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Influence of light on the *Monilinia laxa*–stone fruit interaction

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Light represents a signal for the regulation of virulence in many microbial pathogens. Two stone fruits, nectarines and cherries, were used to investigate the influence of light on brown rot caused by *Monilinia laxa*. Three single-spore strains were inoculated on nectarines and incubated under different white lights, red light, blue light, greenlight, and black light with two photoperiods. In addition, to understand the effect of daylight irradiance on brown rot, *M. laxa* was inoculated on different cherry cultivars and incubated under two simulated solar irradiations. Significantly higher disease severity and

sporulation were reported on inoculated nectarines incubated under 58 W white light for 12 hr light/12 hr darkness than on nectarines incubated in continuous darkness. Only red light caused a significant increase in the incidence and severity of the disease in nectarines inoculated with the three *M. laxa* isolates, compared to fruit incubated under white light. High light intensity (185.45 W/m²), caused greater brown rot severity in all cherry cultivars, both early and late varieties, than low irradiance (145.85 W/m²). Significant up-regulation of the pathogenicity-related *MIPNL2* gene was observed as an early response after cherry inoculation under high-intensity light, especially in late cherry cultivars, while *MIPG1* expression did not show any changes under different light irradiances. *M. laxa* was shown to be a light-responsive fungal pathogen, and light seemed to play an active role in brown rot development.

1 Introduction

Monilinia laxa is a worldwide pathogen on stone fruit, such as peaches, nectarines, cherries, plums, apricots, and almonds (Byrde and Willetts, 1977). The disease caused by *M. laxa* has been reported from almost all European countries and is generally known as European brown rot, although it is also present in Asia, America, Africa, and Australia (Di Francesco and Mari, 2018). The most important losses caused by *M. laxa* are always recorded during postharvest, storage, and transport periods (Martini and Mari, 2014). As a necrotrophic fungus, *M. laxa* secretes at least one phytotoxin (molar mass 329–387 g/mol) and also has different enzymes that degrade the plant cell wall (cutinase, α -glucosidase, pectin lyase, polygalacturonase, protease, and xylanase) to penetrate the fruit surface, and invade and colonise their tissues (Garcia-Benitez et al., 2018). Indeed, the genome of the strain *Monilinia laxa* 8L shows the presence of 403 putative carbohydrate active enzyme (CAZyme) genes (4.21% of its total predicted genes): 221 glycosyl hydrolases, 88 glycosyltransferases, 55 auxiliary activities, 30 carbohydrate esterases, and 7 polysaccharide lyases (Rodríguez-Pires et al., 2020).

Brown rot incidence increases with preharvest rains, excess moisture accumulation on flowers or fruit, hail, wind, or fruit injury caused by insect pests (Holb, 2019). Growth and sporulation of *M. laxa* also depend on temperature, relative humidity (RH), and water availability (Casals et al., 2010), as well as fruit maturity (Garcia-Benitez et al., 2016). However, even though light seems fundamental for the production of conidia by *M. fructigena* (Bannon et al., 2009), little is known about its effect on brown rot development or the pathogen's ability to detect and respond to different wavelengths, as has been described for other organisms (Corrochano, 2019).

Quantitative or qualitative (wavelength) changes in light represent a signal for the regulation of virulence in many microbial pathogens. Fungal pathogen responses to light often include changes in spore germination, mycelial growth, sporulation, and reproductive structures (Corrochano, 2019). Most fungi respond to the light range between blue and ultraviolet (wavelengths 440–490 nm; Tan, 1974; Kumagai, 1989). In fact, these wavelengths produce increased sporulation and faster growth in *M. fructigena*, as do light–dark cycles (de Cal and Melgarejo, 1999). Mycelial growth under long-wave UV light could be a useful tool to distinguish *Monilinia* spp., where *M. laxa* and *M. fructigena* show less colony growth than *M. fructicola* (de Cal and Melgarejo, 1999). These growth differences could indicate the presence of different photoreceptors in these three species.

