Phenotypic plasticity of *Monilinia* spp.in response to light wavelengths: from *in vitro* development to virulence on nectarines

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Abstract

The development of brown rot in stone fruit caused by the necrotrophic fungus *Monilinia* spp. is influenced by many abiotic factors, such as temperature, humidity, and light. Specifically, filamentous fungi perceive light as a signal for ecophysiological and adaptive responses. We have explored how specific light wavelengths affect the *in vitro* development, the regulation of putative development genes and the virulence of the main species of *Monilinia* (*M. laxa*, *M. fructicola* and *M. fructigena*). After subjecting *Monilinia* spp. to different light wavelengths (white, black, blue, red, far-red) for 7 days, several differences in their phenotype were observed among light conditions, but also among species. These species of *Monilinia* exhibited a different phenotypic plasticity in response to light regarding pigmentation, growth, and specially conidiation of colonies. In this sense, we observed that the conidial production was higher in *M. laxa* than *M. fructicola*, while *M. fructigena* showed an inability to produce conidia under the tested conditions. Growth rate among species was significantly lower in *M. fructicola* under red light wavelength while among light conditions it was increased under far-red light wavelength for *M. laxa* and under black light for *M. fructicola*; in contrast, no statistical differences were observed for *M. fructigena*. Gene expression analysis of 13 genes involved in fungal development of *Monilinia* spp. revealed a significant difference among the three species of *Monilinia*, and especially depended on light wavelengths. Among them, a high expression of *OPT1*, *RGS2, RGS3* and *SPP1* genes was observed in *M. laxa*, and *LTF1* and *STE12* in *M. fructicola* under black light. In contrast, a high expression of *REG1* and *C6TF1* genes occurred in both *M. fructicola* and *M. laxa* subject to red and far-red light wavelength, respectively. When nectarines were artificially infected with *M. laxa* and *M. fructicola* subjected to black light, the virulence was clearly reduced, but not in *M. fructigena*. Overall, results presented herein demonstrate that light wavelengths are

a key abiotic factor for the biology of *Monilinia* spp., specially modulating its capacity to form conidia, and thus, influencing its spreading and the onset of the disease on nectarines during postharvest.

Keywords: abiotic factor, brown rot, asexual reproduction, development genes, *Prunus persica*, stone fruit.

1. Introduction

Brown rot is caused by the fungus *Monilinia* spp., which belongs to the Sclerotiniaceae family and comprises several species. Most of them are considered pathogens and present a necrotrophic lifestyle, characterized by colonizing the fruit tissues causing cell death. Among them, *Monilinia laxa* (Aderhold and Ruhland), *M. fructicola* (G. Winter) and *M. fructigena* (Honey) are considered the main species responsible of brown rot in stone and in pome fruit, causing important economic losses in the field and at postharvest (Obi et al., 2018). Specifically, *M. laxa* was the predominant causal agent of brown rot in stone fruits, until *M. fructicola* increased its presence in Spain since its appearance in (De Cal et al., 2009), coexisting then with *M. laxa*. On the other hand, *M. fructigena* is the predominant fungus in pome fruit (Gril et al., 2008), although it is also able to infect stone fruit. The susceptibility of stone fruit to brown rot development is variable and depends on the host but also on several environmental conditions. In this sense, the most studied factors that influence *Monilinia* spp. occurrence are temperature and humidity (Bernat et al., 2018), but there are also other abiotic factors such as light that are less studied, affecting the development and virulence of fungi (Schumacher, 2017). In general, organisms use different light wavelengths to generate energy (e. g. photosynthesis) and also to protect themselves from its harmful effects. Recent advances have shed light on various aspects of fungal virulence highlighting the role of light wavelengths on modulating virulence factors (John et al., 2021). The responses of pathogenic fungi to light wavelength include changes in vegetative growth, reproductive structures, circadian clock and metabolic processes (Roden and Ingle, 2009; Tisch and Schmoll, 2010), both at transcriptional and metabolic level, displaying a phenotypic plasticity (Kronholm et al., 2016). Although some studies already exist for other pathogens such as *Botrytis cinerea* determining the morphological and metabolic changes in response to light (Schumacher,

2017), scarce information is currently available regarding *Monilinia* spp. Recent studies have characterized the light-sensing machinery to evaluate the effect of light wavelength on colony growth and conidiation of several *Monilinia laxa* isolates (Rodríguez-Pires et al., 2021b). Furthermore, a study from (Balsells-Llauradó et al., 2021), aimed to decipher the effect of exposing both *M. laxa* and *M. fructicola* under lighting treatments (full-spectrum light) and its capacity to infect fruit. In the case of *M. fructigena*, Bannon et al., 2009 assessed the ability to sense and react to light and light/dark cycles. This information, together with the literature already available on other fungi, points out to the crucial role of light on the development of the three species of *Monilinia* and their ability to cause brown rot in stone fruit. Thus said, the main objective of this study was to evaluate the effect of the different light wavelengths on: i) *in vitro* ecophysiology and gene expression of several development-related genes of *M. laxa*, *M. fructicola* and *M. fructigena*, ii) *in vivo* studies related to the infectivity of the three species of *Monilinia* in nectarines.

