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Effect of LED Blue Light on *Penicillium digitatum* and *Penicillium italicum* Strains

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ABSTRACT

Studies on the antimicrobial properties of light have considerably increased due in part to the development of resistance to actual control methods. This study investigates the potential of Light Emitting Diodes (LED) blue light for controlling *Penicillium digitatum* and *Penicillium italicum*. These fungi are the most devastating postharvest pathogens of citrus fruit and cause important losses due to contaminations and to the development of resistant strains against fungicides. The effect of different periods and quantum fluxes and of delaying light application on the growth and morphology of *P. digitatum* strains resistant and sensitive to fungicides and of *P. italicum* cultured at 20 °C was examined. Results showed that blue light controls the growth of all strains and that its efficacy increases with the quantum flux. Spore germination was always avoided by exposing the cultures to high quantum flux (700 µmol m⁻² s⁻¹) for 18 h. Continuous light had an important impact on the fungus morphology and a fungicidal effect when applied at a lower quantum flux (120 µmol m⁻² s⁻¹) to a growing fungus. Sensitivity to light increased with mycelium age. Results show that blue light may be a tool for *P. digitatum* and *P. italicum* infection prevention during handling of citrus fruits.
INTRODUCTION

Citrus fruits are subjected to various postharvest diseases that cause important economic losses. These losses are mostly due to pathogen contaminations in different strategic points in the packing-houses, such as drenchers, dip tanks, and cold storage rooms. Therefore, reducing inoculum of pathogens and maintaining good water quality in postharvest facilities is critical to reduce the incidence of decay. *Penicillium digitatum* (Pers.:Fr.) Sacc. (green mold) and *P. italicum* (blue mold) are the major pathogens of citrus fruits after harvesting. Green mold is most commonly responsible for postharvest decay of citrus, contributing up to 90% of total losses (1). The use of fungicides constitutes the most common method to control postharvest diseases in citrus fruits. At present, *P. digitatum* and *P. italicum* are controlled by synthetic fungicides. However, due to the growing concern about human health and the environment, the use of chemicals is becoming increasingly restricted and there is a trend to develop alternative methods to control postharvest diseases (2-6). In the case of citrus fruits, the search of new strategies is also relevant because of the increase of fungicide resistant strains (1) and also because of low residue tolerance, disposal of used fungicide solutions in packinghouses, chemical costs and development of organic marketing programs (7). Induction of natural resistance in the fruit by using physical and chemical treatments (8-12) and biocontrol agents (3,13) is one of these alternatives. Nevertheless, the efficacy of these treatments is variable and is lower than that of synthetic fungicides. Therefore, appropriate infection prevention and measures for the reduction of pathogens contamination during postharvest handling and storage of citrus fruits is critical to control disease and reduce the use of fungicides.

