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Effects of Light Quality on Strawberry, *Botrytis cinerea*, and Their Interaction

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Invloed van lichtkwaliteit op aardbei, *Botrytis cinerea*, en hun interactie.

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This dissertation is dedicated to my family.

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List of Abbreviations and Symbols

ABA	abscisic acid
APX	ascorbate peroxidase
CCA1	circadian clock associated 1
ChlIdx	chlorophyll index
CRY	cryptochrome
DAMP	host damage-associated molecular
DASH	drosophila, Arabidopsis, synechocystis, homo
DS	defense syndrome
ELF	early flowering
ET	ethylene
FAD	flavin adenine dinucleotide
FKF	flavin-binding Kelch F-box
FMN	flavin mononucleotide
F_o	minimal fluorescence
Fv	variable fluorescence
F_m	maximal fluorescence
F_v/F_m	quantum efficiency of photosystem II
GA	gibberellins
GABA	γ-aminobutyric acid
GS/GOGAT	glutamine synthetase/glutamate synthase
HR	hypersensitive response
JA	jasmonic acid
LED	light-emitting diode
LKP2	LOV Kelch protein2
LOV	light, oxygen, voltage
LYM2	LysM domain-containing glycosylphosphatidylinositolan-chored protein 2
mAriIdx	modified anthocyanin reflection index
NIR	near infrared
NO	nitric oxide
NUV	near ultraviolet

OG	oligogalacturonide
PA	proanthocyanidin
PAL	phenylalanine ammonia-lyase
PAMP	pathogen-derived microbial-associated molecular pattern
PDA	potato dextrose agar
PDB	potato dextrose broth
PPF	photosynthetic photon flux
PHY	phytochrome
POD	peroxidase
PR	pathogenesis-related class
PRR	pattern recognition receptor
Pfr	active state of phytochrome
Pr	inactive state of phytochrome
RGB	red green blue
RLK1	receptor-like kinase1
ROS	reactive oxygen species
SA	salicylic acid
SAR	systemic acquired resistance
SAS	shade-avoidance syndrome
TCA	tricarboxylic acid
UV	ultraviolet
UVR8	UV resistance locus8
VOC	volatile organic compound
WCC	White Collar complex
ZTL	Zeitlupe
ρ	particle density

Summary

Strawberry *Fragaria* × *ananassa* (Duchesne ex. Rozier) is the economically most important soft fruit all around the world. Nowadays, strawberries are mainly produced in greenhouses. Strawberry yield is affected by various plant diseases. In the greenhouse, one of the most important diseases is grey mold caused by *Botrytis cinerea*, due to the incidental high humidity in this semi-closed environment. Disease control requires an extensive amount of fungicides, but the available number of commercial products is limited, the application of fungicides is expensive, fungicide efficiency differs between *Botrytis* isolates, and fungicide resistance is easily developed by the pathogen.

Since light is the most important environmental factor in a greenhouse production system, supplementary light is often applied to increase light intensity and to extend the day length leading to an all-year-round growing season to maximize strawberry yield. LEDs are the newest type of artificial lighting and are increasingly used in the greenhouse to promote plant growth and development during autumn and winter. In addition, LEDs with specific wavelengths (light quality) can also affect plant resistance against *B. cinerea*. But also a direct effect on this pathogen exists as *Botrytis* responds to different light conditions through its eleven photoreceptors. This opens possibilities to perform environmental-friendly disease control via the application of specific light wavelengths in the greenhouse. In this doctoral research, we explored the effects of light quality, mainly blue and red light, on strawberry, *B. cinerea* and their interaction.

Plant and leaf developmental age are important determining factors of the biotic stress responses as they affect parameters such as primary and secondary metabolites and antioxidative capacity. First, the effects of leaf age and light quality on leaf resistance to *Botrytis*, and on metabolites, antioxidative enzymes, and leaf pigments were studied. Leaf age affected leaf resistance. This resistance increased from 1-week-old to 4-week-old leaves and decreased again in 5-week-old leaves independent of light quality. Based on this variation in leaf resistance, two-week-old leaves with high susceptibility were selected for further research. Generally, red light pretreatment improved leaf resistance to *B. cinerea*, while blue light decreased leaf resistance. This improvement in leaf resistance was negatively correlated with hydrogen peroxide level, and positively correlated with levels of chlorophyll and carotenoids.

Though red light pretreatment increases leaf resistance to *B. cinerea*, crops that develop under monochromatic red light might display a dysfunctional photosynthesis. Light presence is important in the outcome of the plant-*Botrytis* interaction, and the circadian clock also has a role in the regulation of leaf susceptibility. Therefore, we studied the effects of light

presence, timing of light quality as well as timing of inoculation on leaf susceptibility to *B. cinerea*.

Light presence post-inoculation significantly decreased leaf susceptibility to *Botrytis*, irrespective of different light qualities or light conditions. The effects of 4 h of red/blue light followed with white light and 12 h of red/blue light followed by white light in a 16 h photoperiod on leaf susceptibility were compared. Four hours of red/blue light irradiation in the photoperiod did not alter leaf resistance, while 12 h blue or 12 h red light increased or decreased leaf susceptibility, respectively. This again showed the positive effect of red light on leaf resistance. Timing of inoculation revealed circadian variation in leaf susceptibility to *B. cinerea*. Susceptibility was highest in leaves inoculated in the evening (12 h after the start of photoperiod) followed by incubation in the dark. This coincided with the highest level of hydrogen peroxide, regulated by the circadian clock, at the moment of pathogen penetration (8 hours post-inoculation). When disease development took place under the light regime (12 h W + 4 h W or B or R/8 h Dark), the shorter the light period was after inoculation, the stronger the disease symptoms were, which again confirmed disease inhibition by the presence of light. The output of the circadian clock can be affected by environmental light signals. Twelve hours of red/blue light irradiation in the photoperiod resulted in the alteration of the circadian variation of leaf susceptibility, which was correlated with changes in leaf metabolites, such as hydrogen peroxide, total phenolics, and hexoses.

Botrytis cinerea, the fungal pathogen causing grey mold, displays a high degree of phenotypic diversity as well as variation in virulence. The last experimental chapter focused on the effects of light quality on the phenotypic variation of *B. cinerea* isolates. Virulence variation based on image analysis was assessed on strawberry leaves that had developed under white, blue, or red lights. *B. cinerea* differently responded to different light qualities in phenotypes such as mycelial growth, sporulation, and sclerotia formation. The *Botrytis* isolates showed significant variations in virulence, which was positively correlated to the production of oxalic acid by *B. cinerea in vitro*. Strawberry leaves that had grown under red light had improved resistance to all the tested *Botrytis* isolates, while blue light decreased leaf resistance to some isolates, again showing that red light improves leaf resistance to grey mold. Image analysis based on changes in chlorophyll fluorescence (F_v/F_m), chlorophyll index (ChlIdx), and modified anthocyanin content (mAriIdx) showed that disease development caused by different *B. cinerea* isolates was highly correlated with F_v/F_m. This indicator can be used for the early detection of grey mold on strawberry leaves.

In conclusion, this study clearly showed the high potential of red LED light in disease control of grey mold on strawberry leaves, irrespective of various *Botrytis* isolates. Red light

combined with short irradiation of white light in the photoperiod is beneficial not only for leaf resistance but also for normal plant growth. This study also provides more information about the phenotypic response of *Botrytis* isolates to different light conditions. Moreover, dark-adapted F_v/F_m is a useful indicator for early detection of grey mold based on image techniques.