2. Materials and Methods

2.1. Fungal strains and culture conditions

In this study three species of *Monilinia* were evaluated: *M. laxa* (ML8L), *M. fructicola* CPMC6) and *M. fructigena* (GENA6). *Monilinia laxa* and *M. fructicola* were deposited in the Spanish Culture Type Collection (CECT 21100 and CECT 21105, respectively), and the Bioproject code for *M. fructigena* is PRJNA707424.

Conidial suspensions were prepared from 7–9-day-old cultures grown on PDA supplemented with 25 % tomato pulp (PDA-T). For both *M. laxa* and *M. fructicola* species, inoculum was prepared as described by Baró-Montel et al. (2019b). Due to the inability of *M. fructigena* to produce conidia, the fungal suspension composed predominantly of filamentous structures and scarce conidia, was obtained by recovering

7–9-day-old culture surface and introducing it in a sterile full-page filter bag (BagPage, interscience) with 5 mL of sterile water containing 0.01 % (w/v) Tween-80. The bag was then placed in the homogenizer (MiniMix 100 P CC) for 4 minutes and the obtained fungal suspension was recovered for further assays.

2.2. Light conditions

To analyse the light effect on the phenotypic plasticity of *Monilinia* spp., different light wavelengths were evaluated: white light (Ta = 5500K- 6000K, 3350 wl, 400 – 700 nm) was generated using a spectrum Research Philips LED Modules; Black light (UV-A) (370 nm) was generated with 5 fluorescent Philips LED tubes of F20W/T9/BLB; Blue light (460 nm), red light (660 nm) and far-red light (740 nm) were generated by using a spectrum Research Philips LED Modules based on a platform of 9 tubes with 5 LEDs each. A photoperiod regime of 16-h light and 8-h darkness at 20 °C was stablished for each light wavelength. Finally, a constant darkness condition at 20 °C was included as a control.

2.3. *In vitro* assays

Monilinia spp. were grown on PDA-T and experiments were conducted by applying one drop of 10 µL of the fungal suspension at 10^5 conidia mL^{-1} and subjected to the different light wavelengths for 7 days. Different phenotypic and developmental parameters for each species and light conditions were measured as further explained. All experiments were carried out twice with three replicates per condition.

2.3.1. Colony morphology and conidia characterization

A visual inspection of colony features according to EPPO standard PM 7/18 (3) (Oepp and Bulletin, 2020) were performed. For microscopic visualizations, the inoculum of *Monilinia* spp. was prepared as described in section 2.1. Images of each conidia were taken at 40x magnification in an optical microscope (Leica DM5000B, Leica

129 Microsystems CMS GmbH, Germany). Images were acquired with a Leica digital colour
130 camera (Leica DFC 420).

131 **2.3.2. Conidial quantification**

132 Quantification of total conidia was performed by preparing the conidial suspensions as
133 described in section 2.1, with a known volume of sterile water containing 0.01 % (w/v)
134 Tween-80. Data represents the conidia concentration (conidia mL^{-1}) for each species of
135 *Monilinia* and light condition.

136 **2.3.3. Cell viability**

137 Cell viability was assessed as follows: the conidial suspension was obtained as explained
138 above (section 2.1) and prepared at 10^4 conidia mL^{-1}. Three-fold serial dilutions in PDA
139 medium were performed to assess the colony forming units (CFU) after incubating the
140 plates for 3 to 4 days at 20 °C in the darkness. Cell viability for each species and light
141 wavelength was expressed as conidia mL^{-1}.

142 **2.3.4. Growth rate**

143 The growth rate of the colony (cm day^{-1}) was determined as the slope of linear equation
144 obtained from the individual measurements of the mean of the diameter of the colony in
145 two perpendicular directions by plotting the growth diameter (cm) against the time (days).

146 **2.3.5. Analysis developmental *Monilinia* spp. genes**

147 **2.3.5.1. Identification of *Monilinia* spp. candidate genes**

148 A total of 13 genes involved in fungal development were selected based on previous
149 literature on other necrotrophic fungi **(Suppl. Table S1)**.

150 Candidate genes from other fungi were used as query sequences for a BLAST analysis
151 **(Suppl. Table S2)** to search for homologies within *M. laxa* (ML8L) (Naranjo-Ortíz et al.,
152 2018), *M. fructicola* (CPMC6) (Vilanova et al., 2021), and *M. fructigena* (GENA6)
153 (Marcet-Houben et al., 2021) genomes using NCBI Genome Workbench software v.

