal conductance and transpiration rates in cucumber plants affected by increasing in CO_2 level, meaning that the $V_{c_{max}}$, J_{max} and TPU values not always correlate with higher photosynthesis rate.

LED lighting affects both plant morphology and metabolic reactions and as a result is verified an increase of the total yield (Pettersen et al., 2010b; Cocetta, 2017). In this study, the higher net assimilation CO_2 rate was correlated with higher commercial yield in plants submitted to LED interlighting than control, independently of the year season. Between 0.7 and 1% of increase in cucumber production is expected in response to each additional 1% of light supply (Marcelis et al., 2006). A higher light absorption in leaves and a more homogeneous light distribution contributes to an increase in cucumber yield when LED interlighting is used (Trowburst et al., 2011b). A better production and distribution of photoassimilates in the lower plant canopies treated by LED interlighting results in higher standard quality. Hovi-Pekkanen and Tahvonen (2008) also reported increase on first class production and reduced unmarketable cucumber yield in plants with interlighting treatments.

Many researchers recommend the supplemental lighting for plants grown, in the winter season of temperate climates (Ciolkosz, 2008; Dorais, 2003; Hao and Papadopoulos, 2005). But, in subtropical conditions we could not obtain increase on the total yield and the number of fruits per plant on this season. Most of the greenhouses in high latitude countries, where those researches are conducted, have heating systems that help on the temperature regulation maintaining more favorable climatic conditions for the cucumber plant production. This greatly differs from the greenhouses structure reality on tropical and subtropical regions, as the modules used in this study. In the second half of the experimental period was registered colder temperatures in WP (Fig. 1), coinciding with an important part of the plant productive phase, that affected the number of fruits produced per plant and the total yield. In average was presented a reduction of 48% in the total yield of plants growing in WP as compared to SP. Cucumber plants are not indicated to growth in temperatures below 15° C. Chilling in tropical and subtropical plants as cucumber, under low light conditions characteristic of WP, disrupts the major photosynthetic components altering essential process as electron transport, carbon reduction cycle, stomatal conductance, enzyme destruction and water balance (Zhou et al., 2004). This may cause an irreversible inhibition of CO_2 assimilation and compromise the production.

3.5. Conclusions

The results showed that LED interlighting affect the parameters and mechanisms involved in the plant photosynthetic response, increasing the net CO_2 canopy assimilation rate. Mini-cucumber leaves, with the exposition to LED radiation, present higher photosynthetic rates. This contributes with the increase observed in the commercial

yield of cucumber in any season of the year when compared with plants without lighting. However, colder temperatures in the winter season, affected the production and a yield reduction was observed.

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4. SUPPLEMENTAL LED INTERLIGHTING ENHANCE MINI-CUCUMBER PRODUCTION IN SUBTROPICAL REGION

Abstract

Optimal light environments were created with artificial light, especially through the LED technology lamp utilization. Commercial LED lamps devices for horticultural greenhouses are used to supply light quantity and quality, particularly at the winter season in high northern latitude countries. Increasing the light at the canopy level, the photosynthetic process is stimulated and more yield is expected. This work aimed to compare the influence of the LED interlighting on the yield at summer and winter seasons, and analyze the economic cost of its implementation for the mini-cucumber crop in Brazil. At Piracicaba, Sao Paulo, mini-cucumber plants hybrid Larino grew in a greenhouse, which was given supplemental LED interlighting. Those devices have an intensity of $220 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and emit 80% of red (662 nm) and 20% of blue light (452 nm). Three seasons were installed, one each year: Winter 2015, Winter 2016 and Summer 2017. Growth periods were 120 days for winter season and 90 days for summer season. At winter seasons, the LED bars were on for 12 hours; at summer season for 10 hours. LED interlighting resulted in an increase of commercial yield between 13% and 30% for the three cycles. Fruits in the refused category decreased significantly in plants treated with LEDs over the three seasons. The energy use efficiency and the light use efficiency increased approximately 64% in the Summer 2017 as compared to winter seasons. Benefit cost ratio was positive (1.15) only for the Summer 2017 due to the higher productivity obtained with LED interlighting and the less energy consumption.