Based on our findings, recommendations can be given to growers and scientific researchers. Red LED light is recommended for growers as the main lighting source in combination with white light to control *B. cinerea* disease in strawberry in greenhouse production. Leaf age and time of inoculation should be taken into account by researchers when doing assessments of plant disease severity. The use of young strawberry leaves is recommended owing to their high susceptibility. Studies on *Botrytis* should consider differences in sporulation capacity and virulence among isolates. For the development of grey mold early detection techniques, the F_v/F_m indicator is highly recommended.

Samenvatting

Aardbei *Fragaria x ananassa* (Duchesne ex. Rozier) is economisch het belangrijkste zacht fruit in de wereld. Teeltsystemen in kassen voorzien tegenwoordig in de grootste productie van aardbeien wereldwijd. Echter, de oogst van aardbeien wordt beïnvloed door verschillende plantenziekten. De meest belangrijke in kassen is vruchtrot, veroorzaakt door *Botrytis cinerea*, vanwege de hoge luchtvochtigheid in deze gesloten omgeving. Ziektebestrijding vereist een uitgebreide hoeveelheid fungiciden, maar het beschikbare aantal producten is gelimiteerd, toepassing van fungiciden is duur, fungicide efficiëntie verschilt per *Botrytis* isolaat en fungicide resistentie is makkelijk opgebouwd door het pathogeen. Omdat licht de belangrijkste omgevingsfactor is in een kas teeltsysteem, wordt assimilatiebelichting vaak toegepast om lichtintensiteit te verhogen, de daglengte alsook het groeiseizoen naar het hele jaar te verlengen om de aardbeioogst te maximaliseren. LED's worden tegenwoordig, als nieuwste type kunstmatig licht, in toenemende mate gebruikt in kassen, omdat ze effectief zijn in het bevorderen van plantengroei en -ontwikkeling. Daarnaast kunnen LED's met een specifieke golflengte (lichtkwaliteit) ook plantenresistentie tegen *B. cinerea* beïnvloeden. Maar, deze pathogeen reageert ook op verschillende lichtcondities door middel van zijn elf fotoreceptoren. Dit geeft de mogelijkheid om ziektebestrijding milieuvriendelijk uit te voeren via toepassing van specifieke golflengte in kassen. In dit doctoraatsonderzoek hebben we effecten van lichtkwaliteit, voornamelijk blauw en rood licht, verkend op aardbei, *B. cinerea* en hun interactie.

Plant en blad ontwikkelingsleeftijd is een belangrijke bepalende factor van biotische stressrespons door het beïnvloeden van parameters als primaire en secundaire metabolieten, en antioxidantcapaciteit. Ten eerste werden de effecten van bladleeftijd en lichtkwaliteit op bladresistentie, metabolieten, antioxiderende enzymen en bladpigmenten bestudeerd. Bladleeftijd beïnvloedde bladresistentie. Deze resistentie verhoogde van één tot vier weken oude bladeren en verlaagde weer in vijf weken oude bladeren, onafhankelijk van lichtkwaliteit. Gebaseerd op deze variatie in bladresistentie werden bladeren die twee weken oud waren met een hoge vatbaarheid geselecteerd voor verder onderzoek. In het algemeen, voorbehandeling met rood licht verbeterde de bladresistentie tegen *B. cinerea*, terwijl blauw licht de bladresistentie verminderde. Deze verbetering in bladresistentie was negatief gecorreleerd met het waterstofperoxideniveau, en positief gecorreleerd met de niveaus van chlorofyl en carotenoïden.

Hoewel voorbehandeling met rood licht bladresistentie verhoogde tegen *B. cinerea*, kunnen gewassen die ontwikkelen onder monochromatisch rood licht een disfunctionele fotosynthese tonen. Aanwezigheid van licht is belangrijk in de uitkomst van de plant-*Botrytis*

interactie, en de circadiaanse klok heeft ook een rol in de regulatie van vatbaarheid van het blad. Daarom hebben we de effecten van aanwezigheid van licht, timing van lichtkwaliteit alsook inoculatie op vatbaarheid van het blad voor *B. cinerea* bestudeerd.

Aanwezigheid van licht na inoculatie verlaagde de vatbaarheid van het blad voor *Botrytis* significant, onafhankelijk van lichtkwaliteit of lichtcombinaties. De effecten op bladvatbaarheid van 4 uur rood/blauw licht gevolgd door wit licht werden vergeleken met 12 uur rood/blauw licht gevolgd door wit licht in een 16 uur fotoperiode. Vier uur rood/blauw belichting in de fotoperiode veranderde de bladresistentie niet, terwijl 12 uur blauw of 12 uur rood licht de bladvatbaarheid verhoogde of verlaagde, respectievelijk. Dit toonde weer het positieve effect van rood licht op bladresistentie. Timing van inoculatie onthulde de circadiaanse variatie in bladvatbaarheid voor *B. cinerea*. Vatbaarheid was het hoogst in bladeren die ’s avonds geïnoculeerd werden (12 uur na de start van de fotoperiode), gevolgd door bladeren die in het donker geïnoculeerd werden. Dit viel samen met het hoogste niveau van waterstofperoxide, gereguleerd door de circadiaanse klok, op het moment van pathogeen penetratie (8 uur na inoculatie). Wanneer het ziekteverloop onder verschillende lichtregimes plaatsvond, bleek dat hoe korter de lichtperiode na inoculatie hoe sterker de ziektesymptomen waren, wat opnieuw remming van de ziekte door aanwezigheid van licht bevestigde. De output van de circadiaanse klok kan beïnvloed worden door lichtsignalen uit de omgeving. Twaalf uur van rood/blauw belichting in de fotoperiode resulteerde in een verandering van de circadiaanse variatie in bladvatbaarheid, wat gecorreleerd was met veranderingen in bladmetabolieten, zoals waterstofperoxide, fenolen en hexosen.

Botrytis cinerea, de schimmelpathogeen die vruchtrot veroorzaakt, toon een hoge mate van fenotypische diversiteit alsook variatie in virulentie. Het laatste experimentele hoofdstuk focuste op de effecten van lichtkwaliteit op fenotypische variatie van *B. cinerea* isolaten, en variatie in virulentie gebaseerd op beeldanalyse, werd beoordeeld van aarbeibladeren die zich hadden ontwikkeld onder wit, blauw of rood licht. *B. cinerea* reageerde verschillend op de verschillende lichtkwaliteiten in fenotypen zoals myceliale groei, sporulatie en sclerotia formatie. De *Botrytis* isolaten toonden significante variaties in virulentie, wat positief gecorreleerd was aan de productie van oxaalzuur van *B. cinerea in vitro*. Aardbeibladeren die onder rood licht gegroeid waren, hadden een verbeterde resistentie tegel alle geteste *Botrytis* isolaten, terwijl blauw licht bladresistentie verminderde tegen sommige isolaten, opnieuw tonend dat rood licht bladresistentie verbetert tegen vruchtrot. Beeldanalyse gebaseerd op veranderingen in chlorofylfluorescentie (F_v/F_m), chlorofylindex (ChIIdx), en aangepaste anthocyaaninhoud (mAriIdx), toonden dat het ziekteverloop veroorzaakt door verschillende *B. cinerea* isolaten sterk gecorreleerd met F_v/F_m. Deze indicator can gebruikt worden voor vroege detectie van vruchtrot op bladeren van de aardbei.