The antimicrobial properties of light is a research area of increasing interest, not least due to the emergence of resistance to a number of other control methods including the use
of pesticides, disinfectants and antibiotics (14-18). Different reports have shown the potential of UV-treatments for reducing postharvest decay caused by different fungi (2,5,10,19,20). However, UV light has limitations because of its detrimental effects to workers, such as injury to the skin or eye on direct exposure. Lighting based on Light Emitting Diodes (LEDs) is one of the main emerging technologies in agriculture. In contrast with other illumination systems, LEDs possess features making them unique for industrial applications: higher irradiation with less heat dissipation, making easier control of humidity and temperature in closed environments such as storage rooms, easy installation and lower energy costs. Besides, a narrower bandwidth in the light spectrum achieved by this technology allows better control of the amount of light beam actually exciting photoreceptors in cells (21). Studies on the potential of LED blue light on different processes of agronomic interest has considerably increased during the last decade (21). Moreover, it has been shown that LED blue light may control food relevant fungi (22) and other pathogens that are harmful for consumers, such as Listeria monocytogenes (23), that has caused important problems derived from fresh fruit contaminations during current postharvest practices (24). Such contaminations can occur in different strategic points in the packing-houses, such as drenchers, dip tanks or hydrocooling systems. Therefore, this technology might constitute an appropriate strategy to reduce not only postharvest decay caused by fungi but also the development of pathogens that can be harmful for consumers during postharvest handling of horticultural crops. Nevertheless, the potential of LED blue light to avoid losses of perishable crops during postharvest handling is almost unknown. In fact, to our knowledge, only two postharvest studies have been performed until now that suggest the industrial relevance of LED blue light reducing postharvest citrus fruit losses. On one hand, it has been shown that exposure of citrus fruit to this light emerging
technology, at a quantum flux of 40 µmolm$^{-2}$s$^{-1}$ reduces infection by *P. digitatum* (25,26) although this quantum flux did not induce any change in mycelium growth and sporulation of the fungus *in vitro*. This finding indicated that blue light is able to induce resistance against *P. digitatum* in citrus fruit. On the other hand, Liao et al. (2013) showed that a quantum flux treatment of 120 µmolm$^{-2}$s$^{-1}$ reduced *in vitro* fungal development. This result suggests, therefore, the potential of blue light for controlling mycelial growth and/or inhibiting conidia production of *P. digitatum* and that the efficacy of this physical treatment may increase with the quantum flux. Considering these results, that sanitation of packing- and store-houses is essential to reduce postharvest decay (11) and the high abundance of *P. digitatum* strains resistant to the two fungicides (thiabendazole (TBZ) and imazalil) most commonly used to control decay in citrus fruit (1), the aim of this study has been to investigate the potential of LED blue light for controlling sensitive and resistant strains of *P. digitatum* and by *P. italicum* during postharvest handling of citrus fruits. To that end, we have evaluated the effect of increasing blue light quantum flux and also time of exposure to such radiations on *in vitro* *P. digitatum* and *P. italicum* development and morphology. Conidial suspensions of three strains from *P. digitatum*, two of them resistant to fungicides, and one from *P. italicum* cultured on potato-dextrose agar (PDA) were used. Furthermore, as the effectiveness of chemical and physical treatments on controlling postharvest disease may vary if the treatment is applied after spore germination (27), the efficacy of the most effective blue light treatments was evaluated at different periods after inoculating the PDA plates with the conidial suspensions.

**MATERIALS AND METHODS**
Collection of *P. digitatum* and *P. italicum* isolates: *P. digitatum* and *P. italicum* strains used in this study were provided by Dr. González-Candelas and isolated from citrus fruit with typical green or blue mold collected from different orchards or packing-houses. Most strains are deposited in the Spanish Type Culture Collection (CECT). Petri plates containing 20 ml sterile PDA (39 g l\(^{-1}\)) were inoculated with *P. digitatum* (Pers.:Fr.) Sacc. isolates PHI-26 (CECT 20796), which is sensitive to TBZ and imazalil, and Pd1(CECT 20795) and PHI-41, resistant to both fungicides, and with *P. italicum* isolate PHI-I (CECT 20909) and incubated at 20 ºC for at least 7 days (8). Conidia from each isolate were rubbed from the agar surface by scrapping them with a sterile spatula and transferred to 10 ml of sterile water. The resulting conidial suspensions were filtered and the concentration titrated with a hemacytometer and adjusted to the desired final concentration (8).

**Infection and evaluation of fungal growth:** Petri plates of 9 cm diameter were inoculated in triplicate for each light treatment and pathogen strain with 5µl of \(10^6\) conidia ml\(^{-1}\). To determine the effectiveness of the LED blue light treatments reducing fungal growth, petri plates were treated with different light regimes: 1) immediately after being inoculated (0 days post-inoculation, 0dpi), 2) at 1 and/or 2dpi, after germination of spores begun, and 3) at 4 dpi, when the mycelium of each strain was abundant. Temperature was maintained constant at 20 ºC, both during the light treatments and during fungal growth in the plates under darkness, and monitored with an electronic datalogger. To evaluate how the light treatments may affect fungal growth, colonies diameters were measured from the reverse side in cm with a ruler in two directions along the time at 20 ºC for at least 7 days. For each experimental condition, three colonies were evaluated. Control samples consisted of inoculated plates maintained always under darkness at the same temperature.
The percentage of growth inhibition was also calculated using the following formula:

\[
\text{Percentage of growth inhibition} = 100 \times \frac{(\text{GC}-\text{GSL})}{\text{GC}},
\]

where GC is growth of the control (continuous darkness) and GSL is the growth of the fungal colony of the sample exposed to the light treatment (28).

**Degree of germination:** The degree of germination was determined in three replicate samples using a Neubauer chamber. The number of germinated (already showing a germ tube) and ungerminated spores were counted and the degree of germination was expressed as the percentage of germinated spores. A total of 100 spores were counted in each replicate sample.