Keywords: *Cucumis sativus*; Artificial light; Yield; Economic costs

4.1. Introduction

Light is a critical factor that defines plant growth and yield due to many metabolic light dependent reactions. In the photosynthetic process, light energy is converted in chemical energy producing photoassimilates that contribute to the production performance (Radin et al., 2003). LEDs (Light-emitting diodes) have been introduced in the last years as an alternative to supply extra quality light in greenhouse commercial horticulture industry. The growers have possibilities to use LEDs with specific wavelengths, supplying the plant requirements needed to promote photosynthesis. Thus the biomass production is increased and better response in yield is expected (Darko et al., 2014).

Usually protected environments have higher plant density and light competition is common especially on high wire crops with self-shading leaves. Studies with vegetable crops proved significant effects of LED on plant growth and yield (Hidaka et al., 2014; Hogewoning et al., 2010; Hovi-Pekkanen and Tahvonen, 2008; Lin et al., 2013; Lu et al., 2012; Pettersen et al., 2010).

Typical commercial lighting equipment includes red (600 to 700 nm spectrum visible wavelength) and blue LEDs (400 to 500 nm) because these wavebands are the most efficient for photosynthetic process (Mitchell, 2015). Specific photoreceptors act for each region of the spectrum, phytochromes for red and cryptochromes for the blue section (Kopsell et al., 2015).

LED technology allows programming and modifying the light quantity and quality emitted according to the crop requirement. In addition, the use of LED light has other advantages. Compared with other lamp types for greenhouse industry, LEDs are more efficient in the energy conversion process than high-pressure sodium lamps (HPS). This means that less electrical energy is required to convert photon energy (Mitchell et al., 2012). This is particularly interesting since energy may represent the main cost for many greenhouse industries. With LEDs, economy in energy consumption may be up to 90% comparing with HPS considering the same lamp effect in the plants, with a lengthy lifetime estimated in the order of 20.000 to 30.000 hours (Singh et al., 2015). LED lamps also are low emitting temperature, which allows near contact with the canopy plant without causing injuries to the foliage (Cocetta et al., 2017). Therefore, concerning LEDs commercial use in horticulture, even with a very promising future, it may not be forgotten their economic viability (Massa et al., 2008).

In the horticultural greenhouse industry, new plant genotypes appear to accompany the development of the sector. The mini-cucumber of snack type was introduced in many countries, with high market value that may contributes with the investments return made on technologies. According with Shaw et al. (2000), mini-cucumber is part of cucumber group, developed in Israel for greenhouse production and introduced in USA at early of 2000. Mini cucumbers are gynoecious and parthenocarpic hybrids, with a prolific fruit production. The fruit has thin skin and is seedless. This type of cucumber represents to the growers an alternative for innovation, attending the demands and trend for the vegetable production sector.

Some factors and consuming patterns are stimulating and increasing the consumption of fresh products. A better diet is necessary to attend a healthy lifestyle expectative or a more conscious buyer. This part of the population increases notoriously and associate benefits of fruits and vegetables consumption to avoid chronic diseases (e.g. cancer, diabetes, cardiovascular diseases) and as a source of vitamins, fibers and other important natural compounds. Also, changes in the socio-cultural composition of the society, promote the use of convenience food, ready-to-cook and ready-to-eat (ideal for office's workers or students as healthy snack lunch) for a time-pressed consumer and families with less members (Schreiner et al., 2013).

The objective of this work was to study the influence of the LED interlighting on the yield at summer and winter seasons, and analyze the economic cost of its implementation for the mini-cucumber crop.