Tot slot, deze studie heeft duidelijk het grote potentieel van rood LED licht in ziektebestrijding van vruchtrot in aardbeibladeren aangetoond, onafhankelijk van verschillende *Botrytis* isolaten. Rood licht gecombineerd met korte bestraling van wit licht in de fotoperiode is voordelig niet alleen voor de bladresistentie maar ook voor normale plantengroei. Deze studie biedt ook meer informatie over de fenotypische respons van *Botrytis* isolaten op verschillende lichtcondities. Bovendien, F_v/F_m is een nuttige indicator voor vroege detectie van vruchtrot gebaseerd op beeldanalyse.

Op basis van onze bevindingen kunnen aanbevelingen gegeven worden aan telers en wetenschappelijke onderzoekers. Rood LED licht wordt aangeraden voor telers als de belangrijkste lichtbron in combinatie met wit licht om ziekte veroorzaakt door *B. cinerea* te controleren in aardbei in kasproductie. Er zou rekening gehouden moeten worden met bladleeftijd en moment van inoculatie door onderzoekers tijdens beoordelingen van ernst van de plantenziekte. Het gebruik van jonge bladeren wordt aangeraden vanwege hun hoge vatbaarheid. Studies naar *Botrytis* zouden verschillen in sporulatie capaciteit en virulentie tussen isolaten moeten meenemen. Voor de ontwikkeling van vroege detectie technieken voor vruchtrot wordt de F_v/F_m indicator sterk aangeraden.

Problem Statement and Thesis Outline

Strawberry, *Fragaria* × *ananassa* is the most economically important soft fruit around the world. The worldwide yield of strawberry ranks first within berry crops and its production increased from 4.5 million tonnes in 2000 to 8.3 million tonnes in 2018 worldwide (FAOSTAT, 2020). More than 70 countries produce strawberry and organic fruit production is becoming increasingly important (Wilbois et al., 2012). However, strawberry yield is mostly hampered by plant diseases, one of the most important being grey mold disease caused by *B. cinerea*. The control of this disease requires an extensive amount of fungicides, but fungicide applications are expensive and fungicide resistance is easily developed by the pathogen (Leroch et al., 2011, 2013; Leroux et al., 2010). In 2001 the total fungicide cost to control *Botrytis* was calculated to be *c.* €540 million, occupying 10% of the world's fungicide market (Dean et al., 2012). Moreover, many of these synthetic fungicides are being restricted because of their negative influence on the environment, and human and animal health (Petrasch et al., 2019). Therefore, the available number of products with active substances against fungal diseases has been steadily decreasing due to harsh regulations imposed by the government. On the other hand, using biocontrol agents to manage *Botrytis* has received much attention (Elad & Stewart, 2004; Elmer & Reglinski, 2006). However, effective commercial use of biocontrol agents is more problematic. Many studies reported that application of these biocontrol agents alone is not efficient in controlling strawberry grey mold in commercial horticulture (Boff et al., 2002; Gullino et al., 1989). Furthermore, the efficiency of biocontrol is often variable and inconsistent between studies.

Light is an important environmental factor that is perceived by both plants and pathogens through various photoreceptors and will affect the outcome of plant-pathogen interactions. Yet this interaction is hardly studied. Strawberry is increasingly produced in soilless production systems in greenhouses with the application of supplementary lighting during the winter months. More recently multilayer production in greenhouses (up to 4 layers) is investigated. In the lower layers, supplementary lighting with light-emitting diodes (LEDs) is necessary. Additionally, LEDs are already commercially used for extending the photoperiod thus optimizing flowering and fruit production in strawberry. Commercial LED lamps in greenhouse production typically combine blue and red wavelengths as these are highly absorbed by chlorophylls and thus promote photosynthesis and biomass production.

Given the increased use of LED lights in horticultural production, including strawberry, we need to gain insight into how light quality influences the strawberry-*Botrytis* interaction. In this thesis we focus on the two wavelengths that are dominantly applied in the LED lighting sources: red, blue and its combination.

The main objective of this thesis is to acquire a better understanding of the effects of blue and/or red wavelengths on the strawberry-*Botrytis* interaction.

Therefore we formulated following research questions:

RQ1: What is the effect of light quality on vegetative morphology and antioxidant properties of strawberry leaves?

RQ2: Does leaf age have an effect on *B. cinerea* resistance in strawberry?

RQ3: Which of these antioxidants are correlated with changes in leaf resistance under the influence of light quality?

RQ4: What is the effect of pre- and/or post-inoculation treatment with different light qualities and their combinations on the interaction of strawberry with various *B. cinerea* isolates? Is the circadian clock involved in these regulations?

RQ5: How does light quality affect phenotype and pathogenicity of different *B. cinerea* isolates?

RQ6: Is it possible to use image-based technology to detect *Botrytis* infection at an early stage in strawberry?

The layout of the thesis is as follows:

In **chapter 1** we introduce the photoreceptors in *B. cinerea* and plants, as well as their responses to different light qualities. We also give an introduction to grey mold disease caused by *B. cinerea* in greenhouse plants, as well as plant defense mechanisms against *B. cinerea*, with the main focus on strawberry.

Chapter 2 studies effects of leaf age and light quality (monochromatic blue and red and dichromatic blue+red) on leaf metabolism and changes in basal resistance to *B. cinerea*. Levels of hydrogen peroxide, antioxidants (phenolics and flavonoids), anti-oxidative enzymes (ascorbate peroxidase, peroxides), and leaf pigments were measured in 1-week-old to 5-week-old leaves. Furthermore, correlations between leaf resistance and leaf metabolite levels were explored to understand their roles in influencing leaf resistance.

Since light affects the outcome of plant-pathogen interactions, in **Chapter 3**, the impact of pre- and post-inoculation light quality on *Botrytis* disease development was investigated in strawberry leaves. The effect of inoculation time, four hours end-of-day and 12 h start-of-day light quality treatments on leaf resistance were studied. Furthermore, circadian variations in leaf metabolism developed under 12 h light quality radiation were measured to explain changes in susceptibility to *B. cinerea*.

B. cinerea is a fungal pathogen that displays a high degree of phenotypic diversity and responds to a broad light spectrum via eleven photoreceptors. **Chapter 4** studies light quality effects on phenotypes of fifteen *B. cinerea* isolates, including mycelial growth rate, sporulation, and sclerotia formation. Pathogenicity of *Botrytis* isolates was tested in strawberry leaves from plants grown under white, blue, and red LED lights. Furthermore, we used image analysis to quantify virulence of the different *Botrytis* isolates based on changes in the photosynthetic performance of the strawberry leaves: chlorophyll fluorescence (F_v/F_m), chlorophyll index (ChlIdx) and anthocyanin content (modified anthocyanin reflection index, mAriIdx).

Chapter 5 generally discusses the experimental chapters and gives some future perspectives.

Chapter 1

General Introduction

1.1 Light in greenhouse production

The greenhouse production system is nowadays a major cropping system supplying fresh soft fruit, vegetables, and ornamentals throughout the year all across the world. Most of the world's greenhouses are built between 25° and 65° latitude. At the higher latitudes periods of low solar irradiance and short days often limit crop production for a longer period. Hence, supplementary light is needed to extend the day length as well as to increase the daily light integral for a given crop thus ensuring a qualitative all-year-round production.

Light is a crucial environmental factor that drives plant growth, development and defense. Light is not only the energy source for photosynthesis but also acts as an external signal for regulating plant architecture, triggering developmental processes and influencing qualitative parameters of the horticultural product. So light control is increasingly used as an environment-friendly tool to manage horticultural crops produced in greenhouses. Artificial light sources have long been used in greenhouses to supplement natural light (Krizek et al., 1998). However, the traditional lighting sources such as fluorescent or high-pressure sodium lamps are neither spectrally optimal nor energetically efficient for many plant species. Furthermore, excessive heating when lamps are placed close to the plants may result in tissue damage (Dutta Gupta & Jatothu, 2013). With the evolving technology in lighting, new light sources such as LEDs have been developed and further improved over the years.