Extensive and sophisticated light detection machinery has been described in *Botrytis cinerea*, which also belongs to the family Sclerotinaceae as do *Monilinia* spp. *B. cinerea* uses at least 11 potential photoreceptors that cover the range of ultraviolet, blue, green, red, and far-red light (Schumacher, 2017). Photoreceptors could activate multiple response systems, from the tropism of unicellular structures (germ tubes) and multicellular structures (conidiophores, apothecia), to influencing morphogenesis, circadian clock and protection responses like pigmentation (Schumacher and Tudzynski, 2012; Schumacher, 2017). In addition to the different morphological and reproductive

responses described above, light also seems to influence different metabolic pathways in fungi (Tisch and Schmoll, 2010), including cell wall-degrading enzymes (Schmoll, 2018).

Our objective was to determine the effects of changes in the quantity and quality of light on the development of brown rot on nectarines and cherries, as well as its effects on the pathogen's production of conidia and the regulation of two *M. laxa* pectinase genes. Nectarines and cherries provide good models in which to study the infection process and from them we can draw inferences regarding the behaviour of *M. laxa* in any stone fruit.

2 Materials and methods

2.1 *Monilinia laxa* strains and growth conditions

Three single-spore strains from *M. laxa* (5L, 8L, and 25L) belonging to the culture collection of the Plant Protection Department of Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (Madrid, Spain), were used in this study. All strains were originally collected in 2015 from mummified fruit in a commercial plum orchard (cv. Sungold) in Lagunilla, Salamanca, Spain. *M. laxa* 8L is deposited in the Spanish Culture Type Collection (CECT 21100), and the genome sequence was recently published (Naranjo-Ortíz et al., 2018). *M. laxa* strains were maintained as cultures on potato dextrose agar (PDA; Difco) at 4°C in darkness for short-term storage and as a conidial suspension in 20% glycerol at –80°C for long-term storage. For production of conidia, *M. laxa* strains were grown on PDA amended with 20% tomato pulp at 22°C for 7–9 days with a 12 hr photoperiod.

2.2 Plant material and incubation lights used

Experiments were performed on nectarines (cv. Venus) from Ebro Valley (Lleida, Spain) with harvest date between the end of July and middle August. From Jerte Valley (Cáceres, Spain), early cherries (cvs Burlat and Early Lory) with harvest date at the beginning of May, and late cherries (cvs Hudson and Lapins) with harvest date at the beginning of July

were also used. Nectarines and cherries were harvested at optimal commercial maturity and were used in brown rot infection experiments in growth chambers.

Fruit infected with *M. laxa* were incubated at 20–25°C under different white lights, red light, blue light, green light, and black light with different photoperiods and continuous darkness. White light was generated using a set of four fluorescent bulbs of Osram 58W/840 “cool light”, Osram 36W/954 “daylight”, or Osram 36W/840 “cool light”. Red, blue, green, and black light (UV-A) were generated using a set of four fluorescent bulbs of F36W/T8/Red Sylvania, F36/T8/B Sylvania, F36W/T8/G Sylvania, and TL-D Philips 36W, respectively (Table 1). Illuminance at fruit level from each light condition was quantified by lux meter (Testo540, SE&Co, KGaA). Irradiance (W/m²) was calculated as illuminance (lx = lm/m²)/luminous efficacy (lm/W).

2.3 Effect of white lights on nectarine brown rot disease

Nectarines (cv. Venus) were surface disinfected according to Sauer and Burroughs, (1986) and dried in a laminar flow cabinet for 2 hr. Once dried, unwounded fruit were inoculated with three drops of 15 µl of a suspension containing 10⁵ conidia/ml of each *M. laxa* strain (5L, 8L, and 25L). Inoculated fruit were placed on a dry Petri dish in plastic boxes lined with moist paper to maintain high humidity. Inoculated fruit were incubated for 7 days at 20–25°C under the three types of white light described above (Table 1) and a photoperiod of 12 hr light/12 hr darkness or 8 hr light/16 hr darkness, or in continuous darkness. The distance from the nectarines to the fluorescent bulbs was 28 cm. Ten nectarines were used per strain and incubation condition.

Brown rot incidence (the percentage of nectarines with brown rot symptoms), incubation period (the time interval between inoculation and the onset of symptoms from that infection) and latency period (the time interval between inoculation and the onset of sporulation from that infection) were evaluated every day. The daily lesion length (diameter in mm/day) was calculated from the individual measurements of the colony diameter on fruit using regression analysis. Sporulation from each infected fruit was

calculated at the end of the incubation period. For that purpose, infected fruit were individually immersed in 200 ml sterile water, sonicated for 1 min, and shaken for 30 min at 150 rpm in an orbital shaker to promote the release of spores from the fruit. The suspension was filtered through glass wool, centrifuged at $15,191 \times g$ for 20 min at 4°C, and the resultant pellet was resuspended in sterile water. The number of conidia produced per brown rot lesion (represented as conidia/cm²) of each *M. laxa* strain was counted using a haemocytometer and a microscope. The complete randomized block experiment was repeated twice.