2.11.10 (https://www.ncbi.nlm.nih.gov/tools/gbench/), and the BLAST tool implemented therein. The expect (E) value was set at 10^{-3}. The identity (>60 %) and the fraction of query sequences covered by the match region (>50 %) were used as filter criteria to select only reliable hits. Results obtained were checked by carrying out blastx, blastn and tblastn analysis.

2.3.5.2. RNA extraction and qPCR analysis

PDA-T cultures of *Monilinia* spp. grown as described in section 2.2, were collected and immediately frozen. RNA was extracted using TRI reagent (Sigma, MO, USA) as described by Baró-Montel et al. (2019b), using 3 biological replicates for each light condition.

cDNA synthesis was performed on 5 µg of DNase-treated RNA samples using the commercial Superscript IV First-Strand reverse transcriptase cDNA Synthesis Reaction kit (Invitrogen, Carlsbad, CA, USA).

Gene expression analysis were performed as described by Baró-Montel et al. (2019a). Primers used for gene expression analysis **(Suppl. Table S3)** were designed *de novo* using the Primer-BLAST tool (Ye et al., 2012). For each selected gene, primer sequences were common for the three *Monilinia* species. Elongation Factor 1*α* (*EF1-α)* was selected based on its constant expression among conditions. Primer efficiency was determined using 3-fold cDNA dilutions in triplicate and primer specificity was checked by analysing the melting curves at temperatures ranging from 60 to 95 °C. A non-template control (NTC) was included using water instead of DNA. Relative gene expression was expressed as Mean Normalized Expression (MNE) and calculated using the method described by Muller et al (2002).

2.4. *In vivo* assays

2.4.1. Plant material

Brown rot development was assessed on 'Extreme 563' nectarines (*Prunus persica* (L.) Batch) harvested at commercial maturity from an organic orchard located in Vilanova de Segrià (Lleida, Catalonia, Spain). Once harvested, nectarines were separated into two batches according to the single index of absorbance (DA index) using a DA-Meter (TR-Turoni, Forli, Italy); the first batch comprised a DA index from 0.17 to 0.81, while the second batch presented a DA index from 0.82 to 1.71.

2.4.2. Fruit inoculations

Fruit was inoculated with a conidia suspension of each *Monilinia* spp. (*M. laxa*, *M. fructicola* and *M. fructigena*) subjected to darkness condition (control) and the different light wavelengths as described in section 2.2. Fungal suspensions of each pathogen were obtained as explained in the section 2.1. Fruit was inoculated by applying one drop of 10 µL at 10^5 conidia mL^{-1} and incubated in a chamber under darkness and a relative humidity of 97 ± 3 % at 20 ± 1 °C. The experiments were conducted twice, using 20 nectarines for each light wavelength and species. Fruit was examined daily for 7 days to record the incidence of brown rot (percentage of fruit with brown rot symptoms), the severity (lesion diameter length in cm of rotted fruit), the incubation period (number of days to the observation of the onset of brown rot symptoms), and the latency period (number of days to the observation of conidiation).

2.5. Statistical analysis

All data were collated and subjected to analysis of variance (ANOVA) using JMP® 14 (v. 14.2.0, Cary, NC: SAS Institute Inc.). When the analysis was statistically significant, the Tukey's HSD test at the level $p \leq 0.05$ was performed for comparison of means between light wavelengths for each species or between species for each light wavelength. In the case of conidiation results, the comparison between the two *Monilinia* species was performed using the Student's T-test at the $p \leq 0.05$ level.

Results

3.1. *In vitro* assays

3.1.1. Effect of light wavelengths on the *in vitro* development of *Monilinia* spp.

Growing the three *Monilinia* spp. under the different light wavelengths demonstrated a clear difference among species based on the morphology of colonies, which was dependent on the light wavelength. Specifically, *M. laxa* **(Figure 1A)** displayed differences in the colour of growth rings, highlighting olivaceous colours with hazelnut margins. Under darkness, a white colour of the whole colony predominated (mycelium). At the microscopic level, the characteristic ovoid shape but also cylindrical-like shapes were observed for this species irrespective of the light wavelength. Colonies of *M. fructicola* **(Figure 1B)** under white, blue, and red light wavelengths induced different hazel tones. In addition to the presence of concentric rings under white and blue conditions. In contrast, far-red and darkness lead to a grey colour. In the case of black condition, different olivaceous tones conforming the growth rings were observed. None of conditions induced changes in the conidia morphology.