Now, LEDs are the newest type of artificial lighting being used in greenhouse production systems. These light sources have certain advantages over conventional light sources such as high-pressure sodium lamps. LED is a type of semiconductor diode that can easily be integrated into digital control systems. This allows the control of spectral composition and the adaptation of light intensity to match plant photoreceptors in order to optimize plant growth and to influence morphogenesis as well as different physiological processes over a course of plant developmental stages (Yeh & Chung, 2009). LED units can be placed close to plants, as they do not emit radiant heat (Dutta Gupta & Jatothu, 2013). Their long lifespan and high energy efficiency can reduce the electricity cost and investment in long-term operations in greenhouse industries (Singh, et al., 2015). Therefore, LEDs as a source of plant lighting is increasingly favored in greenhouse production by the producers.

LEDs emitting monochromatic light spectra or different spectral combinations have been used to study the effect of light on plant growth and development. It was shown that plants display a high degree of morphological and physiological plasticity when responding to different light qualities. The red/far-red and blue wavelengths of the light spectrum are widely used in vegetable and ornamental horticulture. Red/far-red light is responsible for branching, leaf elongation and orientation and can indirectly influence photosynthesis at the scale of the

whole plant (Demotes-Mainard et al., 2016). Blue light is of high importance for leaf expansion, petiole elongation, leaf orientation, pigment synthesis and stomatal opening (Huché-Thélier et al., 2016; Olle & Viršile, 2013). Supplemental LED lights based on blue and/or red light also increase transplant growth and quality in vegetable and forest nursery greenhouses (Landis, et al., 2013; Olle & Viršile, 2013). Moreover, the spectral quality of LED lights induces changes in primary and secondary plant metabolites leading to more resilience to diseases and pests (Meng, et al., 2019; Wang et al., 2010). Therefore, the use of LED technology for greenhouse horticulture is attractive and promising not only for plant growth and development but also for plant health.

Strawberry is a popular soft fruit crop worldwide and global strawberry consumption is on the rise. Strawberries can be grown in open field, under high tunnels covered by plastic, or in greenhouses. Nowadays, greenhouses provide the main production of strawberries around the world. In Belgium, greenhouse production is increasing year after year, from around 200 ha in 2000 to 545 ha in 2019 (STATBEL, 2020). In 2016, the greenhouse production of strawberry in the Netherlands exceeded the yield from open field for the first time, and in 2019 the production of strawberry under protection contributed to 68% of overall production (Centraal bureau voor de statistiek, 2018; 2020). In China, the biggest strawberry producer in the world, greenhouses contributed to almost 80% of strawberry production in 2009 (Mordini et al., 2009). Taken together, the introduction of the light-emitting diodes in greenhouses benefits the strawberry production during the winter months and also opens possibilities for innovation such as multi-layer production in greenhouses or strawberry production in plant factories. However, strawberry is susceptible to various pathogens. The grey mold fungus *B. cinerea* is considered one of the most important pathogens and causes impactful economical losses to the strawberry industry worldwide.

1.2 *Botrytis cinerea*, the cause of grey mold

1.2.1 Variability of *B. cinerea* isolates

Botrytis cinerea (Teleomorph *Botryotinia fuckeliana)* is a filamentous plant fungus with a necrotrophic lifecycle . The pathogen has a certain degree of host specialization and can cause grey mold on over 1000 plant species worldwide. *B. cinerea* isolates from different plants and regions display important variability in phenotype, pathogenicity and host range (Acosta Morel et al., 2019; Asadollahi et al., 2013; Corwin et al., 2016; Johnston et al., 2014; Karchani-Balma et al., 2008; Kuzmanovska et al., 2012; Mirzaei et al., 2009; Rasiukevičiūtė et al., 2018).

1.2.1.1 Phenotypic variability

Phenotypic variability is mainly attributed to differences in colony morphology, mycelial growth, intensity of sporulation, size of conidia and sclerotia formation.

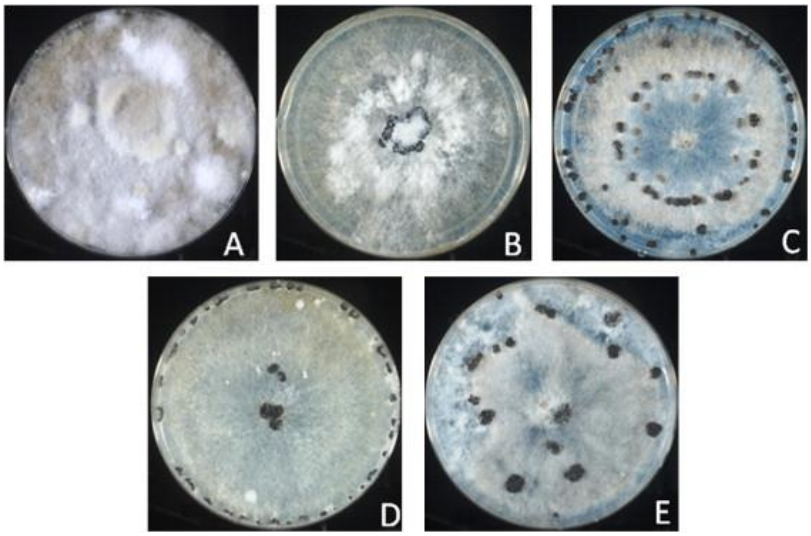


Figure 1.1 Patterns of sclerotia distribution in different isolates of *B. cinerea* (A) sclerotia absent; (B) centrally placed large sclerotia; (C) sclerotia arranged in concentric rings; (D) sclerotia arranged on the periphery; (E) sclerotia arranged irregularly (Kumari et al., 2014).

The colonies of *B. cinerea* are primarily grouped in two main classes: mycelial type and sclerotial type based on the absence/presence of sclerotia. The mycelial type can be further divided into four subclasses mainly according to mycelial morphology and includes short, aerial, mycelial masses, and thick and woolly mycelium. The sclerotial type is subgrouped based on the pattern of sclerotia distribution in Petri plates, such as centrally placed large sclerotia, sclerotia arranged in concentric rings or on the periphery, and sclerotia placed irregularly (Figure 1.1) (Kumari, et al., 2014; Kuzmanovska et al., 2012; Martinez et al., 2003; Mirzaei, et al., 2009). One hundred and eleven strains that were isolated from grapevine in France and one hundred and twenty-three *Botrytis* isolates that were collected from tomato in 7 different regions in the Republic of Macedonia were studied for their phenotypic diversity. Eight different morphological types were identified with four mycelial and four sclerotial classes in the isolates from France, while one more class was found in the tomato isolates, which was a transient type between mycelial and sclerotia morphological classes (Kuzmanovska et al., 2012; Fabian Martinez et al., 2003). Additionally, forty-four *B. cinerea* isolates from different hosts and regions in Iran were also grouped into the same eight different classes (Mirzaei, et al., 2009). Furthermore, Kuzmanovska et al. (2012) also noted morphological variations between *B. cinerea* strains from different regions in mycelium color (from dirty white to dark grey) and conidia size. Mycelial growth rate correlated negatively with the virulence of *B. cinerea* isolates from grapevine in France. Therefore, morphological

differences between isolates from different hosts and regions could be related to their aggressiveness and life-strategy such as the survival role of sclerotia in an unfavorable environment (Fabian Martinez et al., 2003).