2.4 Effect of coloured lights on nectarine brown rot disease

Disinfected nectarines (cv. Venus) were inoculated with each *M. laxa* strain (5L, 8L, and 25L) as described above and incubated for 7 days at 20–25°C under red, blue, green, or black light (UV-A) (Table 1), with a photoperiod of 12 hr light/12 hr darkness or 8 hr light/16 hr darkness. Inoculated fruit incubated under white fluorescent lighting of 36 W were used as a control. The distance from the nectarines to the fluorescent bulbs was 28 cm. Ten nectarines were used per strain, and incubation conditions, including photoperiod and colour of light. Brown rot development was measured as described above, and the complete randomized block experiment was repeated twice.

2.5 Effect of daylight irradiance on cherry brown rot disease

The mean global irradiance for the Jerte Valley (Cáceres, Spain) during May (293.9 W/m²) and July (372.04 W/m²) was reported by Sancho et al. (2013). July is generally the month in which the maximum global irradiance values are recorded in Spain. Solar irradiation was simulated using five fluorescent bulbs of Osram 58W/840 “cool light” at 36.5 cm or 24.5 cm distances from fruit with photoperiods of 14 hr light/10 hr darkness or 15 hr light/9 hr darkness, respectively. The first photoperiod used simulated that observed in the Jerte Valley at the beginning of May, and the second one corresponded to the beginning of July in the same valley. Irradiance observed under bulbs with 14 hr light/10 hr darkness and 15 hr light/9 hr darkness was 145.85 W/m² and 185.45 W/m²,

respectively. These were the maximum irradiances that could be achieved with the fluorescent lighting available in our incubation conditions, while maintaining temperatures compatible with the development of the disease. Although the irradiances reached in the Jerte Valley during May and July were 293.9 W/m², and 372.04 W/m², the ratio of 1.27 between these irradiances in the valley is the same as between the two irradiances under our artificial light conditions.

Two early cherry cultivars (Burlat and Early Lory), and two late cultivars (Hudson and Lapins) were harvested on 7 May 2019 and 27 June 2019, respectively in organic orchards in the Jerte Valley (Navaconcejo, Cáceres, Spain). Both early and late fruit were disinfected (Sauer and Burroughs, 1986), left to dry in a laminar flow cabinet for two hours, and then inoculated with 50 µl of a suspension containing 10⁴ conidia/ml of *M. laxa* strain 8L by fruit pedicel insertion. All inoculated fruit were incubated for 6 days at 23–25.8°C and 23–26.5°C under 145.85 W/m² and 185.45 W/m², respectively. Five repetitions were carried out per irradiance and cherry cultivar, with three fruit per repetition. Fifteen fruit were used per irradiance and cultivar. Aggressiveness factors were recorded as described above for brown rot incidence, incubation period, and latency period for each fruit. A brown rot progress curve was obtained for each fruit. The area under the disease incidence progress curve (AUDPC), in units of percent-days, was calculated by trapezoidal integration.

2.6 Effect of daylight irradiance on the expression of two pectinase genes in infected cherries

Total RNA was extracted from two cherry cultivars (Burlat and Lapins) that had been inoculated with *M. laxa* 8L and each incubated under one of two daylight irradiances, as described above. RNA was isolated from cherry tissue according to the rapid CTAB-based protocol described by Gambino et al. (2008) with modifications. Briefly, frozen inoculated fruit were ground in liquid nitrogen using a prechilled mortar and pestle. The powdered inoculated fruit (2–4 g) were extracted with 12 ml of preheated extraction