4.2. Materials and methods

4.2.1. Experimental site

Three experiments were carried out in greenhouse conditions in the University of São Paulo (USP), Piracicaba, São Paulo, Brazil (22°42' South Lat.; 546 m altitude; altitude tropical climate, type Cwa according with Köppen). The first experiment (120 days) was conducted from April to August 2015 corresponding to a winter season (Winter 2015); the second one (120 days), also winter season, from April to August 2016 (Winter 2016) and the last one (90 days) from October 2016 to January 2017 in the summer season (Summer 2017). The greenhouse structure had the following dimensions: 12.8 m width, 27 m length and 3.4 m height.

4.2.2. Climate conditions

The module was equipped with a climate control evaporative cooling system (pad and fan) automatically activated when temperature raised 25 °C. The greenhouse structure did not have heating systems for the winter season. A meteorological station (WatchDog 2400 Mini Station External Sensor, Spectrum® Technologies, Inc.; Aurora, Illinois, USA) was placed at 1.5 m of height inside the greenhouse in order to register continuously temperature, humidity and PAR radiation. Table 1 summarizes the climatic conditions that prevailed in the three seasons.

Table 1. Air temperature, relative humidity and photosynthetically active radiation (PAR) registered inside the greenhouse on three mini-cucumber seasons.

Variable	Winter	Winter	Summer
	2015	2016	2017
Absolute maximum temperature (°C)	27.4	29.7	31.1
Average maximum temperature (°C)	25.3	25.9	26.9
Absolute minimum temperature (°C)	10.7	3.1	18.5
Average minimum temperature (°C)	16.3	14.3	20.4
Absolute maximum relative humidity (%)	99.5	100	95.7
Average maximum relative humidity (%)	96.0	96.2	89.4
Absolute minimum relative humidity (%)	52.0	33.0	34.1
Average minimum relative humidity (%)	68.4	60.2	57.9
Average radiation (mol m ⁻² day ⁻¹)	9.80	9.34	13.89
Accumulate radiation (mol m ⁻² cycle ⁻¹)	1196.10	1139.10	1264.05

4.2.3. Lighting system

Interlighting red:blue (R:B) LED lamp bars (Philips GreenPower LED, Philips Lighting Holding B.V., The Netherlands) were used to perform the light supplementation to the crop. Bars were placed in a horizontal position at 1.5 m height. R:B lamps (80:20) produced at light intensity of 220 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. The peaks wavelength, measured with a portable radiation spectrum (LI-1800, LI-COR; Lincoln, Nebraska, USA) at 15 cm distance of the lamp, were 662 nm in the red light and 452 nm in the blue light spectrum.

Supplemental lighting started 28 days after transplanting (DAT) to the end of the growth periods. The LED arrays were turned on for a total photoperiod of 12 hours (from 9:00 AM to 9:00 PM) at Winter 2015 and Winter 2016, and 10 hours of photoperiod (from 9:00 AM to 7:00 PM) for the Summer 2017.

4.2.4. Plant material and management

The selected genotype for the experiments was the F1 hybrid Larino (Rijk Zwaan®). This is a mini-cucumber of snack type, typically for greenhouse production. It requires a vertical conduction system (high wire

crop) and stands out for its performance (can produce three to five fruits per bud in a period of two or four months). The fruits are commercialized between 9 and 11 cm length.

High quality mini-cucumber seedlings were planted into 8 L capacity plastic containers. Coconut fiber substrate was used as soil less growth medium. The cucumber plants were placed in a double lines system, with a spacing of 0.4 m between plants, 0.8 m between lines and 2.0 m between the double lines. It resulted in a total density of 2.5 plants m⁻².

A drip irrigation system was provided and moisture-sensors (Irrigation controller-MRI, Hidrosense, Jundiai, Brazil) were placed into substrate at 15 cm depth to monitor the moisture. The plants were irrigated when the water tension of the substrate reached 3 kPa. The nutrient solution volume was calculated to supply water to reach the water tension at field capacity (1 kPa). The daily fertigation program was (mg L⁻¹): 120 N, 42 P, 120 K, 108 Ca, 32 Mg and 60 S for vegetative phase; and 150 N, 105 P, 200 K, 133 Ca, 50 Mg and 84 S for reproductive phase. Micronutrients were also applied from a fertilizer containing 7.26% Fe, 1.82% Cu, 0.73% Zn, 1.82% Mn, 0.36% Mo and 0.36% Ni. The average E.C (dS m⁻¹) and pH nutrient solution were 2.2 and 6.5, respectively.