1.2.1.2 Pathogenic variability

B. cinerea is a fungal pathogen that exhibits a high degree of pathogenic variability. Despite its wide host range, a certain degree of host specialization exists in this pathogen (Muñoz et al., 2002; Thompson & Latorre, 1999). For example, Cotoras and Silva (2005) reported that *B. cinerea* strains isolated from tomato were more virulent on tomato leaves than isolates from grapes. Furthermore, a study of 490 isolates from open-field crops based on microsatellite loci suggested the occurrence of host-specific divergence of *B. cinerea* in perennial hosts (Asadollahi et al., 2013).

Pathogenic variability results from variations in the production of a series of enzymes and phytotoxic factors by *Botrytis*. The production of various enzymes helps penetration into plant cells, including pectic enzymes, such as endopolygalacturonase, methylesterase, and pectin lyases. In *B. cinerea*, at least six endopolygalacturonase genes are found, and the expression of these genes during plant infection differs depending on plant tissues, plant species and incubation conditions, suggesting a certain degree of functional diversity (Ten Have et al., 2001). Movahedi and Heale (1990) revealed that the production of polygalacturonase, pectin lyases and pectin methylesterase varied between different *B. cinerea* isolates with respect to the time of production during the primary stages of infection. A study on 79 *B. cinerea* isolates showed variations in activities of these enzymes depending on the isolate (Kumari et al., 2014). However, no differences were observed by Reignault et al., (1994) in pectin methylesterase profile between 25 *B. cinerea* strains from different hosts, different geographical regions, and/or different years of isolation. The different profile of these enzymes in causing cell death leads to the variability of virulence in *B. cinerea* isolates.

Oxalic acid, as a potential virulence factor, is synthesized and secreted by *B. cinerea* in large amounts into host tissue to stimulate the infection. High levels of oxalic acid production by *B. cinerea* are correlated with enhanced pathogen invasion, while lower oxalic acid levels are associated with lower virulence (Kumari et al., 2014; Sun et al., 2019). Therefore, the extensive deviations in virulence between *B. cinerea* isolates are the consequence of differences in secretion of virulence factors, such as cell wall invasive enzymes, oxalic acid, toxins, reactive oxygen species and so on (Nakajima & Akutsu, 2014).

1.2.1.3 Genetic variability

B. cinerea is known as a pathogen that has a very high degree of genetic variability. The study of its genetic diversity is based on various molecular tools, including PCR detection of transposable elements (Martinez et al., 2008), RFLP analysis of PCR-amplified loci (Baraldi et al., 2002; Muñoz et al., 2002), PCR amplification of microsatellite loci (Asadollahi et al., 2013; Isenegger et al., 2008), and randomly amplified polymorphic DNA (RAPD) analysis (Alfonso et al., 2000; Pande et al., 2010). Molecular markers have elucidated the considerable variation in DNA content per nucleus, high gene flow and recombinations among *B. cinerea* populations from different geographical regions as well as different host plants (Büttner et al., 1994; Kumari et al., 2014; Kuzmanovska et al., 2012; Mirzaei et al., 2009). This can explain the genetic diversity and phenotypic instability in most isolates of this pathogen.

On the other hand, sexual reproduction and meiotic recombination may be a potentially significant source of genetic variation in this pathogenic fungus. Sexual bodies formed after fertilization of sclerotia of one isolate with the microconidia (spermatia) from another isolate, generate new genotypes, and contribute to the genetic diversity and evolutionary potential (McDonald & Linde, 2002). Its complexity, variability and adaptive potential hamper disease management based on fungicide and host resistance (Muñoz et al., 2002).

1.2.2 Responses of *B. cinerea* to light quality

B. cinerea responds to near-UV/blue (350-500 nm), green (around 540 nm), red and far-red light (650–780 nm) by using eleven photoreceptors (Figure 1.2). The photoreceptors in this fungus are composed of an apoprotein and a chromophore that binds with different domains. Upon the light signals sensed by the photoreceptors, *B. cinerea* makes decisions for its growth, survival and protection (Schumacher, 2017).

1.2.2.1 Near-UV/blue light photoreceptors and opsins

In *B. cinerea*, near-UV light and blue light are absorbed by cryptochromes (CRY)/photolyases and LOV (light, oxygen, voltage) domain-containing proteins. CRYs are proteins with similarity to photolyases (PLs) and both of them exhibit similar domain structures, i.e. amino-terminal PHR (PL-related) and carboxy-terminal FAD-binding domains. In total, six proteins have been identified in *B. cinerea*, including BcCry 1 and 2 for near-UV light sensing, and BcWcl1, BcVvd1, BcLov3 and BcLov4 for blue light absorption. Generally, near-UV light stimulates conidiation in *B. cinerea*, whereas blue light represses both spore and sclerotia production (Tan & Epton, 1973). Hence, near-UV light is used to induce sporulation in laboratory conditions, and blue light is investigated for its potential to inhibit

disease development caused by *B. cinerea*. For example, the development of grey mold was significantly inhibited by blue light irradiance in lettuce (Kook et al., 2013). Moreover, blue LED light suppressed mycelial growth of a *B. cinerea* isolate from a tomato plant in China, and this led to a slight (3.6%) inhibition of tomato lesion development in comparison to the dark control (Xu et al., 2017). However, high disease suppression by blue-LED was observed on tomato leaves caused by a *B. cinerea* strain collected in South Korea (Kim et al., 2013).

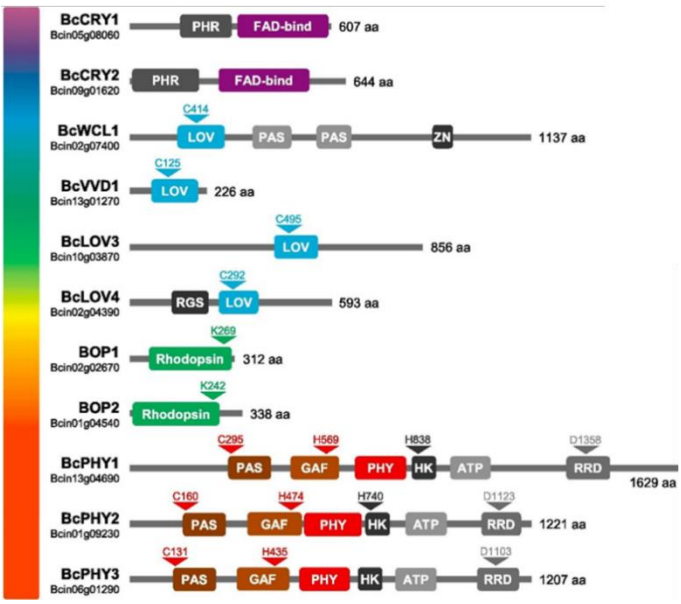


Figure 1.2 The eleven potential photoreceptors in *B. cinerea*. GeneIDs refer to the revised *B. cinerea* B05.10 genome sequence (Van Kan et al., 2017). Conserved domains are: PHR (DNA photolyase); FAD-binding; LOV (light-oxygen-voltage); PAS (Per-Arnt-Sim); ZN (GATA-type zinc finger); RGS (regulator of G protein signaling); Rhodopsin (Bacteriorhodopsin-like); GAF (cGMP-specific phosphodiesterases, Adenylyl cyclases and FhlA); PHY (phytochrome region); HK (His Kinase A phospho-acceptor); ATP (Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase); RRD (response regulator receiver). Conserved residues involved in chromophore binding are indicated in color, those required for HK activity in PHYs in gray (Schumacher, 2017).