buffer (2% CTAB, 2.5% PVP-40, 2 M NaCl, 100 mM Tris.HCl pH 8.0, 25 mM EDTA pH 8.0, 2% β -mercaptoethanol) and incubated at 65°C for 10 min. An equal volume of chloroform isoamyl alcohol (24:1) was added, vortexed to mix, and centrifuged at $2,860 \times g$ for 20 min at 4°C. The supernatant was recovered, and the chloroform isoamyl alcohol extraction was repeated for a second time. Nucleic acids, mainly RNA, were precipitated by adding LiCl (3 M final concentration), followed by incubation on ice for 30 min. Total RNA was pelleted by centrifugation at $2,860 \times g$ for 60 min at 4°C, resuspended in 500 μ l SSTE buffer (10 mM Tris.HCl pH 8.0, 1 mM EDTA pH 8.0, 1% SDS, 1 M NaCl) preheated to 65°C, and an equal volume of chloroform isoamyl alcohol (24:1) was added. The supernatant was recovered by centrifugation at $25,750 \times g$ for 10 min at 4°C. RNA was precipitated by adding 0.7 volumes of cold isopropanol and centrifuging at $57,950 \times g$ for 15 min at 4°C. The pellet was subsequently washed with 70% ethanol and centrifuged at $25,750 \times g$ for 15 min at 4°C. The dry RNA pellet was resuspended in RNase-free Milli-Q water, immediately frozen in liquid nitrogen, and maintained at -80°C until further use. RNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer, and RNA integrity was checked by 1.2% agarose gel electrophoresis. RNA was treated with DNase I (Invitrogen) according to the manufacturer's specifications to remove any remaining genomic DNA. cDNA was synthesised from 2 μ g of RNA using the SuperScript First-Strand Synthesis System for RT-PCR and oligo(dT) primer (Invitrogen). Real-time PCR was performed with a 7500 Fast Real-Time PCR (Applied Biosystems) using GoTaq qPCR Master Mix (Promega). Each reaction was carried out in triplicate, with a total volume of 20 μ l, containing 10 μ l of 2 $\times$ GoTaq qPCR Master Mix, 7.8 μ l of nuclease-free water, 0.6 μ l of each primer (10 μ M) and 1 μ l of cDNA. The cycling programme was 2 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. After the amplification reaction, a melt curve analysis was performed to check the specificity. Primers for polygalacturonase 1 (*MIPG1*), pectin lyase 2 gene (*MIPNL2*), and histone H3 gene (*MIH3*) were used from Rodríguez-Pires et al. (2020), and the primers for the gene encoding the 60S ribosomal protein L5 (*MIRPL5*) were designed based on the *M. laxa* 8L genome sequence using Vector NTI (Thermo

Fisher) (Table S1). The relative expression levels of genes were calculated using the $2^{-\Delta\Delta C_t}$ method with *MIH3* and *MIRPL5* used as reference genes (Schmittgen and Livak, 2008). Three technical replicates were analysed for each biological replicate for both the target and the reference genes. Nucleotide sequence data reported are available in the Third-Party Annotation Section of the DDBJ/ENA/GenBank databases under the accession numbers TPA: *MIH3*, accession no. BK012065; *MIPG1*, accession no. BK012078; *MIPNL2*, accession no. BK012082; and *MIRPL5*, accession no. BK013068.

2.7 Data analysis

Data were analysed by analysis of variance (ANOVA) using the STATGRAPHICS program (XVII Centurion. v. 17.2.00). When the *F* test was significant ($p \leq .05$), comparison of means was made by Duncan's multiple range test. Experiments were conducted twice within each host species.

Data from *MIPG1* and *MIPNL2* gene expression were calculated using the $2^{-\Delta C_t}$ method and normalized to the expression of the constitutive genes of *MIH3* and *MIRPL5*. The expressions relative to *MIPG1* and *MIPNL2* were analysed using ANOVA analysis for cherry cultivar analysis and time-course expression. When the *F* test was significant ($p \leq .05$), a comparison of means was made by Duncan's multiple range test.

3 Results

3.1 Effect of white lights on nectarine brown rot disease

Significantly higher disease severity was reported on inoculated nectarines incubated under 58 W cool white light for 12 hr light/12 hr darkness than inoculated nectarines incubated in continuous darkness, for any of the three *M. laxa* isolates used (Figure 1). The maximum irradiance, luminous flux and illuminance were recorded under 58 W fluorescent tubes (Table 1). All infected nectarines incubated under 58 W cool white or 36 W daylight tubes with 12 hr light had the highest pathogen sporulation. However, with

an 8 hr photoperiod, increased sporulation was observed only on infected fruit incubated at 58 W (Figure 1). For 12 hr light and 8 hr light photoperiods respectively, the average temperatures were 23.6 and 23.9°C for 36 W daylight, 26.8 and 27.6°C for 36 W cool white, 22.8 and 23.5°C for 58 W, and for darkness the average temperature was 20.5°C (Table 1).