Plant conduction was made with vertically plastic strips supported by 3 m height horizontal wire. The plants were maintained only with the main stem over the cycle. The harvests were realized three to four times per week for three months in the winter seasons (Winter 2015 and Winter 2016) and two months in the summer season (Summer 2017).

4.2.5. Yield classification

At each harvest, mini-cucumber fruits from each plot were counted, weighted and classified into the categories commercial, non-commercial, refused and total. Fruits with sizes between 9 and 11 cm of length and without any curvature or injury were considered commercial or marketable. Non-commercial yield was composed by fruits outside the minimum standard (9 cm), and refused yield was formed by fruits with excessive curvature and other shape defects. Finally, total yield was calculated by sum of both categories.

4.2.6. Economic considerations

The electric energy and light use efficiency and the cost performance (return/cost) of the LED lamps were calculated. The following formulas for these variables were considered according to Tewolde et al. (2016):

Electric energy use efficiency (kg kWh⁻¹) = increase in yield with LED (kg m⁻²) / electric consumption (kWh⁻¹ m⁻²).

Light use efficiency (g MJ⁻¹) = Electric use efficiency (kg kWh⁻¹) / 0.4 (conversion coefficient for LEDs lamps).

Cost performance = [fruit price (USD kg⁻¹) x increase in yield with LED (kg m⁻²)] / [electricity used per crop season (kWh⁻¹ m⁻²) x price of electricity (USD kWh⁻¹) + LED linear depreciation per crop season (USD m⁻²)]. LED linear depreciation was calculated by the following formula:

initial investment cost of LED module (USD m⁻²) / lifetime of LED module in number of crops (number of crops = lifetime of LED module in hours / LED lighting hours per crop).

For these calculations were considered the next assumptions for the LED device and electricity cost in Piracicaba, Sao Paulo, Brazil:

Lamp initial cost: 484 USD

Lifetime: 25 000 hours

Lamp electric consumption: 0.0158 (kWh⁻¹m⁻²)

Electricity rate: 0,089 USD/ kWh⁻¹ (winter); 0,085 USD/ kWh⁻¹ (summer)

Exchange rate of R\$3.1 for one American dollar (USD)

A cost analysis was also considered calculating the gain with LED interlighting utilization over the three seasons. Growth and management conditions for the plants in the area were the same for the three cycles, excepting the supplemental light for treatments with or without LED light. The investment and cost of the greenhouse and infrastructure associated were not considered. Other assumptions for these calculations follow:

Number of plants: 36 (in double-row system)

Required LED lamps: 3

Season duration: 120 days (winter); 90 days (summer).

Grower cost production per plant (considering supplies, plant requirements and management, and direct labor): 8.00 USD (winter); 6.60 USD (summer)

Grower harvest income: 3.9 USD kg⁻¹

4.2.7. Experimental design and statistical analysis

The greenhouse had two environments: LED interlighting and non-LED interlighting (as control). Each plot had 12 plants, both in winter and in summer seasons. The yield data parameters were analyzed using the SAS ANOVA procedure (SAS/STAT release 9.3 ® program, SAS Institute Inc., Cary, NC, USA). Variance analyses were made and treatments means were compared by Tukey test at $P < 0.05$.

4.3. Results

The number of commercial fruits (CFN) and commercial yield (CY) increased between 10% and 30% with LED interlighting for all the cycles. However, the higher increases of CFN and CY were reached in the summer season (2017) as compared to winter seasons (2015 and 2016). For the total yield (TY), plants treated with LED light produced 14% more than control in the summer season. For the winter season, LED did not improve the TY (Table 2).