On the other hand, germ tube growth of *B. cinerea* exhibits negative tropism under near-UV. Under blue light, a further slight reduction was observed. A study on onion and broad bean epidermal strips showed negative phototropism caused by near-UV and blue light which contributed to the increased number of penetrating germ tubes from *B. cinerea* spores (Islam et al., 1998).

Rhodopsins are transmembrane proteins that use retinal to sense green light. *B. cinerea* produces two opsins BOP1 and BOP2, which are responsible for the inhibition of mycelial growth and conidial germination rate caused by green light (Schumacher, 2017).

1.2.2.2 Phytochromes

Phytochromes (PHY) are histidine kinases using bilin to sense red/far-red light and three phytochromes (BcPHY1, BcPHY2, and BcPHY3) exist in *B. cinerea*. Sclerotia production is promoted by both red and far-red lights. Conidiation is slightly stimulated by red and far-red light (Tan & Epton, 1973). The inhibition of sporulation by blue light can be overcome by far-red light, and this effect of far-red is nullified by further exposure to red and blue light. Based on these observations, a two-receptor model has been postulated. In this model, the red/far-red absorbing photoreceptor interacts closely with the near-UV/blue reversible photoreceptor (Tan, 1975). Although no near-UV/blue light-reversible phytochromes have been found in a genome study of *B. cinerea*, red light and blue light-sensing systems are interdependent as the expression of PHY-encoding genes depends on the blue-light photoreceptor (Canessa et al., 2013). Additionally, red light promotes the growth of germ tubes, and induces positive phototropism in *B. cinerea*, while far-red light inhibits elongation and causes negative phototropism. Therefore, red light decreases the number of penetrating germ tubes on onion and broad bean epidermal cells, resulting in a large number of germ tubes merely growing across the surface of plant tissue (Islam et al., 1998).

1.2.2.3 General photomorphogenesis

In general, the presence or absence of light induces different responses in *B. cinerea*. In this fungus, sporulation is triggered in the light, but short darkness is also important for proper conidiation, as more conidia are formed on medium plates when they are submitted to light-dark cycles compared to constant light (Tan & Epton, 1973). Sclerotia formation for survival exclusively occurs in cultures in complete darkness, but this can be affected by the light dosages. Fewer sclerotia were formed when irradiated for 30 min compared to 15 min, and none were observed when irradiated for more than 60 min. Furthermore, sclerotia formation is promoted by red and far-red light (Tan & Epton, 1973). However, blind *B. cinerea* strains were observed showing the same phenotypes in both light and darkness, they always produce mycelia, or sclerotia, or conidia irrespective of the surrounding light environment (Canessa et al., 2013). For example, a nonpathogenic mutant A336, obtained via insertional mutagenesis, always gives conidia, independent of light or darkness (Kunz et al., 2006). Thus, *B. cinerea* can be classified into light-responsive strains and blind strains. Canessa et al. (2013) studied 72 wild *Botrytis* strains isolated from different hosts, 79% of the strains formed conidia and sclerotia in a light-dependent fashion, 15% of isolates were blind ones. Therefore, both light-responsive and blind strains are present in nature (Canessa et al., 2013).

1.2.3 Grey mold symptoms caused by *B. cinerea*

1.2.3.1 *B. cinerea* disease in greenhouse crops

B. cinerea causes disease in almost all major crops produced under protected cultivation, such as lettuce, sweet basil, tomato, cucumber, strawberry, kiwifruit, rose, gerbera, and most flowering potted plants. The symptoms of grey mold caused by *B. cinerea* are very different in greenhouse crops in comparison to crops in open field and orchards, as the disease development is more predictable and less weather-dependent in greenhouses. Greenhouse production can protect the crops from bad weather, such as rain, wind, and cold though here high humidity enhances the risk of *Botrytis* infection.

B. cinerea in greenhouse crops occurs in different organs and at different stages depending on the plant. In vegetables, this fungus may infect leaves, stems, and fruits. In sweet basil, *B. cinerea* usually starts its infection through stem wounds caused by harvesting and then develops on stems, finally killing leaves and new buds until plant death (Sharabani et al., 1999). However, in lettuce plants, *B. cinerea* usually grows as an endophyte, this is often symptomless and may arise from seed infection (Sowley et al., 2010). In tomato, this pathogen develops infection on flowers, fruits, leaves, and stems in a non-heated greenhouse, while the infection is limited to stems when grown in a heated greenhouse. This stem infection can also lead to plant death by girdling the plant (Jarvis, 1989; Shtienberg et al., 1998). *B. cinerea* is an important disease in strawberry, which will be introduced later. In cut flowers such as rose and gerbera, grey mold and botrytis blight are very damaging diseases, as the fungus causes lesions on petals thus greatly reducing the market value of the flowers (Dik & Wubben, 2007). In potted plants such as *Pelargonium*, the susceptibility of flowers rapidly increases with age after anthesis (Sirjusingh & Sutton, 1996).

B. cinerea disease in greenhouse crops is influenced by several factors, including greenhouse climate, light, carbon dioxide enrichment, sanitation, cultivar, plant spacing, cropping methods, fertilizer, and irrigation. Amongst these factors, the greenhouse climate and lighting system are the most important. Temperature and relative humidity both have a direct outcome on *B. cinerea* epidemics, and temperature greatly influences relative humidity and leaf wetness in the greenhouse. Infection is most successfully at 15-20 ˚C with free water or relative humidity above 90% for at least 4 h (Carisse, 2016). Water droplets on the surface of plants or high humidity enable nutrient leaching from the conidia by water, this benefits the germination of conidia (Brodie & Blakeman, 1977). Light not only affects the temperature and relative humidity, but also has direct effects on this pathogen (see 1.2.2). Finally, the host plants also affect the virulence of the pathogen (Dik & Wubben, 2007).

1.2.3.2 Grey mold in strawberry

B. cinerea is a very important disease-causing fungal pathogen in strawberry. It causes grey mold in senescing flowers and on fruit at different stages, but it also can infect vegetative tissues, such as leaves and petioles (Figure 1.3). It is considered the primary pathogen for strawberry production that leads to serious yield reduction and postharvest losses.