No photoperiod or white light effects were observed on nectarine brown rot incidence (Figure 1). The incubation period was similar for all conditions, with a range between 4.5 to 6 days (data not shown). Sporulation was only observed for 58 W cool white and 36 W daylight in all isolates, with a latent period from 5 to 7 days (data not shown). No sporulation was detected for 36 W cool white or darkness, except that nectarines infected with the 5L isolate showed some low sporulation after 7 days in continuous darkness (data not shown).

3.2 Effect of coloured lights on nectarine brown rot disease

Some differences were observed between light colours in terms of brown rot incidence, severity, and sporulation of the three *M. laxa* isolates on inoculated nectarines (Figure 2). Red light significantly increased the brown rot incidence of all *M. laxa* isolates, either with a 12 hr light/12 hr darkness photoperiod on isolate 5L (Figure 2a), or an 8 hr light/16 hr darkness photoperiod on 8L (Figure 2d) and 25L (Figure 2g), compared to those inoculated fruit incubated under 36 W daylight fluorescent. Moreover, red light also increased the severity of disease caused by isolates 5L and 8L with 12 hr light/12 hr darkness photoperiod and 8 hr light/16 hr darkness photoperiod, respectively (Figure 2b,e). Increased sporulation was observed only on nectarines inoculated with isolate 8L incubated under red light, regardless of photoperiod (Figure 2f).

Only the 5L isolate showed a significant increase of brown rot severity on infected nectarines incubated under blue, green, and black lights, especially with a photoperiod of 12 hr light/12 hr darkness, compared to those incubated under daylight (Figure 2b).

The highest irradiance was observed under blue light, while the highest luminous flux and illuminance were recorded under green light (Table 1). However, lowest illuminance was observed under red and black light (Table 1). For the 12 hr light and 8 hr light photoperiods, respectively, average temperatures were 22.4 and 22.8°C for blue light, 26.3 and 26.7°C for green light, 22.8 and 23.3°C for red light, 23.0 and 23.1°C for black light (UV-A), and 23.6 and 23.9°C for 36 W daylight (Table 1).

3.3 Effect of daylight irradiance on cherry brown rot disease

Brown rot developed on all the cherry cultivars used. The early cherry varieties were more susceptible than the late ones (Figure 3a). In all the varieties used in the two trials, the AUDPC on cherries incubated under high irradiance was significantly higher than under low irradiance (Figure 3a), while the incubation and latency period were shorter (Figure 3b,c).

3.4 Effect of daylight irradiance on the expression of two pectinase genes in infected cherries

The expression of *MIPNL2* after 6 days of incubation was higher (>3.7-fold) under low irradiance (145.85 W/m²) in the cherry cultivar most susceptible to brown rot (cv. Burlat), than that observed at high irradiance (185.45 W/m²; Figure 4). In Lapins cherries, no significant differences in *MIPNL2* expression were found after 6 days of incubation at either irradiance. There were also no significant differences in *MIPG1* expression between irradiances in either cultivar (Figure 4).

Immediate induction of *MIPNL2* expression was observed in Lapins cherries after 1 day incubation at both irradiances. However, significantly higher expression of the *MIPNL2* gene occurred under high irradiance (>8-fold; Figure 5). After 2 days incubation at either irradiance, *MIPNL2* expression in Lapins cherries was low, with no significant difference between the irradiances (Figure 5). Expression of *MIPG1* was not significantly different either over time or between irradiances (Figure 5).

4 Discussion

Light is an essential environmental factor with daily and seasonal fluctuations. *M. laxa* is a light-responsive fungal pathogen, affected by the intensity and quality of light, as well as light duration, which could thus influence the development of brown rot. Recently, in addition to the model filamentous fungi *Neurospora crassa* and *Aspergillus nidulans*, light has been established as an important modulator of biological processes in plant pathogenic fungi, such as pathogenesis (Schumacher, 2017), reproduction (Gadoury et al., 2012), or metabolism (Kim et al., 2011).