**Blue light treatments:** To test whether the effectiveness of blue light in reducing the pathogens’ growth infective capacities may depend on both light quantum flux and time of exposure to light, inoculated plates were exposed to light quantum fluxes ranging from 30 to 700 µmolm$^{-2}$s$^{-1}$ during periods ranging between 5 min and 3 days. The first experiment was performed with the Pd1 strain, since *P. digitatum* is the major pathogen of citrus fruits and this strain is highly resistant to the fungicides TBZ and imazalil. The matrix blue light quantum flux/exposure time to light first ranged between 30 and 120 µmolm$^{-2}$s$^{-1}$ and between 5 min and 6 h. On the basis of the results obtained, we further tested the effect of applying 120 µmolm$^{-2}$s$^{-1}$ for 6, 24 and 72 h using the Pd1 and the PHI-41 strains, both resistant to imazalil and TBZ. Moreover, we investigated the effect of the most effective treatment on the infective capacity of the strain Pd1 when the light was applied at different dpi (0, 1, 2 and 4), and whether the effectiveness of the treatments was maintained after removing the light stimulus. Finally, we studied whether light treatment duration may be
reduced by sharply increasing quantum flux to 700 µmolm$^{-2}$s$^{-1}$. Under such light regime, the effectiveness of time periods equal or lower than 24 h was tested and the effect of the selected treatment on fungal growth and morphology of the 4 strains was studied at 0 and 4 dpi.

Petri dishes were treated with the lid covers to avoid contaminations under the different light regimes described above in Mammoth Pro dark growth tents (60 x 60 x 160 cm) (Mammoth Pro 60, Eltac Hidrofarm, Spain) equipped with velcro-sealable ventilation panels (300mm x 200mm) and tough fabric lined with 95% reflective mylar to assure uniform light quantum flux. The light source was a LumiGrow Pro 650$^{\text{TM}}$ LED array (LumiGrow, Novato, CA, USA) that emitted blue light at a center wavelength of 450 nm with a full width at half-maximum of 20 nm. Light quantum flux was measured and adjusted using a spectroradiometer (GL Spectics, Stuttgart, Germany). The growth tents had sufficient capacity for air exchange and were placed in a temperature and humidity controlled room.

Statistical analysis: A mean comparison using the Tukey’s test was performed to determine if means values were significantly different ($P \leq 0.05$) between blue light-treated and control samples (constant darkness) for each experimental light regime. The results were the means of three replicated samples ± S.E.M.

RESULTS AND DISCUSSION

Effect of LED blue light on growth of $P.\ digitatum$ strains resistant to fungicides
Recently, it has been reported that LED blue light at a quantum flux of 40 µmolm$^{-2}$s$^{-1}$ induces resistance against *P. digitatum* in citrus fruit (25) and also that blue light reduced fungal development when *P. digitatum* culture was exposed to a higher quantum flux (120 µmolm$^{-2}$s$^{-1}$) (26). Therefore, we hypothesized that the effect of blue light inhibiting fungal growth may increase with light quantum flux and that the duration of light treatment may be shortened by increasing the quantum flux. Moreover, an interesting possibility would be that blue light would even have a fungicidal effect that could be used in the packing-houses as a mean of sanitation. This would be especially relevant since decay in citrus fruit is mainly controlled by two fungicides and strains resistant to them are abundant. To test such hypotheses, we first examined whether the growth of the *P. digitatum* Pd1 strain, resistant to TBZ and imazalil, was affected by exposing the cultured plates from 5 min to 6 h to light quantum fluxes ranging between 30 and 120 µmolm$^{-2}$s$^{-1}$. Pd1 fungal growth was only slightly reduced when the plates were treated with a quantum flux of 120 µmolm$^{-2}$s$^{-1}$ for 6 h (see Supplementary Materials Fig. S1), and such reduction was not statistically significant. Therefore, in a subsequent experiment, this light treatment was extended for 72 h.