Finally, *M. fructigena* **(Figure 1C)** showed a greater difference in its morphological characterization in relation to *M. laxa* and *M. fructicola*, predominantly displaying white and grey tones among all light wavelengths. Unlike the other species, *M. fructigena* is unable to produce conidia under the conditions tested in this study. Hence, at the microscope level, almost no conidia were visualized.

Conidiation was only quantitatively examined for *M. laxa* and *M. fructicola* **(Figure 2A**). Results among species demonstrated significant differences, producing *M. laxa* a greater conidiation compared to *M. fructicola*, except in far-red light wavelength. Regarding wavelengths, *M. laxa* produced the highest number of conidia when grown under white light (7.94 conidia mL-1) and the lowest in darkness conditions (7.04 conidia mL-1). In the case

of *M. fructicola*, far-red light wavelength induced the highest number of conidia (7.26 conidia mL-1) and white light was the lowest (6.45 conidia mL-1).

Concerning cell viability **(Figure 2B)**, results revealed that *M. laxa* had, in all the light conditions tested, a reduced viability compared to *M. fructicola* and *M. fructigena*. In contrast, when comparing the different light wavelengths for each species, no significant differences were observed for any of the three *Monilinia* spp.

The analysis of the growth rate data **(Table 1)** demonstrated that under our *in vitro* conditions, *M. laxa* grew significantly faster under far-red light wavelength, while the growth under blue light wavelength was reduced compared to the other conditions. In the case of *M. fructicola*, black light wavelength induced a faster growth if compared to red light. In contrast, the growth rate of *M. fructigena* did not show significant differences among conditions. Results among species for each light condition, denoted no significant differences for any of the light wavelengths analysed, except for red light wavelength, which caused a lesser growth rate in *M. fructicola* compared to the other species.

3.1.2. Effect of light wavelengths on the transcriptional pattern of developmental genes

A deeper study was carried out by means of a gene expression analysis of *Monilinia* spp. candidate genes with potential role in fungal development. A total of 13 genes belonging to several families were selected **(Suppl. Table S1)**.

The family of Regulators of G-protein Signaling (RGS) was selected since it is involved in the negative regulation of G-protein signaling to control developmental processes such as conidiation and appressorium formation **(Figure 3 and Suppl. Figure 1)**. Both regulator of *G-PROTEIN SIGNALING* 1 (*RGS1)* and *G-PROTEIN SIGNALING* 4 (*RGS4)* genes were more expressed in *M. fructicola* if compared to the other species, although no significant differences were obtained among light conditions.

For *G-PROTEIN SIGNALING* 2 (*RGS2)*, all light wavelengths showed significant differences for all *Monilinia* spp. For *M. laxa*, a significant overexpression was observed under black light wavelength if compared to the rest of conditions. In turn, in *M. fructicola* a significant up-regulation occurred under white (0.041 MNE) and black (0.037 MNE) conditions in relation to blue light wavelength (0.015 MNE). In contrast, *M. fructigena* presented a significantly lower expression of *RGS2* gene compared to *M. laxa* and *M. fructicola* for all the light conditions tested.

G-PROTEIN SIGNALING 3 (*RGS3)* gene showed a similar pattern to *RGS2* in both *M. laxa* and *M. fructigena*. However, *M. fructicola* displayed a lower expression level in all conditions, specially, under blue light wavelength.

For *RGS4*, no significant differences were observed between conditions for any of the species. However, expression levels were significantly higher in *M. fructicola* than in the other two species.

The expression of developmental regulatory genes corresponding to different subfamilies of Transcription Factors (TF) was also analysed. Among them, *REGULATOR 1* (*REG1)* gene from Gti1/Pac2 subfamily, *STE12* corresponding to Ste12 subfamily, and *C6 TRANSCRIPTION FACTOR (C6TF1)* and *ACTIVATING TRANSCRIPTION FACTOR* (*ATF1)* genes from bZIP subfamily were analysed **(Figure 4 and Suppl. Figure 1)**. The *REG1* gene showed differences among light conditions for each species. In the case of *M. laxa*, far-red light wavelength induced a significant increase compared to darkness (2.105-fold) and red (3.089-fold) conditions. This behaviour was also observed in *M. fructigena*, highlighting a significant increase under far-red light wavelength in related to the other conditions. In the case of *M. fructicola*, the red light wavelength induced the highest expression in relation to black and darkness conditions.

Regarding *STE12*, an overexpression in *M. fructicola* occurred under black (0.152 MNE) and red (0.146 MNE) if compared to blue light wavelength, while in *M. laxa* and *M. fructigena*, the expression levels of this gene remained low.

Lastly, bZIP subfamily comprising both *ATF1* and *C6TF1* genes, also showed a species-dependent expression pattern. *ATF1* expression in *M. laxa* was significantly induced after exposure to black and far-red light wavelengths if compared to the other conditions. In the case of *M. fructicola*, the greatest effects were reported after subjecting the fungus to white and black light. In contrast, *M. fructigena* only showed a significant increase under darkness compared to red, white, far-red and blue light wavelengths.