In the current study, white light intensity and its photoperiod had a significant effect on the severity, but not the incidence, of brown rot caused by *M. laxa* isolates on nectarines. An increase in growth rate of *M. laxa* on fruit was observed under 58 W cool white and 36 W daylight with the 12 hr photoperiod and 58 W cool white with an 8 hr photoperiod, which resulted in greater disease severity in these fruits than in those under 36 W cool white light or continuous darkness. In the closely related *B. cinerea*, wild type isolates (T4 and 1750) that were “blind” to light were associated with reduced virulence on bean plants (Schumacher, 2017). The observed effect on growth of *M. laxa* may have been caused by the higher light illuminance and irradiance. However, it is feasible that those responses were not observed under 36 W cool white light because the temperature recorded under these lights was slightly higher than the optimum for growth of *M. laxa*, beyond which *M. laxa* fungal decay rates and mycelium development have been found to decrease (Bernat et al., 2017). A higher conidia production by *M. laxa* was also recorded with more hours of 58 W white light exposure. It is known that light is essential for conidiation in *A. nidulans* (Mooney and Yager, 1990) as are light–darkness cycles in light-responsive *B. cinerea* isolates (Schumacher and Tudzynski, 2012). This increased conidial production could be an advantage for dispersal because asexual reproduction is the primary mechanism for reproduction of *M. laxa* in Spain (Villarino et al., 2010).

High sunlight intensity was associated with greater brown rot severity in all cherry cultivars (both early and late) than was low irradiance, a similar effect to that shown in nectarine assays under 58 W cool white light. Furthermore, the lowest incubation and latency periods of *M. laxa* were observed on cherries incubated under high light irradiance. Our results suggest that higher light irradiance increased the growth rate of *M. laxa* on fruit and its dispersal. Light and other environmental factors (pH, carbon and nitrogen sources) regulated secondary metabolism and morphogenesis in *Cercospora zae-maydis* (Kim et al., 2011). Like other necrotrophic plant pathogens, *M. laxa* produces extracellular enzymes (Rodríguez-Pires et al., 2020). In this study, we explored the genetic expression of two related pathogenicity-related lytic enzyme genes of *M. laxa*, *MIPG1* and *MIPNL2*, under two different irradiances. *MIPNL2* expression in cherries inoculated with *M. laxa* was dependent on the intensity of light, while *MIPG1* expression did not show any changes under the two different light irradiances. However, *MIPG1* expression is highly induced by pectin, while *MIPNL2* is not carbon source-dependent although its protein is detected after 6 hr in the exoproteome, as described by Rodríguez-Pires et al. (2020). The effect of light on *MIPNL2* expression decreased drastically over time during fruit incubation, although, in the cherry cultivar more susceptible to brown rot (cv. Burlat), expression at lower irradiance remained significantly higher than at high irradiance after 6 days of incubation. This could be due to a delay in the infection process. Thus, differences in sunlight throughout the year could cause different adaptive responses in *M. laxa* and play an active role in brown rot development.

Considering that the full spectrum of white light had an effect on *M. laxa*, different wavelengths of white light such as blue, green, red, and black light were studied. Different wavelengths of light have previously been shown to affect the behaviour and growth of pathogenic fungi (Corrochano, 2019). Our results showed a specific role for red light in increasing incidence and severity of brown rot by *M. laxa* compared with fruit incubated under white light. In contrast, reduced virulence of a *B. cinerea* isolate on broad bean has been observed after incubation in red light compared to near-UV and blue light (Islam et

al., 1998). Red light-sensing photoreceptors have been discovered in the two model fungal species, *A. nidulans* (Blumenstein et al., 2005) and *N. crassa* (Froehlich et al., 2005). The effect of the longer wavelength of red light on photoreceptors could be responsible for the increased incidence and severity caused by *M. laxa* on nectarines.