The number of non-commercial yield (NCFN), number of refused yield (RFN), non-commercial yield (NCY) and refused yield (RY) are shown on the table 3. Plants treated with LED interlighting showed lower RFN and RY as compared to control in all years. The NCFN was 17% reduced with LED light use only in winter season of 2016. However, this decrease of NCFN was not enough to reduce the NCY.

In all mini-cucumber cycles, at least 71 to 72 % of the fruits were commercial for control plants. When plants were treated with LED interlighting, there was an increase of fruits commercial category that represented 77% to 82% of total yield (Figure 1). This is important for the grower because this category offers the best economic return.

Table 2. Number of commercial fruits (CFN), number of total fruits (TFN), commercial yield (CY) and total yield (TY) of mini-cucumber in plants cultivated with LED interlighting (LED) and without supplemental light (control) for three cycles (2015, 2016 and 2017).

	CFN (fruits plant ⁻¹)	TFN (fruits plant ⁻¹)	CY (kg plant ⁻¹)	TY (kg plant ⁻¹)
Winter 2015				
Control	73.3 b	116.5 a	3.71 b	5.20 a
LED	80.9 a	117.8 a	4.21 a	5.48 a
CV%	5.48	4.43	7.02	5.42
Winter 2016				
Control	42.3 b	65.4 a	1.87 b	2.60 a
LED	49.6 a	67.4 a	2.23 a	2.81 a
CV%	9.58	9.21	9.80	9.39
Summer 2017				
Control	57.0 b	87.0 b	3.50 b	4.84 b
LED	72.7 a	97.0 a	4.54 a	5.50 a
CV%	3.60	2.26	1.58	2.37

^z Means followed by the same letter in the column did not differ from each other by Tukey range test at

P≤0.05.

Table 3. Number of non-commercial fruits (NCFN), number of refused fruits (RFN), non-commercial yield (NCY) and refused yield (RY) of mini-cucumber in plants cultivated with LED interlighting (LED) and without supplemental light (control) for three cycles (2015, 2016 and 2017).

	NCFN (fruits plant ⁻¹)	RFN (fruits plant ⁻¹)	NCY (kg plant ⁻¹)	RY (kg plant ⁻¹)
Winter 2015				
Control	10.17 a	33.08 a	0.29 a	1.20 a
LED	10.25 a	26.58 b	0.30 a	0.95 b
CV%	16.79	5.00	17.68	4.16
Winter 2016				
Control	10.75 a	12.33 a	0.32 a	0.42 a
LED	8.92 b	8.92 b	0.27 a	0.30 b
CV%	15.21	18.53	14.48	20.99
Summer 2017				
Control	7.25 a	22.75 a	0.22 a	1.12 a
LED	7.50 a	16.75 b	0.24 a	0.71 b
CV%	11.41	4.26	31.59	12.86

^z Means followed by the same letter in the column did not differ from each other by Tukey range test at

P≤0.05.

Figure 1. Percentage distribution by categories of mini-cucumber yield produced in plants cultivated with LED interlighting and without supplemental light (control) throughout three cycles.

Economic considerations about the use of LED technology are showed on the tables 4 and 5. Higher energy consumption was reached on the winter season, accompanying a more extended plant cycle and more hours per day light requirements. Increased yield of mini-cucumber was reached with LED interlighting, but this increase was higher in the summer season. As a result, the efficiency of energy and light use were 64% higher for the summer period than winter seasons. The cost performance of LED for summer season was 77% higher as compared to the

winter season. At winter season, the value was lower than 1.0, which means that LEDs do not pay the cost production.

Table 4. Electric energy use and light use efficiency of LED interlighting in both winter and summer season, for a mini-cucumber greenhouse production.

	Energy consumption per crop ^z (kWh ⁻¹ m ⁻²)	Total yield increased (kg m ⁻²)	Energy use efficiency (kg kWh ⁻¹)	Light use efficiency (g MJ ⁻¹)
Control	-	-	-	-
Winter LED ^y	17.10	0.6 ^x	37.97	94.92
Summer LED	9.48	1.7	107.59	268.98

^z Accumulated electric energy used by m² per crop cycle.