The effectiveness of the 120 µmolm$^{-2}$s$^{-1}$ treatment applied for 6, 24 and 72 h was tested for Pd1 and also for PHI-41, which is another *P. digitatum* strain resistant to both fungicides. Results confirmed that exposing the plates for 6 h to this quantum flux barely reduced fungal growth of both pathogens and showed that the efficacy of the light treatment may be increased by extending the time of exposure to blue light (Fig. 1). By day 7, the efficacy of the 72 h treatment inhibiting fungal growth of the PHI-41 strain was c.a. 3-fold higher than that of the 24 h treatment (Fig. 1). Results also showed that differences afforded by the 72 h treatment inhibiting fungal growth of the Pd1 and the PHI-41 strains were initially low, and such differences were lost as time progressed (Fig. 1). Therefore, results
indicate that susceptibility to blue light of strains from the same fungus genus may be similar, while the effect of blue light varied when applying to different food relevant fungi (22) or to various *Penicillium* fungal species that cause postharvest decay in citrus fruit (26). Since the 72 h light treatment was only partially effective controlling fungal growth, the question was whether increasing light exposure or quantum flux would be able to control *Penicillium* growth and if such control might be even achieved if the light is applied at different dpi.

**Effect of delaying LED blue light application on growth and morphology of *P. digitatum***

Considering the points highlighted above, we first focused on studying the effect of exposing continuously, or for 3 days, the *P. digitatum* culture to the 120 µmolm$^{-2}$s$^{-1}$ blue light treatment at different dpi. The experiment was performed using the Pd1 strain. At 0 dpi, Pd1 fungal growth inhibition was about 45 % after 3 days, when the plates were continuously incubated under light (Fig. 2, 0 dpi). The diameter of the Petri plates used was of 9 cm. Therefore, this was the maximum fungal growth detected in the control plates continuously maintained under darkness. This maximum was reached by day 11 in control plates under dark conditions, but the experiment was prolonged to know whether this light quantum flux may have a fungistatic effect although it was not possible to monitor growth in control plates after that day. As shown in this graph (Fig.2, 0 dpi), by day 9, Pd1 fungal growth was about 2-fold lower in the cultures held continuously under light than in those transferred from light to darkness (non-continuous light). The above result reinforces the idea that the efficacy of the treatment increases with its duration for the same light quantum flux, and further suggests that *P. digitatum* fungal growth might be stalled by increasing
exposure to blue light radiation for a given dose. Therefore, an interesting possibility is that blue light might exert a fungistatic effect on *P. digitatum* if appropriate light quantum flux is applied. This result is in concordance with data indicating that increasing radiation intensity may in some cases maximize the benefits of UV-C on reducing decay caused by *Botrytis* (20). However, mechanisms by which blue light may have a fungistatic effect on *Penicillium* or other postharvest pathogens should be at least in part different. In this regard it has to be considered that differences in sensitivity and responses of different fungi or plants species to light of different wavelengths might be related to the different sensitivity of light receptors (22, 29-31). The relevance of cryptochromes in the responses of plants and fungi to blue light has been described (31, 33). However, to date it has not been shown that cryptochromes can perceive UV-B or UV-C irradiations (34).

Delay in application of postharvest fungicides as well as of other alternative treatments controlling decay caused by pathogenic fungi may alter their efficacy reducing both decay incidence and *in vitro* growth (9,35,36). Therefore, we also investigated whether delaying LED blue light application may influence the growth of the fungus. Results revealed the potential of blue light for reducing *P. digitatum* fungal growth if the cultured plates are light-treated at different time points after pre-inoculation with the fungus since blue light (120 µmol·m⁻²·s⁻¹) was able to restrict growth of Pd1 *P. digitatum* strain regardless of time of light treatment delay (Fig 2). Fungal growth was almost stopped always after 8 days of continuous light application and was c.a. the 50 % that of the colonies held under darkness. These results indicate that the 120 µmol·m⁻²·s⁻¹ light quantum flux did not avoid spore germination although it may stall fungal growth. Moreover, it is interesting to note that the time required to achieve such effect when applying continuously this light quantum flux barely depended on the developmental stage of the fungus.
Results further showed that if the colonies treated at 0, 1, 2 or 4 dpi for 3 days with a 120 μmolm$^{-2}$s$^{-1}$ blue light quantum flux were shifted to dark before reaching the stalling growth they still grew and that, after light removal, the fungal growth rate depended on the day in which the light was applied (Fig. 2). Thus, the slopes found for 0, 1, 2, and 4 dpi were 0.859, 0.802, 0.630 and 0.2418, respectively. Such slopes were calculated from the day it finished the light treatment for each dpi trial and during the period the growth was still linear. These results indicate that the loss of light efficacy inhibiting fungal growth when the cultures were transferred to darkness was faster at 0 dpi and lower at 4 dpi, which suggests that the already grown mycelium loses the ability to cope with the damage inflicted by the light treatment.