In contrast, in nectarines, only those inoculated with *M. laxa* isolate 5L and incubated under black, blue, and green lights showed a significant increase in brown rot incidence or severity compared to fruit incubated under white light. These results suggest that *M. laxa* 5L had greater sensitivity to different wavelengths than other isolates, resulting in increased brown rot incidence and severity. *B. cinerea* was also found to respond to different wavelengths of light: UV light promoted conidia production, red light promoted sclerotial development, and blue light promoted undifferentiated mycelial growth (Tan and Epton, 1973; Schumacher, 2017). The only light to cause an increase in *M. laxa* sporulation on fruit was red light on *M. laxa* isolate 8L. In *B. cinerea*, red light promoted germ tube elongation and a positive phototropic response, without infection hyphae (Islam et al., 1998). Although red light also promoted conidiation in *Alternaria alternata*, unlike *M. laxa*, conidiation was equally promoted by darkness (Pruss et al., 2014). In nectarines inoculated with *M. laxa* isolate 25L, incubation under green light led to a significant reduction in conidia. The role of light as a modifier of asexual development is a common theme in studies of filamentous fungi (Gadoury et al., 2012). Conidiation of *B. cinerea* was effectively promoted by black light (near-UV) and by an alternation of black light and white light, whereas almost no conidiation could be observed in darkness or after irradiation with blue, green, yellow, or red light (Tan and Epton, 1973). Blue light had an inhibitory effect on conidiation and could partially reverse the black light effect and vice versa, indicating that the conidiation response of *B. cinerea* is photoreversible (Tan, 1974). Photoinduction of conidiation by blue light has also been demonstrated in *A. alternata* on tomato, and in *Podosphaera pannosa* on strawberry (Kumagai, 1989; Suthaparan et al., 2010). Cryptochromes are blue-light photoreceptors initially identified in plants and closely related to light-dependent DNA repair photolyases (Thompson and

Sancar, 2002). In addition to photorepair in fungi, cryptochromes are involved in the regulation of conidiation and secondary metabolism (Idnurm et al., 2010).

Filamentous fungi have a sophisticated regulatory network of photoreceptors and signal transduction pathways that enable them to use the different light characteristics (direction, quantity, and quality) as a signal to regulate development (tropic growth and morphogenesis), to induce adaptive responses (protective pigments and proteins), and to set a circadian clock (Rodriguez-Romero et al., 2010; Corrochano, 2019). Photoreceptor proteins require the presence of a chromophore to sense light, providing sensitivity to a specific range of wavelengths. Fungal photoreceptors also perceive different wavelengths of light and serve as a signal to activate or silence pathways related to the pathogen life cycle (Rodriguez-Romero et al., 2010; Corrochano, 2019). *B. cinerea* conidiation is improved by far-red light, while it is repressed by red light (Schumacher, 2017); in addition, the absence of one of its phytochromes reduces pathogenicity and decreases its vegetative growth (Hu et al., 2014). Phytochromes act as red/far-red light photoreceptors through a linear tetrapyrrole chromophore and are important in regulating the transition between sexual and asexual reproduction in a select number of fungi (Blumenstein et al., 2005). However, in *C. zeae-maydis* pathogenesis, the potential role of a red-light photoreceptor was not clear, as a red light-specific morphological or metabolic phenotype was not observed (Kim et al., 2011).

Daylight and darkness, which are repeated in a daily rhythm, could also be important factors for the development of brown rot. Fungal daily light detection seems decisive to help pathogenic fungi in their adaptation to environmental responses, organized in many cases by circadian clocks (Schumacher, 2017). Taking advantage of a circadian clock to regulate melanization and/or sporulation could allow *M. laxa* to optimize the timing of asexual reproduction to avoid harmful UV light. The role of melanin in improving *M. laxa* survival when exposed to UV light and even its pathogenesis capacity has been demonstrated by de Cal and Melgarejo (1993). Melanin-like pigments in *M. laxa* hyphal walls increase the fungal resistance to autolysis (de Cal and Melgarejo, 1994). It is

possible that light-dependent processes among *M. laxa* isolates are circadian, in which molecular clocks entrained by light maintain an endogenous rhythm (Bell-Pedersen et al., 1996). However, the existence of an endogenous molecular clock in *M. laxa* has not been confirmed, and only red light affected the development of brown rot among the *M. laxa* isolates used in the present study.

Thus, our results showed that light seems to be an important factor in the adaptive capacity of *M. laxa* isolates. This behaviour may be even greater in field conditions where sunlight, with greater irradiance than that used in the present study, affects brown rot development. *M. laxa* can survive in daylight, as well as infecting and colonizing the fruit, adapting to daily light cycles; hence the increased sensitivity of *M. laxa* to red light that could be due to the adaptive adjustment of the pathogen at sunset.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1 Primers for use in quantitative reverse transcription PCR (RT-qPCR) for the analysis of genes for pectin degradation in *Monilinia laxa*.