^y Energy consumption and efficiency values were the same for Winter 2015 and Winter 2016.

^x Average of two winter seasons.

Table 5. LED lighting use per day and crop cycle, LEDs lifetime per crops and cost performance of LED interlighting in both winter and summer season, for a mini-cucumber greenhouse production.

	Use per day (hours)	Use per crop cycle (hours)	Number of crops	Cost performance
Winter LED ^z	12	1080	23	0,41
Summer LED	10	750	33	1,79

^z values for winter LED were the same for Winter 2015 and Winter 2016.

A cost analysis made from the yield data of this study (Table 6), confirms that production of mini-cucumber with LEDs in the summer season is profitable. This was reflected on the favorable benefit cost ratio. Operating cost is lower at summer, while total net income was higher at this same stand, when compared with both winter stands. Total net income was negative only for Winter 2016, affected by low productivity.

Table 6. Cost analysis for a mini-cucumber greenhouse production illuminated with LED interlighting in both winter and summer season (values on USD kg plant⁻¹).

	Winter 2015	Winter 2016	Summer 2017
Operating cost with LED	2.05	3.87	1.54
Operating net income	1.85	0.03	2.36
Total cost with LED	2.80	5.28	2.23
Total net income	1.10	- 1.38	1.67
Benefit cost ratio (BCR)	0.51	0.39	1.15

The break-even point analysis is showed in the Figure 2. LED interlighting utilization forces to a higher yield performance with LEDs in all the stands. Thus, concerning the feasibility of the production, it is required 32% higher yield at winter and 35% at summer, comparing with a production without LED technology. Yield was higher than break-even point for the Winter 2015 and Summer 2017 stands.

Figure 2. Break-even point analysis, with and without LED interlighting, for a mini-cucumber greenhouse production supplemented with LEDs through three stands.

4.4. Discussion

The use of supplemental lighting in greenhouses contributes to compensate lack of day light in winter (with less day hours) and on cloudy days (Ciolkosz, 2008). On those poor light conditions, luminous interception declines especially at the middle and lower parts of the canopy, when mutual leaf shading affects the use of light. Consequently improving the light distribution it is possible to get a higher leaf photosynthetic capacity, resulting in higher yield performance (Tewolde et al., 2016). Furthermore, energy required for lighting is used more efficiently by the crop (Hemming, 2011). Hovi-Pekkanen et al. (2004) reported the increase on cucumber total and commercial yield when using supplemental lighting. In our study, the commercial yield enhanced with LED interlighting in the three cycles of mini-cucumber. At subtropical climate conditions, supplemental light seems to potentiate yield when temperature is more favorable for cucumber production. Summer season at the Brazilian southeastern is hot and rainy, and cloudy days with limited natural light are common. In the winter, days are cold and dry; the clear sky prevails. Different from high latitude countries, days are not so short at winter of Piracicaba, SP, Brazil (11 light hours).

The refused fruit number and yield were consistently lower under LED interlighting than control in the three cycles. Hao et al (2012) reported that intracanopy lighting increased marketable yield of cucumber fruits. Hovi-Pekkanen and Tahvonen (2008) found that interlighting increased first class yield and decreases refused fruits, agreeing with our results. This increase on yield of bigger fruits than other categories may be explained by the lighting effect inside the canopy (Dorais, 2003). Marcelis (1993) also stated that greater irradiance is directly related

with higher cucumber production. Continuous exposure to higher irradiance favors a substantial increase in fruit load. The same author indicated that with high available light, the source strength is higher affecting directly the sink capacity of the plant and indirectly the biomass distribution. With an increase of light intensity, the photoassimilate translocation from leaves is promoted in cucumber plants (Robbins and Pharr, 1987). Light intensity positively affects indirectly the stomata opening and directly the activity of enzymes related to photoassimilate translocation, decisive process in carbohydrate transport (Hashimoto et al., 2013; Yonekura et al., 2013; cited by Wang et al., 2014). Thus, interlighting may contribute well the photoassimilate movement, including the supply from the leaves at the bottom layer (Wang et al., 2014), that stimulates a better fruit load favoring commercial yield.