We further examined whether the 120 μmolm$^{-2}$s$^{-1}$ blue light quantum flux was able to stop the colonies’ growth if they were shifted to the dark after reaching the stalling growth. This experiment was performed at 0 and at 4 dpi since these two post-inoculation periods showed the highest difference in growth rate when the plates were transferred to darkness after the 3 days light treatment. Moreover, the incubation period was considerably extended. Results confirmed the behavior found in the previous experiment since continuous light induced a delayed fungistatic effect after 8 days of fungal growth and this effect was maintained until the end of the experiment (22 days) (Fig. 3). Furthermore, the loss of light efficacy of the 3 days light treatment was faster at 0 dpi, (Fig. 3). Results also showed that no further growth occurred if the colonies treated with light at 0 or 4 dpi were transferred to darkness after the mycelium growth was stalled (Fig. 3). That is, the efficacy of the treatment was not reversed after removing the light stimulus.

Morphology of Pd1 P. digitatum strain grown under different light regimes is shown in Fig. S2 (see Supplementary Materials) and Fig. 4. In the 0 dpi trial, slight changes in the
mortality of the colonies occurred when this light quantum flux was applied for 3 days and then the plates were transferred to dark, but extending the light exposure period clearly affected the mycelium. Sporulation was already observed by day 4 in control plates held under darkness (Fig. S2a). In contrast, it was not observed in the cultures treated for 3 days with light and then transferred to darkness for 1 additional day (Fig. S2b) or in cultures held under continuous light (Fig. S2c). By day 7, the colonies held under darkness showed the characteristic green conidia and white mycelium of *P. digitatum* (Fig. 4a). The morphology of the cultures exposed to non-continuous light (Fig. 4b and c) was similar, although the 3 days light treatment delayed the fungal growth (Fig. 3). These results indicate that non-continuous light (3 days light) at a quantum flux of 120 µmolm$^{-2}$s$^{-1}$ has only a slight effect retarding growth and sporulation when the light is applied at 0 dpi. However, extending light exposure (continuous light) had a noticeable effect (Fig 4d-f). By 7 days, the presence of brownished mycelium was already observed, although white mycelium still predominated (Fig. 4d). At day 11 under continuous light, the older part of the colony was mostly brown (Fig. 4e) but the remaining white mycelium was still able to sporulate as green conidia were very evident after shifting the plates to darkness for 4 days (Fig. 4f).

Overall results indicate then that exposing *P. digitatum* to a 120 µmolm$^{-2}$s$^{-1}$ blue light for a short period (3 days) at 0 dpi slightly affects growth and sporulation and, therefore, the effect of the treatment on reducing fungal growth may be reversed after removing the light. However, longer light exposure (11 days) may have a dramatic effect on fungal viability, which may explain, at least in part, the lack of reversibility of the effect of the 11 days light treatment on fungal growth after shifting the plates from light to darkness.

Examination of changes induced in morphology by continuous and non-continuous light at 4 dpi (Fig. 4g-l) in cultures treated with the same light quantum flux further
supported that older mycelium was more sensitive to this light quantum flux. Treating *P. digitatum* with light for 3 days at 4 dpi caused brownish on the center and older part of the colony (Fig. 4g), a fact not found at 0 dpi. Thus, by 7 days of fungal growth, colony appearance was very different depending on when the 3 days light treatment was applied, either at 0 or 4 dpi (Fig. 4b and g). This differential effect was maintained after further incubation in the dark (Fig. 4c and 4h). As shown in Fig. 4i, further incubation in the dark allowed the outer younger part of the colony treated at 4 dpi with light for 3 days to sporulate, however, colony growth was stalled (Fig. 3). Therefore, the 3 days light treatment did not completely avoided sporulation although it had an important inhibitory effect on fungal growth when it was applied at 4 dpi (Fig. 3). In contrast, it barely affected the fungus morphology and had only a transient inhibitory effect when it was applied at 0 dpi (Fig. 3). The multiple effects of UV treatments both in pathogens and fruits have been widely studied (10,37,38). Information related to blue light-emitting diodes induced responses against pathogens causing diseases in fruits is less abundant (39). As far as we know, there is only one report related to the study of the mechanisms of blue light effects on *P. digitatum*. This study showed that low blue light quantum flux (40 µmol m\(^{-2}\)s\(^{-1}\)) reduces the activity of the pectolytic enzyme polygalacturonase produced by the fungus (26).