On the other hand, expression levels of *M. fructigena C6TF1* gene remained low and invariable regardless of the conditions. For *M. laxa*, expression levels significantly increased under far-red light wavelength (0.057 MNE) if compared to red light wavelength (0.028 MNE). In contrast, in *M. fructicola*, red light wavelength was the condition that induced the greatest and significant increase compared to white, darkness, far-red and blue conditions.

As for Light Transcription Factor (LTF) gene family, involved in the induction of conidiation and repression of sclerotia development, *LIGHT-RESPONSIVE TRANSCRIPTION FACTOR 1* (*LTF1)* and *LIGHT-RESPONSIVE TRANSCRIPTION FACTOR 2 ALPHA (LTF2α)* genes were also selected for study **(Figure 5 and Suppl. Figure 1)**. The expression levels of *LTF1* gene significantly changed among conditions for each *Monilinia* spp. In *M. laxa*, far-red light wavelength induced a significant overexpression in relation to the other conditions. In the case of *M. fructicola*, the black light wavelength significantly increased the expression levels if compared to the rest of conditions. For *M. fructigena*, only an overexpression of this gene was observed when exposed to darkness.

On the other hand, *LTF2α* was highly expressed in *M. fructicola* compared to the other species. For all species the highest expression was observed when the fungi were exposed to white light.

Finally, other genes described to be involved in functions related to conidiation and virulence in other pathogenic fungi, were also studied: *POLYPHENOL OXIDASE* (*PPOA)*, *OLIGOPEPTIDE TRANSPORTER 1 (OPT1)* and *SSP PROTEIN (SSP1)* **(Figure 6 and Suppl. Figure 1)**. Regarding *PPOA* gene, a significant increase occurred in *M. laxa* under black light wavelength (0.033 MNE) if compared to red (0.003 MNE) and blue (0.003 MNE) light wavelengths. In this line, *M. fructicola* also showed an overexpression of this gene when exposed to darkness, black and white conditions in relation to blue light wavelength. In general, low levels of expression were observed in *M. fructigena*.

Concerning the expression of *OPT1* gene in *M. laxa*, a significant increase induced by black light wavelength occurred. In the case of *M. fructicola*, blue and white light wavelengths induced a greater expression than the rest of conditions. On the other hand, in the case of *M. fructigena*, the expression levels remained low, being black, far-red and darkness the conditions inducing the greatest expression.

Finally, *SSP1* gene was remarkably induced by black light in *M. laxa* (0.796 MNE), by white wavelength in *M. fructicola* (2.586 MNE) and by darkness in the case of *M. fructigena* (0.120 MNE) if compared to the rest of light wavelengths for each species.

3.2. *In vivo* assays

3.2.1. Effect of light wavelengths on the virulence of *Monilinia* spp. on nectarines

The brown rot phenotype on 'Extreme 563' nectarines inoculated with *M. laxa* subjected to white, black, blue and red light **(Figure 7A**), showed olivaceous tones, while a whiter tone dominated when subjected to darkness and far-red light wavelengths. In *M. fructicola*, a coloration similar to *M. laxa* was observed, although displaying a more intense olivaceous

coloration in all conditions. Finally, in fruit inoculated with *M. fructigena* a white colour predominated, probably due to the scarce ability of this species to conidiate.

Brown rot incidence on nectarines infected with *M. laxa* subjected to black light wavelength **(Figure 7B)** revealed a significant reduction up to 30 % of disease incidence compared to darkness. When *M. laxa* was subjected to the rest of conditions also slightly reduced the incidence in relation to darkness, although without significant differences. In contrast, the brown rot caused by *M. fructicola* subjected to all light wavelengths showed a significantly higher percentage of incidence in relation to black and darkness conditions (35% and 40 % less, respectively). In the case of *M. fructigena,* no significant differences were observed among conditions. Results obtained from comparing species for each light condition (**Suppl. Table S4),** revealed that only *M. fructicola* presented a significant reduced incidence when grown under black light and darkness.

Regarding to the severity results **(Figure 7B),** when *M. laxa* was subjected to far-red light wavelength significantly increased the severity, if compared to the other conditions. In turn, *M. fructicola* subjected to far-red and red light showed the highest severity. In the same line, *M. fructigena* showed a significant reduction of severity when was subjected to red and darkness conditions. When comparing severity results at 3 days post-inoculation among species for each light wavelength **(Suppl. Table S5)**, significant differences were observed among species for all light conditions except for blue light. At 7 days post-inoculation **(Suppl. Table S6)**, *M. fructigena* displayed the highest severity when grown under darkness, black, white and blue conditions in relation to the other two species. However, no significant differences were obtained among species when exposed to far-red light.