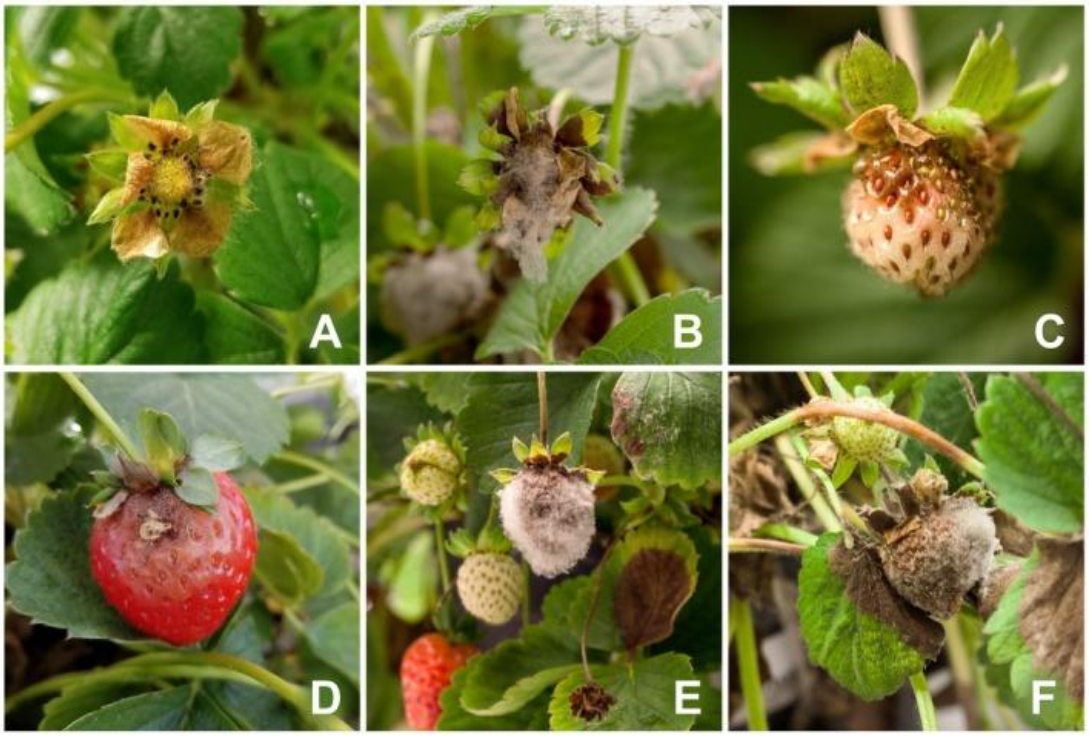


Figure 1.3 Infections of *B. cinerea* in strawberry. A: a senesced flower with *B. cinerea* mycelium growth; B: an advanced floral infection; C: infection of fruit at an early stage; D: infection of fruit at mature stage; E and F: *B. cinerea* infection on leaves (Petrasch et al., 2019).

B. cinerea enters the host via wounds or natural openings, but it is also able to penetrate directly into host tissue by appressoria (Holz, et al., 2007; Salinas & Verhoeff, 1995). Grey mold in strawberry can arise from the primary infection of open flowers or from secondary infections by penetrating fruit receptacle tissues (Bristow et al., 1986).

In primary infection, flower organs such as petals, stamens, and calyxes are getting infected by the pathogen during or right after flowering (Figure 1.4). The sources of inoculum for primary infection come from overwintering sclerotia to conidia or mycelium from neighboring diseased plants (Jarvis, 1962). The infected flower organs allow *B. cinerea* mycelium to develop into the receptacle, thus facilitating the primary infection in fruit. Following this primary infection in unripe receptacle fruit, *B. cinerea* mycelial growth is usually retarded and a symptomless quiescent phase occurs. This quiescence in unripe fruit is proposed to be caused by several reasons, such as lack of nutrients from the host, presence of antifungal

compounds, and an unsuitable environment for the development of infections (Prusky & Lichter, 2007).

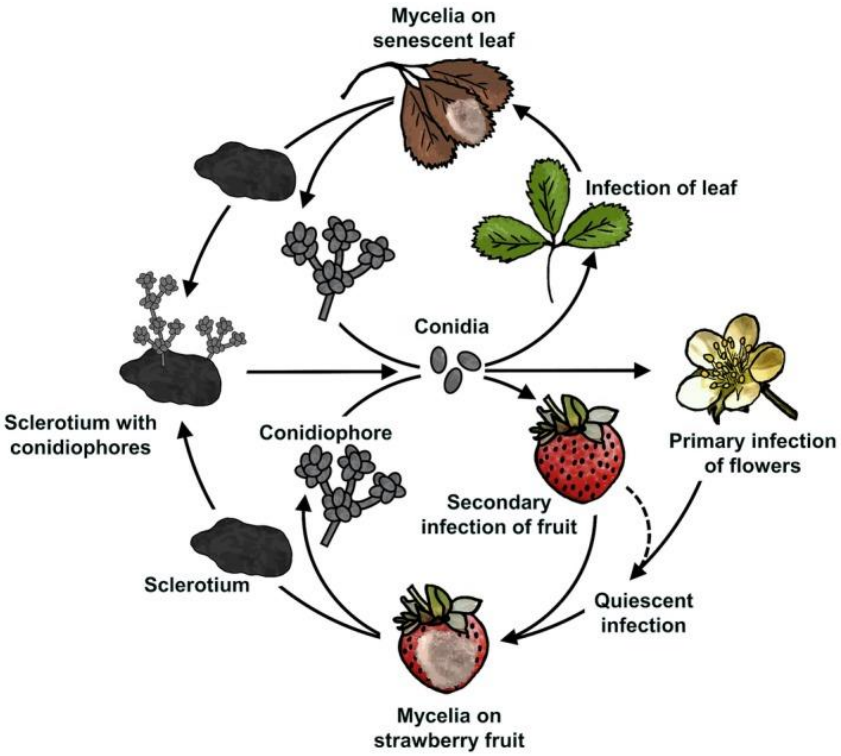


Figure 1.4 *Botrytis cinerea* disease cycle in strawberry (Petrasch et al., 2019).

In secondary infections, *B. cinerea* initiates its necrotrophic infection without latency (Holz et al., 2007). The sources of spores for these infections range from *B. cinerea*-infected leaves to infected fruit or from infected flower parts (Figure 1.4). The infected flower organs are the main source for secondary infection, as more than 64% of the strawberry infections result from their contact with fruit. Senescent flower parts often contact with strawberries long enough to create a humid environment for at least 8 h, which gives the needed time for conidia germination (Jarvis, 1962). Secondary infections can also arise from nesting, which corresponds to direct penetration by mycelium growing from infected leaves and fruits of neighboring plants (Braun & Sutton, 1988). Generally, secondary infections develop quickly in fruit and the pathogen can finish its germination and invasion as fast as 16 h after inoculation (Mehli et al., 2005).

1.2.3.3 Disease management for *B. cinerea* in strawberry

Many disease management methods have been applied for grey mold control in strawberries, such as cultural practices, chemical and biological control, and resistance breeding (Petrasch et al., 2019).

Several horticultural practices have historically been used to prevent *B. cinerea* infection in strawberry production, such as removal of senescent plant parts to avoid inoculum production, and non-contacting of fruit with soil (Daugaard, 1999). Drip irrigation and micro-sprinkler irrigation systems have been selected to limit the incidence of grey mold by reducing inoculum spreading and water film formation on the fruit (Dara et al, 2016; Terry et al., 2007). These cultural practices are necessary to limit *B. cinerea* infection in strawberry before harvesting. In modern production systems, fungicide implementations are the most common method to control *B. cinerea*. The frequency and timing of fungicide applications are important for grey mold control. Due to multi-resistance of different *B. cinerea* isolates to fungicides, rotation and combination of different fungicides with different modes of action or testing local isolate resistance are recommended for disease control (Hahn, 2014; Wedge et al., 2007). To date, biological products for *B. cinerea* control are applied, but their use is limited in commercial strawberry production due to their poor applicability in the field or the supply chain as well as high cost in comparison to conventional grey mold control (Pertot et al., 2017). On the other hand, improving strawberry resistance against *B. cinerea* is the best way for disease control. However, complete resistance genotypes of *F.* × *ananassa* have not been observed, nor cultivars from breeding programs. Currently, genetic techniques such as trans- or cis-genesis are used to modify strawberry cultivars. Several studies showed these approaches greatly improved strawberry tolerance to grey mold, however, no genetically modified strawberry cultivars are grown yet for commercial production (Bestfleisch et al., 2015; Petrasch et al., 2019). In greenhouse production, heating and fan ventilation, and opening of the greenhouse side vents are usually used to dry the foliage and reduce the leaf wetness, thus reducing *Botrytis* incidence. Additionally, red LED light has been reported to improve leaf basal resistance to *B. cinerea* in strawberry, which can be a promising method for disease control in the greenhouse (Meng et al., 2019, see Chapter 2, 3 and 4). In summary, although combined management strategies cannot completely prevent *B. cinerea* in strawberry (Feliziani & Romanazzi, 2016), novel breeding approaches and new lighting technology (LEDs) can be helpful to achieve maximum control of grey mold.