Changes in morphology found when continuous light was applied further support the higher susceptibility of the fungus to light at 4 dpi as compared to 0 dpi. As shown in Fig 4j-l, applying the light for 7 days at 4 dpi led to abundant brownish mycelium and completely inhibited sporulation. This effect was not reversed after shifting the plates to darkness. This might indicate severe hyphae damage or even cell death. In this regard, it should be noted the participation of oxidative stress in cell death and that the accumulation
of singlet oxygen is involved in the inhibitory effect of 450 nm blue light generated by LED on growth of *B. cinerea* (40). Therefore, focusing on oxidative stress would be an interesting approach to decipher the mode of action of blue light inhibiting *P. digitatum* growth. Likewise, it should be considered that blue light-mediated damage to bacteria cells has been related to the development of vacuoles within the cytoplasm, implying that damage was related to intracellular chromophores excited by the blue light, to the release of cytoplasmatic material to the surrounding environment and to cytoplasmic disruption (41).

**Fungicidal effect of blue light on *Penicillium digitatum* strains resistant and sensitive to fungicides and on *Penicillium italicum***

The above results suggest that blue light might reach a fungicidal effect on *P. digitatum* if the quantum flux is substantially increased and also that increasing the quantum flux might favor a drastic shortening of the treatment. If these were the cases, then blue light might be a useful tool for sanitation or for reducing contaminations even if it is applied after spore germination. These ideas were tested by increasing c.a. 6-fold the light quantum flux (700 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). The effect of this light dose was assayed in four different strains, three of them from *P. digitatum* (Pd1, PHI741 and PHI726) and one from *P. italicum* (PHI71). Two of the *P. digitatum* strains (Pd1 and PHI741) are resistant to the fungicides imazalil and TBZ.

The effect of shortening the 700 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) light treatment was first investigated in the PHI-41 (*P. digitatum*) and PHI-1 (*P. italicum*) strains. Results show that the effectiveness of this high light quantum flux also increases with duration of the treatment and that applying the light for 18 h had already a fungicidal effect on both strains because the fungi did not resume growing after the plates were returned to darkness (Fig. 5). After this light treatment, the percentage of germination was 0 %, while c.a. 81 % of the spores germinated.
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in the control plates held under darkness. Reducing time of light application to 3 h had no inhibitory effect on PHI-41 growth and slightly inhibited the growth of PHI-1 (data not shown).

In a subsequent experiment, we extended the incubation period of the inoculated plates and further confirmed that applying 700 µmol m$^{-2}$ s$^{-1}$ blue light for 18 h has a fungicidal effect on all the studied strains if the light is applied at 0 dpi (Fig. 6). Growth of these strains was also reduced when this treatment was applied at 4 dpi (Fig. 6). Therefore, it appears that the fungal spores are more susceptible to the higher quantum flux doses but this different effect is not observed with 4 days old hyphae. Applying 700 µmol m$^{-2}$ s$^{-1}$ blue light for 18 h affected the mycelium of the 4 strains grown for 4 days. Changes in morphology of the PHI-26 strain grown for 4 days at 20 ºC and then exposed to this treatment are shown in Fig. 7. Patterns of changes in the rest of strains assayed were similar (not shown). Brownish areas were already present after finishing the treatment (day 5) and also 2 days after transferring the cultures to darkness (day 7), although the white mycelia predominated and was able to further sporulate when the light was applied at 4 dpi for just 18 h. This would explain further fungal growth (Fig. 6 and 7) when this treatment was applied. As shown above, continuous light had a clear and not reversible detrimental effect on fungal growth even though it was applied at a lower quantum flux (Fig 4). Therefore, global results indicate that fungal spores cannot germinate under short high light quantum flux (0 dpi) and that a continuous lower light quantum flux is able to suppress both sporulation and mycelium growth when applied to already grown mycelium (4 dpi). Since blue light may avoid sporulation of Penicillium strains sensitive and resistant to the fungicides used to reduce disease incidence in citrus fruits, this new strategy might constitute a useful
alternative method for sanitation within integrated control programs for postharvest pest management of citrus fruits. Blue light would be of interest to reduce the use of fungicides by reducing contaminations of the fungicide solutions, which might allow reducing the concentrations applied or, at least, further re-utilization of such solutions. Although these solutions may be turbid, it is noticing the development of light processing methods for highly turbid fluids (42). Moreover, blue light could be used as a mean to minimize contaminations in cold storage rooms and other strategic points in the packing-houses. This would be especially relevant since decay in citrus fruit is mainly controlled by two fungicides (imazalil and TBZ) with different mode of action, whose use must be rotated in the packing- and store-houses for avoiding the development of strains resistant to them. Therefore, this study encourages further research simulating industrial conditions to implement blue light within integrated control programs for pest management of citrus fruits. Results indicate that the development of technologies able to induce high quantum fluxes of blue light would be helpful to shorten the light treatments. This study was limited by the light output of the LED array used but development of systems that use high-power LED arrays/light sources could enable the delivery of very high LED blue light doses for decontamination applications. This innovative emerging technology could be useful to the industry to reduce citrus fruit losses, and probably of other fruit and vegetable crops, caused by pathogens sensitive and resistant to fungicides. Moreover, it could help to open new markets demanding fruits non-treated with fungicides, while minimizing energy cost and toxic residues of fungicides.