Results for the incubation and latency periods **(Figure 7B)** corroborated the previous results. Thus said, brown rot symptoms firstly appeared in those nectarines inoculated with *M. laxa* subjected to far-red condition, while in *M. fructicola* all lights exhibited earlier brown rot

symptoms, compared to darkness. However, *M. fructigena* subjected to white and blue lights induced an earlier onset of the disease if compared to darkness condition. Regarding the latency period, the first symptoms of conidiation appeared in nectarines infected with *M. laxa* subjected to far-red, red and darkness, while *M. fructicola* grown under white and darkness delayed the appearance of conidiation on nectarines. In contrast, the presence of mycelium on nectarines firstly appeared when *M. fructigena* was subjected to blue and far-red light wavelengths. A similar virulence pattern was obtained for the second batch of 'Extreme 563' nectarines for all the parameters analysed (data not shown).

4. Discussion

Light is an important factor determining the fungal biology and involved in several physiological responses, metabolic processes, conidiation and circadian clock (Bannon et al., 2009; Canessa et al., 2013; Van Leeuwen and Van Kesteren, 1998). Some studies have been carried out on *M. laxa*, *M. fructicola* and *M. fructigena* under UV-A (black) light wavelength in *in vitro* conditions (De Cal and Melgarejo, 1999), different lighting treatments with *M. laxa* and *M. fructicola* in both *in vitro* conditions and nectarines (Balsells-Llauradó et al., 2021), and different combinations of photoperiod and light wavelengths for different strains of *M. laxa* in stone fruits (Rodríguez-Pires et al., 2021b). However, no studies aimed to analyse the effect of the different light wavelengths on the three main species of *Monilinia*.

4.1. *Monilinia* spp. display a different phenotype dependent on the light wavelengths

Previous studies on *B. cinerea* have shown a broad spectrum of action in response to light that covers the entire visible spectrum and beyond (Schumacher, 2017). Different phenotypes were also obtained in the study carried out with different strains of *M. laxa* by Rodríguez-Pires et al. (2021b), and the ability of this pathogen to detect light through photoreceptors Rodríguez-Pires et al. (2021a). Once sensed, the signal is rapidly generated and propagated to

374 stimulate cellular responses such as an induction of pigmentation (i.e. biosynthesis of
375 carotenoids, mycosporins (Fuller et al., 2015) and melanin (Rehnstrom and Free, 1996)),
376 either as protective molecules or acting in developmental functions. However, their specific
377 role on *Monilinia* spp. remains still elusive. Our results referring to the morphological
378 characterization showed a different colour colony among species under the different light
379 wavelengths, suggesting that *Monilinia* spp. responded differentially by accumulating
380 pigments (Villarino et al., 2011). Hence, studies aiming to determine the nature and function
381 of such accumulation are encouraged. In turn, different lines of evidence support that near-
382 UV light wavelength induces oxidative stress in fungal cells (Schumacher, 2017), causing an
383 alteration of homeostasis in ROS levels (Canessa et al., 2013), but also in the adjustment of
384 turgor of conidia and total virulence to infect stone fruits (Yu et al., 2020). In our results, *M.*
385 *laxa* presented ovoid conidia under darkness growing conditions, but both ovoid and
386 cylindrical conidia morphologies under light conditions. Although these morphologies are
387 common when grown under light (Balsells-Llauradó et al., 2021), the mechanism underlying
388 this dual phenotype has not been elucidated yet.
389 Light can markedly affect asexual reproduction of fungi and, therefore, an appropriate
390 response of fungi regarding conidiation to this abiotic factor can ensure the dispersion of the
391 species. Our results suggest the ability of *Monilinia* spp. to respond to light, express a
392 phenotype with higher conidial production at certain wavelengths if compared to darkness.
393 Accordingly, it has been shown that in *B. cinerea*, light wavelengths promote the production
394 of conidia, the development of sclerotia and the growth of mycelia. Interestingly, blue light
395 wavelength increased the conidia production of both *M. laxa* and *M. fructicola* **(Figure 2A)**.
396 In fact, blue light wavelength has also been shown to induce an increase in conidial production
397 and mycelial growth in *Alternaria* sp. (Kumagai, 1989). The reduced viability of *M. laxa*

compared to the other species **(Figure 2B)** could be in part explained by alterations in the morphology or turgor of *M. laxa* conidia as previously reported (Yu et al., 2020).