In interlighting absence, a reduced carbohydrates translocation affected negatively the marketable yield while the number of refused fruits increased, as demonstrated over the three cycles of growth mini-cucumber. The less production of photoassimilates results in competition of carbohydrates and other important metabolic products between fruits and leaves, factor that may cause fruit abortion, curvatures and yield decrease (Marcelis, 1992). Marcelis et al. (2006) reported positive effect of light supplemental on fruit yield. Photoassimilates movement is also light quality dependent, but evidence indicates that this translocation may be specie-specific (Wang et al., 2014). As previously mentioned as LED advantage, these lamps may be programmed to work in specific wavebands length more suitable for the plant. In cucumber leaves, blue light stimulates the accumulation of photoassimilates, while red light promotes photoassimilate exportation (Wang et al., 2009).

The extension of the photoperiod is also critical and determines the movement of photoassimilates between source and sink organs. Under long days, the enzymes involved in the reduction phase of the Calvin-Benson cycle are more abundant (Victor et al., 2010), reason why plants in days with more light-hours show a higher photoassimilate translocation. This fact also contributes to explain higher yield and better fruit commercial distribution at the summer season, with an average of 13 light hours at Piracicaba.

Light is not the only factor that defines the growth and yield of the plants because the temperature also affects strongly the morphological, photosynthetic and physiological characteristics of plants (Marcelis et al., 2006). In this study, the daytime and nighttime temperatures affected differently the yield of mini-cucumber in the seasons.

Lower nighttime temperatures than critical temperatures were registered at the winter season of 2016, with average minimal temperatures between 5 and 10° C for several continuously days, especially at the second half of the plant growth period. Guo et al. (2008) also reported the seasonal temperatures influence on cucumber affecting negatively the yield at autumn-winter season. Under low temperatures (12° C), occurs a low photoassimilate translocation rate, demonstrated by the increase content of sucrose, stachyose and galactinol in mature cucumber leaves, while did not change the content of sucrose, glucose and fructose in fruits (Miao et al., 2007). In the winter season of 2015, average minimum temperatures were higher than temperatures of winter season of 2016, which could explain the higher yield of 2015 than 2016. The similar commercial yields between 2015 and 2017 may be explained by absence of continual minimum temperatures below the critical minimum temperature in the winter season of 2015. In addition, the commercial yield in 2017 was not higher than yield of 2015 because the duration of the plant cycle was 30 days shorter than winter season.

Energy cost must be considered when lighting devices use is included. Lighting and heating the environment are the main cost factors operating greenhouse production systems in countries with limited photoperiod and severe winters (Singh et al., 2015). Several studies showed cost-effective use of LEDs and interlighting lamps in temperate climates for crops as tomato (Tewolde et al., 2016) or cucumber (Hovi-Pekkanen et al., 2004). But, no reports were found for LED lamps use for countries under tropical or subtropical latitudes.

In this study, the increase of total yield due to LED interlighting was 64.7% lower in the winter seasons than the increase of total yield in the summer season. This result explains the lower light and energy efficiency use and the cost performance in the winter seasons than summer season. Winter 2015, even with yield over the breakpoint in the two areas (LED treated and control) did not have economic feasibility with LEDs due to the little yield increase comparing with plants that grew only under natural light conditions. In Winter 2016, scarce production, due to adverse cold conditions, was responsible for the negative economic scenario of that year. Therefore, the economic LED use was identified for the summer crop. Thus, the cost analysis made showed a positive value only for the Summer 2017, with a gain of 15 USD for each 100 USD invested. However, a more significantly increase on yield due to LED use is desirable, and could contribute to a better economic return and benefit cost ratio. This is important to stimulate the use of this technology in countries where artificial light is not a traditional practice.