CONCLUSIONS
Results showed the potential of LED blue light for controlling the growth of *P. digitatum* and *P. italicum*, which are the major pathogens of citrus fruits after harvesting, and that efficacy increases with the duration of the treatment and with the light quantum flux. The older mycelium was more sensitive to blue light. Spore germination of strains sensitive and resistant to fungicides can be completely avoided by exposing them for a short period of time to high blue light quantum flux (700 μmol m⁻² s⁻¹). Lower quantum fluxes (120 μmol m⁻² s⁻¹) have a fungicidal effect when continuously applied to already grown mycelium (4 dpi). Therefore, the combination of high quantum flux followed by a continuous lower quantum flux may reduce both sporulation and mycelium viability. Global results indicate that blue light may be a tool to reduce contaminations and infections caused by *P. digitatum* and *P. italicum* strains in postharvest facilities by reducing pathogen inoculum.

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**SUPPLEMENTARY MATERIALS**

Additional Supplementary Information may be found in the online version of this article:
Figure S1. Growth of the Pd1 *P. digitatum* strain in potato-dextrose agar (PDA) after being treated from 5 min to 360 min with blue light at a quantum flux of 120 µmolm$^{-2}$s$^{-1}$. Results represent the means of three replicates samples ± S.E.M. No significant differences (P < 0.05) were found between the control and any of the light-treated samples for the same storage period.

Figure S2. Morphology of *P. digitatum* in response to the 120 µmolm$^{-2}$s$^{-1}$ LED blue light treatment. The fungus was grown in PDA at 20 ºC for 4 days and photographed: a) 4 days darkness (D, control), b) 3 days light (L) plus 1 day darkness (D), c = 4 days light (L).
REFERENCES


Figure legends

Figure 1. Effect of treating PHI-41 and Pd1 *P. digitatum* strains with 120 µmolm²s⁻¹ LED blue light for 6 (○), 24 (□) and 72 h (▽) on fungal growth and on fungal growth inhibition. Control samples were always maintained under darkness (●). The treatments were applied immediately after inoculating (0 dpi) the plates with 5µl of 10⁶ conidia ml⁻¹. Results represent the means of three replicates samples ± S.E.M. Significant differences (P < 0.05) between the light-treated PHI-41 and Pd1 strains and their respective controls continuously held under darkness for the same storage period were found from day 3 when the samples were treated with light for 72 h. Significant differences were only maintained till day 5 when the light was applied for 24h, and no statistic difference was found when it was applied for 6 h.