Finally, we observed that far-red and black light wavelengths increased the growth rate of *M. laxa* and *M. fructicola*, respectively, in line with that observed in *Aspergillus* spp., which presented an increased growth rate under far-red, UV-A (black) and near-UV light wavelengths (Aziz and Moussa, 1997; Cheong et al., 2016). Overall, in our studies observed that both *M. laxa* and *M. fructicola* grew phenotypically more influenced by light wavelengths than *M. fructigena*. Based on these results, is decided to analyse possible changes in the behaviour of genes related to development that have previously been cited in other fungi but not previously analysed in the three mains of *Monilinia* species by a gene expression analysis.

4.2. Development genes expression pattern is controlled by light wavelengths in a species-specific manner.

Gene expression analysis of development genes showed a wide range of responses depending on: i) the gene function, ii) *Monilinia* spp. and iii) light wavelength. RGS proteins play critical roles in modulating the heterotrimeric G protein signal transduction cascades that allow the pathogen to perceive external signals and elicit appropriate physiological and biochemical responses. Specifically, the role of *RGS1* and *RGS4* proteins has been demonstrated in cell wall integrity, surface hydrophobicity, squeeze formation, mycelial growth, sexual reproduction, conidiation and pathogenicity (Dohlman and Thorner, 1997; Zhang et al., 2011). In contrast, *RGS2* and *RGS3* genes have been demonstrated to have a negative effect on the regulation of these processes, and especially on conidia (Wang et al., 2013). The low level of expression of *RGS3* on *M. fructicola* **(Figure 3)**, together with high expression level of *RGS1* and *RGS4*, could explain the ability of this species to produce conidia, unlike *M. fructigena*, which showed levels of expression low.

Transcription factors are crucial controlling several fungal processes (John et al., 2021). Among all the TFs, the role of a putative transcriptional regulator belonging to the Gti / Pac2 family, *REG1* gene, was analysed taking into account its role in pathogenicity, resistance to osmotic stress, formation of conidia and metabolism (Michielse et al., 2011; Schumacher, 2017). Our results demonstrated an important expression level of *REG1* gene in *M. fructicola*, but specially in *M. laxa* **(Figure 4)**. Besides, the expression of *STE12* gene, was more induced under black and red light wavelengths in *M. fructicola*, pointing out to a change on the phenotypic response related to the colony pigmentation, probably due to an accumulation of melanin pigments. In agreement with our results, *STE12* has been associated with the formation of melanized appressoria in *Magnaporthe grisea* (Park et al., 2002) and with the pathogenicity of *Penicillium digitatum* in oranges (Vilanova et al., 2016). Other TFs studied in this work were *ATF1* and *C6TF1* genes, both belonging to bZIP family. These genes are involved in the formation of sclerotia that occurs in the absence of light, and also conidiation, that is induced by different types of light (Sang et al., 2019; Temme et al., 2012). In fact, the involvement of *ATF1* in pathogenicity comes from its role in coding for an important regulator of conidia dependent on sunlight (Temme et al., 2012). In turn, the virulence related role of *C6TF1* gene is dependent on light and originates from studies proving its implication in conidia formation and suppression of mycelium development (Sang et al., 2019). As describe before for other genes, *M. fructigena* also showed a low expression of both genes in all light conditions **(Figure 4)**. These results would explain its inability to form conidia. On the other side, the higher *ATF1* gene expression under black light wavelength in the case of *M. fructicola* and *M. laxa*, could partially explained the increased conidiation observed under these conditions.

Regarding the group of light-responsive TFs, *LTF1* gene has been already studied in *B. cinerea*, revealing its implication in growth in the presence of near-UV, and the production of

antioxidants required to deal with oxidative stress caused by either prolonged exposure to light or during host infections (Schumacher, 2017). Accordingly, this gene was highly expressed in *M. fructicola* after being exposed to black light wavelength **(Figure 5)**, probably in an aim to face the oxidative stress situation. In the case of *M. laxa*, black light wavelength also induced its expression although interestingly, far-red light wavelength largely increased its expression. Contrary to the other species, *M. fructigena* did not change its expression pattern in response to light **(Figure 5)**. According to assays carried out with *B. cinerea*, *LTF2α* gene is a positive regulator of conidiation and may also be involved in the suppression of sclerotial development under light (Schumacher et al., 2014). In addition, *LTF1* participates in the repression of *LTF2α* transcription under darkness (Schumacher et al., 2014). In this line, our results also demonstrated that in all *Monilinia* spp. the least expression of *LTF2α* gene occurred under darkness, while the highest expression was achieved after exposure to white condition. This fact explains why these fungi showed, in general, low conidiation under darkness **(Figure 5)**.