An investment analysis was not considered for this specific study, but the economic studies indicate that due the LEDs high energy efficiency, low maintenance and longevity in long-term operations (several years) in greenhouse industries, the investment could be returned in a medium-term period (Singh et al., 2015).

The economic feasibility of supplemental lighting is strongly dependent on the yield increase, but other factors as product market price, the energy cost, the interest rate and the lamp cost are also important (Hao and Papadopoulos, 2005). In this study, the cost of import a high quality LED lamp in Brazil affected also our calculations. The dollar exchange rate and other variables are external factors which the grower cannot control. A global trend is that as this technology has been more popular and used in commercial horticultural systems over the last years, a natural reduction in the cost of LED lamp devices should be happen. The first LED arrays appeared in the late 80s and since then, each decade the LED price drops by a factor of 10, while the performance of the LEDs grows by a factor of 20. Due to an economies of scale process, more LED lighting applications installed replacing the traditional lamps will drive significant cost decreases (Morrow, 2008), which is actually happening in more developed countries. Manufacturers are improving lighting devices and the costs continue to decrease as new technologies and product reliability become available (Nelson and Bugbee, 2014).

4.5. Conclusion

LED interlighting to enhance light distribution of lower canopy leaves leaded to higher commercial yield in the winter and summer seasons. However, the total yield was favorable with LED supply only at summer. Low temperature at winter with non-equipped greenhouse for heating the environment is a condition that affected the total performance. Consequently the gain with LED use was not enough to compensate the investment made. Thus, energy use efficiency, light use efficiency and economic parameters as cost performance, profitability over total cost and benefit cost ratio were only favorable to the mini-cucumber growth in the summer season with more favorable climatic conditions. To decide the LEDs bars use, an investment analysis including the calculation of the net present value (NPV) is desirable.

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Appendix

APPENDIX A. Greenhouse equipped with LED interlighting

(A), (B) and (C) Greenhouse module used for the experiments with supplemental lighting and evaporative cooling system; (D), (E) and (F) LED interlighting bars details.

APPENDIX B. Greenhouse internal view of the LED interlighting bars

A) Nocturnal view with LEDs; (D) and (E) Diurnal view with LEDs; (B), (C) and (F) LED bars positioning between double row crops.

APPENDIX C. Fertigation system

A

B

C

D

E

F

(A) and (B) Preparation of nutrient solution; (C) Fertigation injection system; (D) Irrigation branch lines; (E) Polytube with pressure controlled emitters coupled to a four-way splitter; (F) Two microtubes and spikes per pot.

APPENDIX D. Installation of the experiment

(A) Medium texture coconut coir as substrate growth media; (B) 8 L plastic containers; (C) Mini-cucumber Larino seedling; (D) Grafted mini-cucumber Larino seedling; (E) Transplantation process; (F) Experiment view right after transplanting.

APPENDIX E. Plants growth phases

(A) Plants with 30 DAT (days after transplant); (B) 65 DAT; (C) 90 DAT; (D) and (E) Flowering; (F) Fructification.

APPENDIX F. Harvesting process

(A), (B) and (C) Harvest point; (D) Fruit classification by categories; (E) Commercial and non-commercial categories; (F) Refused category; (G) Fruit packing.

APPENDIX G. Photosynthesis and light spectrum measurements

(A) and (B) Photosynthetic determination with LI 6400 XT IRGA; (C) and (D) Light response curve determination with LCpro + IRGA; (E) and (F) Light spectrum measurements with portable spectroradiometer LI-1800.

Appendix H. Meteorological station and postharvest tests

A **B** **C** **D** **E** **F**

(A) and (B) Meteorological station position at the greenhouse for temperature, relative humidity and PAR radiation measurements; (C) Long shelf life test at cold chamber; (D) Sample preparation; (E) Total solid soluble test; (F) Titratable acidity test.