Figure 2. Effect of treating the Pd1 *P. digitatum* strain with 120 µmolm²s⁻¹ LED blue light at different dpi on fungal growth. The cultures were treated continuously with this light quantum flux (○), or for 3 days and then shifted to darkness (non-continuous light, □). Control samples were always held under darkness (●). The arrows indicate when the light treatments started (0, 1, 2 and 4 dpi). All the samples were inoculated with 5µl of 10⁶ conidia ml⁻¹. Results in the upper panels represent the means of three replicate samples ± S.E.M and the asterisks indicate significant differences (P < 0.05) between the treated and the control samples for the same storage period. The statistical analysis was not performed for the 14 days samples since the diameter of the control colony held under darkness was close or higher than 9 cm. Data missing in each plot corresponding to the day 14 were close or higher than 9 cm. Data in the lower panels show the rate of growth when the cultures were treated with non-continuous light at different dpi.
Figure 3. Effect of treating the Pd1 *P. digitatum* strain with 120 µmolm$^{-2}$s$^{-1}$ LED blue light at 0 and 4 dpi on fungal growth. The cultures were treated continuously with this light (L) quantum flux (○), or for 3 days (□) and then shifted to darkness (D), in each dpi trial. Other sets of samples for each dpi were treated with light for 11 days, to reach the mycelium growth stalling, and then shifted to darkness (△). Control samples were always maintained under darkness (●). The arrows indicate when the light treatments started or finished (0 and 4 dpi). The L in the arrows indicates treatment with continuous light, while LD3 and LD11 indicate the days the plates were shifted to darkness after being treated for 3 and 11 days with light, respectively. All the samples were inoculated with 5µl of 10$^6$ conidia ml$^{-1}$. Results represent the means of three replicates samples ± S.E.M. Significant differences (P < 0.05) between the treated and the control samples for the same storage period were found from day 3 at 0 dpi and from day 8 at 4 dpi. The diameter of the control plates (darkness, D) was close or higher than 9 cm after day 11 and, therefore, these values were not included in the plots.

Figure 4. Changes in the morphology of *P. digitatum* during its growth under different light regimes. The quantum flux of the LED blue light was 120 µmolm$^{-2}$s$^{-1}$ light, the light was applied both at 0 (a-l) and at 4 dpi (g-l), and the fungus was grown in PDA at 20 ºC and photographed at different time points. In the photographs, d indicates the number of days, D darkness and L light. The symbol + indicates the shifting from light to darkness or from darkness to light.

Figure 5. Effect of treating the PHI741 (*P. digitatum*) and PHI71 (*P. italicum*) strains with 700 µmolm$^{-2}$s$^{-1}$ LED blue light for 6 (◇), 9 (○), 18 (▽) or 24 h (□) on fungal growth and
fungal growth inhibition. Control samples were always held under darkness (●). The treatments were applied immediately after inoculating (0 dpi) the plates with 5µl of 10^6 conidia ml^-1. Results represent the means of three replicates samples ± S.E.M. Significant differences (P < 0.05) between the control and the samples subjected to any treatment for the same storage period were found from day 2.

**Figure 6.** Effect of treating the Pd1, PHI-26, PHI-41 *P. digitatum* strains and the PHI-1 *P. italicum* strain with 700 µmol m^-2 s^-1 LED blue light for 18 h at 0 (○) and 4 dpi (□) on fungal growth. Control samples were always held under darkness (●). All the plates were inoculated with 5µl of 10^6 conidia ml^-1. Results represent the means of three replicated samples ± S.E.M. Significant differences (P < 0.05) between the control and the samples subjected to the light treatment at 0 and 4 dpi were found along the whole experiment for the same storage period. Data missing in each darkness plot or in the plot corresponding to the PHI-26 culture treated with light at 4 dpi were equal or higher than 9 cm.

**Figure 7.** Changes in the morphology of the PHI-26 *P. digitatum* strain during growth at 20 °C. Cultures were held continuously under darkness (D) (pictures in upper panels), or were treated for 18 h with 700 µmol m^-2 s^-1 LED blue light (L) at day 4 (4 dpi) and then shifted for different number of days (d) to darkness. This light regime avoided sporulation at 0 dpi and therefore, no photograph corresponding to 0 dpi is shown. Pattern of changes in the rest of strains assayed was very similar and, therefore, only the photographs corresponding to the PHI-26 strain are shown to better visualize the effect of blue light.
Figure 2

![Graph showing growth of plants under different conditions]
Figure 3

![Graph showing the effect of different lighting conditions on plant growth over time. The x-axis represents days at 20°C, and the y-axis represents diameter in cm. The graph includes data for 0 dpi, 4 dpi, and 11 dpi, with different lighting conditions indicated as D ( Darkness), L (Light), LD11 (Light followed by Darkness), LD3 (Light followed by Darkness), and 3d L then D (3 days of Darkness followed by Light).]
FIG 4

190x254mm (96 x 96 DPI)
Fig. 5

PHI-41

PHI-1

Diameter (cm)

Fungal growth inhibition (%)

0 2 4 6 8

Days at 20ºC

darkness

6 h light

9 h light

18 h light

24 h light
Figure 6
Fig. 7

254x190mm (96 x 96 DPI)