Several other genes are also important for development and/or virulence of fungi. However, they have never been characterized in neither *Monilinia* spp. nor under environmental factors such as the exposition to light wavelengths. Previous studies on *PPOA* gene revealed that it is involved in the production of oxylipins, directly affecting positively conidia development (Fischer and Keller, 2016; Tsitsigiannis et al., 2004). Accordingly, its expression pattern was also studied, and our results showed a higher expression in *M. fructicola*, and a low expression in *M. fructigena* **(Figure 6)**, mainly characterized by the presence of mycelium. *OPT1* gene has also been studied in *Colletotrichum gloeosporioides*, showing an increased production of conidia, pigmentation and a low mycelial growth in *in vitro* conditions (Chagué et al., 2009). Thus said, a large induction of *OPT1* gene was observed for *M. laxa* under black light wavelength, although not it did not correlate with a high conidiation. Regarding *SSP1* gene,

it was hardly expressed in *M. laxa*, except under black light wavelength **(Figure 6)**. A similar pattern occurred for *M. fructicola*, although the main effect was observed after exposing this species under white light condition. In the case of *M. fructigena*, the highest induction was shown under darkness. A transcriptional study carried out by Angelini et al. (2018) revealed that *SSP1* could help *M. fructicola* to survive under unfavourable conditions.

Based on the results obtained on the effect of light on the development of *Monilinia* spp. in *in vitro* conditions, was decided to evaluate how the incubation conditions of the fungus under different light conditions, affected its ability to produce brown rot in nectarines.

4.3. *Monilinia* spp. subject to light wavelengths differentially alters the capacity to infect stone fruits

Different light wavelengths have been shown to affect the growth, the metabolism, but also the behaviour of pathogenic fungi when infecting fruit (Schumacher, 2017; Xu et al., 2017; Zhang et al., 2021).

The incidence of brown rot caused by *Monilinia* spp. subjected to different light wavelengths, varied depending on both species and growth conditions. *Monilinia laxa* tended to show a reduction in the ability to infect fruit when grown under any light wavelengths compared to darkness **(Figure 7B)**. In fact, a recent study has shown that non-continuous light exposure of *P. digitatum* leads to a decreased ability to infect oranges compared to continuous darkness (Lafuente et al., 2018). However, in other *Monilinia* species light did not affect (*M. fructigena*) or even increased (*M. fructicola*) its ability to infection nectarines. On the other hand, a negative effect on the virulence of *M. laxa* and *M. fructicola* was observed after subjecting these species to black light. These results are in agreement with previous studies which demonstrated that black light had also an inhibitory effect on *B. cinerea*, reducing the development of the pathogen in tomato leaves (Tokuno et al., 2012; Xu et al., 2017).

Attending to the aggressiveness of the species, the far-red light wavelength largely increased the severity of *M. laxa*, in agreement to Rodríguez-Pires et al. (2021b).

4.4. The light-dependent virulence of *Monilinia* spp. could rely on the different modulation of specific genes

Based on the results obtained in this study we could hypothesize which is the condition that most affects the phenotype of *Monilinia* spp. and brown rot development on fruit **(Figure 8)**. In the case of *M. laxa* and *M. fructicola*, *REG1* and *C6TF1* genes showed high expression when grown under long wavelengths (red and far-red). The functions of these genes are related to processes of conidia production. This could explain that the highest conidia production of *M. fructicola* occurred under far-red light. Furthermore, when infecting fruit, an increase in the virulence was observed in both red and far-red light, while a reduced virulence occurred under black light for both *M. laxa* and *M. fructicola*. In the case of black wavelength, a high expression of genes related to pigmentation, *STE12* and *OPT1*, was obtained under *in vitro* conditions, as well as the production of conidia. This could explain the characteristic *in vitro* morphology of *M. fructicola* with growth rings delimited by different tonality. Furthermore, the highest growth rate in *M. fructicola* was under black light. However, for *M. laxa* was observed that both the growth rate and the conidia production were not as significant as in *M. fructicola*. Finally, in the case of *M. fructigena*, hardly any significant differences were observed between different wavelengths and darkness, probably promoted by greater growth in absence of light. Overall, this information indicates that *M. laxa* and *M. fructicola* showed a similar behaviour in relation to light wavelength factor, while *M. fructigena* was not affected by this factor, opting for darkness.

5. Conclusions

M. laxa and *M. fructicola* were clearly influenced by light, showing different phenotypes depending on the wavelength. These differences were also observed on gene expression

analysis of some developmental genes. Consequently, the reaction to light condition of each species can use to gain a better understanding of how environmental factors affect the overall fitness of these pathogens, for instance, the asexual reproduction (conidia) essential for their dispersion. Pathogens with high phenotypic plasticity, as *M. laxa* and *M. fructicola*, exhibit a wide range of morphological, physiological and molecular change in response to different light conditions to reduce this environmental stress and, therefore, could adapt quicker to each situation and cause disease.

Author statement

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Conflict of interests

546 The authors declare no conflict of interest

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