Figure legends

Figure 1 Effect of white light on brown rot incidence (percentage, black bars), disease severity (lesion length in mm/day, grey bars), and *Monilini laxa* sporulation (conidia/cm² fruit, white bars) on unwounded nectarines inoculated with three *M. laxa* isolates (5L, 8L, and 25L), and incubated for 7 days at 20–25°C under three types of white lights: 58 W cool-light, 36 W cool-light, and 36 W daylight for 12 hr light/12 hr darkness or 8 hr light/16 hr darkness, and in continuous darkness. Bars are the mean of 20 nectarines per type of white light and photoperiod, and letters denote significant differences according to the analysis of variance (ANOVA) and Duncan's multiple range test. Different upper case letters indicate a significant difference in disease severity; different lower case letters indicate significant differences in sporulation ; ns indicates no significant difference in brown rot incidence.

Figure 2 Effect of red, blue, green, black, and white fluorescent lighting of 36 W on brown rot incidence (percentage; a, d, g), disease severity (lesion length in mm/day; b, e, h), and *Monilinia laxa* sporulation (conidia/cm² fruit; c, f, i) on unwounded nectarines inoculated with three *M. laxa* isolates: 5L (a, b, c), 8L (d, e, f), and 25L (g, h, i). All fruit were incubated for 7 days at 20–25°C under five types of colour lights for 12 hr light/12 hr darkness (white bars) and 8 hr light/16 hr darkness (grey bars). Bars are the mean of 20 nectarines per colour light and photoperiod, and different letters denote significant differences according to the analysis of variance (ANOVA) and Duncan's multiple range test.

Figure 3 Effect of two light irradiances on cherry brown rot disease: area under disease progress curve (AUDPC) (a), incubation period (b), and latent period (c). Two early cherry cultivars (Burlat and Early Lory), and two late cultivars (Hudson and Lapins) were inoculated with conidia of 8L *M. laxa*. Inoculated fruit were incubated for 6 days at 23.0–25.8°C under 58 W cool light with irradiance of 145.85 W/m² for 14 hr light/10

hr darkness (white bars), or at 23.0–26.5°C under 58 W cool light with irradiance of 185.45 W/m² for 15 hr light/9 hr darkness (black bars). The ratio between irradiances was 1.27, corresponding to the ratio between irradiances reached in Jerte Valley during May (293.9 W/m²) or July (372.04 W/m²). Bars are the mean of 15 fruit per irradiance and cultivar, and letters denote significant differences according to the analysis of variance (ANOVA) and Duncan's multiple range test. Upper case A–C refer to late cultivars; lower case a–c refer to early cultivars.

Figure 4 Differential expression of genes coding for polygalacturonase 1 (*MIPG1*) pectin lyase 2 (*MIPNL2*) of two cherry cultivars (Burlat and Lapins) inoculated with conidia of *Monilinia laxa* isolate 8L, and incubated for 6 days at 23.3–26.5°C under 58 W cool light with irradiance of 145.85 W/m² for 14 hr light/10 hr darkness (white bars), or under 58 W cool light with irradiance of 185.45 W/m² for 15 hr light/9 hr darkness (black bars). The ratio between irradiances was 1.27, corresponding to the ratio between irradiances reached in Jerte Valley during May (293.9 W/m²) or July (372.04 W/m²). The numbers of transcripts for each gene at different time points were compared using the comparative *C_t* method (2^{-ΔC_t}). Each time point represents the mean (*n* = 2), and letters denote significant differences according to the analysis of variance (ANOVA) and Duncan's multiple range test.

Figure 5 Differential expression of genes coding for polygalacturonase 1 (*MIPG1*) and pectin lyase 2 (*MIPNL2*) of cherries (cv. Lapins) inoculated with conidia of *Monilinia laxa* isolate 8L, and incubated for 1–6 days at 23.3–26.5°C under 58 W cool light with irradiance of 145.85 W/m² for 14 hr light/10 hr darkness (May), or under 58 W cool light with irradiance of 185.45 W/m² for 15 hr light/9 hr darkness (July). The ratio between irradiances was 1.27, corresponding to thratio between irradiances reached in Jerte Valley during May (293.9 W/m²) or July (372.04 W/m²). The numbers of transcripts for each gene at different time points were compared using the comparative C_t method ($2^{-\Delta C_t}$). Each time-point represents the mean ($n = 2$), and letters denote significant differences according to the analysis of variance (ANOVA) and Duncan’s multiple range test.