1.3 Plant defense mechanisms to *B. cinerea*

1.3.1 *B. cinerea* infection strategies

Botrytis cinerea, the causal agent of grey mold disease is a ubiquitous filamentous, heterothallic fungal pathogen infecting a wide range of plant species. The pathogen uses modified hyphae as infection structures and can infect all aboveground host organs, causing enormous damage both during plant growth and in the post-harvest phase. Infection of host tissue can be achieved by active penetration or by passive ingresses, such as infection sites

previously infected by other pathogens and infection via open stomata. However, *B. cinerea* is perfectly able to penetrate host surfaces. Before penetration, attachment of conidia on the host surface is firstly required. Following attachment, the conidia germinate and produce germ tubes on the host surface under moist conditions followed by the formation of appressoria. The appressoria produce a high turgor pressure to support the penetration process (Howard et al., 1991). During this penetration, *B. cinerea* enters the host organ by secreting lytic enzymes and phytotoxins in the host plant (van Kan, 2006). Consequently, the accumulation of reactive oxygen species (ROS) occurs at the host-pathogen interface, leading to plant cell death (Tenberge et al., 2002). Both the pathogen as well as host enzymes contribute to the oxidative burst (Mellersh et al., 2002; Tian et al., 2013). Upon the death of an invaded cell, primary necrotic lesions are initially established and the host defense is simultaneously activated in host plants.

1.3.2 Mechanisms of plant defense against *B. cinerea*

The entry of *B. cinerea* to the host occurs by penetration directly through the cell wall, or invasion through natural openings. The first layer of defense or pre-formed defense depends on physical and chemical barriers. Physical barriers include wax layers, hair, epidermal cell walls, and stomata. Chemical barriers comprise a broad range of secondary metabolites, phytoanticipins, and antimicrobial proteins or peptides. The efficiency of these physical and chemical barriers to a particular microbial attacker, as well as the speed and extent at which they are established in inducible reactions, is believed to determine whether a plant becomes infected by the potential pathogen (Ashry & Mohamed, 2011; Bennett & Wallsgrove, 1994; Pusztahelyi et al., 2015). Therefore, the quality of these barriers plays an important role in inhibiting the infection at the initiating stage. The wax and hair help indirectly in resistance to penetration. The abscisic acid (ABA)-deficient tomato mutant *sitiens* exhibits a decrease in leaf trichome number which was associated with higher cuticle permeability, and a significant negative correlation was observed between permeability and susceptibility in *sitiens*, thus higher cuticle permeability resulted in higher resistance to *B. cinerea* (Curvers et al., 2010). The thickness or toughness of cell walls may directly delay the entrance of the pathogen (Akai, 2012). In addition, phenolics, as secondary metabolites, can function as antimicrobial compounds that directly inhibit spore germination and mycelial growth. In kiwifruit cultivar *Actinidia chinensis* “Hort 16A”, higher constitutive concentrations of phenolics contributed to the higher basal resistance against *B. cinerea* (Wurms et al., 2003b). The pathogen that overcomes these defensive barriers activates plant inducible defense responses.

Plants activate various defense mechanisms against *B. cinerea* infection by recognizing pathogen-derived microbial-associated molecular patterns (PAMPs) and host damage-

associated molecular patterns (DAMPs) (Newman et al., 2013). PAMPs/DAMPs bound by plant innate pattern recognition receptors (PRRs) serve as early warning signals to activate the inducible immune system in plants. Chitin, the major constituent of the fungal cell wall act as a PAMP that is recognized by host plasma membrane receptors RLK1 (receptor-like kinase1) and LYM2 (LysM domain-containing glycosylphosphatidylinositolan-chored protein 2) (Faulkner et al., 2013; Miya et al., 2007). DAMPs are endogenous elicitors that are released from plant tissue during pathogen infection or under abiotic stress (De Lorenzo et al., 2011). Oligogalacturonides (OGs), pectin fragments released from the plant cell wall by fungal enzymes, function as DAMPs and contribute to a plant defense against *B. cinerea* (Ferrari et al., 2013).

Following the recognition of PAMPs/DAMPs, plants can respond with a series of defenses to inhibit pathogen growth. These responses include both physical changes and biochemical responses. The physical responses consist of cell wall thickening, callose deposition, or formation of cork layers, biochemical responses comprise production of ROS, and activation of salicylic acid (SA) and jasmonate (JA)-dependent signaling pathways (Chisholm et al., 2006; Jones & Dangl, 2006). For example, hydrogen peroxide (H_2O_2) as reactive oxygen species plays an important role in plant resistance to *B. cinerea*. In plants, H_2O_2 has two sides in regulating defense reaction (Chamnongpol et al., 1998; Govrin & Levine, 2000). Generally, H_2O_2 is involved in the stimulation of several defense reactions, such as induction of defense genes, phytoalexin synthesis, and hypersensitive response (Chen, et al., 1993). In tomato, accumulation of H_2O_2 at the early stage is linked with resistance to *B. cinerea* in the ABA-deficient tomato mutant *sitiens* (Asselbergh et al., 2007). Specifically, upon *Botrytis* infection, accumulation of ROS in epidermal cells triggered the epidermal hypersensitive response (HR)-mediated defense response and cell wall fortification via the phenylpropanoid pathway. Concurrently, in the mesophyll cells, the tricarboxylic acid (TCA) cycle is constantly replenished by overactivation of the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle and the γ-aminobutyric acid (GABA) shunt, thus controlling the defense-associated HR and slowing down the infection-induced senescence (Seifi et al., 2013). On the contrary, H_2O_2 accumulation is involved in successful infection by *Botrytis* at later stages of the infection. In Arabidopsis, H_2O_2 is positively correlated with necrosis produced by *B. cinerea* (Govrin & Levine, 2000). Increased H_2O_2 levels by salicylic acid pretreatment or H_2O_2 pretreatment increased leaf susceptibility to *Botrytis* in broad beans (Khanam et al., 2005). In secondary metabolism, phenolics can contribute to cell wall thickening to physically protect the plant against pathogen attack (Chérif et al., 2007). Increased production of phenolic compounds is one of the best characterized responses to different stresses, and higher content of phenolics leads to a higher resistance to *B. cinerea* (Wurms et al., 2003). The

hormones such as SA, JA and ethylene (ET) have been known to play vital roles in defense against *B. cinerea*. For instance, the effect of SA in immune responses is plant-species dependent. SA application significantly improved the resistance to *B. cinerea* in tomato leaves (Angulo et al., 2015), while accumulation of SA in *Arabidopsis bik1* mutant was shown to have a positive correlation with susceptibility to *B. cinerea* (Veronese et al., 2006). JA signaling pathway has positive effects in inducing plant resistance against *B. cinerea*. Arabidopsis mutants with enhanced endogenous JA levels displayed enhanced resistance to *B. cinerea* (Bonaventure et al. 2007; Coego et al., 2005). In contrast, JA-knockout mutants with decreased JA levels showed increased susceptibility to *Botrytis* species (Thomma et al., 1998). ET signaling pathway is an essential component in plant defense against necrotrophic pathogens, which can be triggered by pathogen invasion or PAMP recognition (Shakeel et al., 2013; Zhu et al., 2011). In *Arabidopsis*, mutations conferring ethylene insensitivity, such as *ethylene insensitive* (*ein2* and *ein3*) and *ethylene receptor1* (*etr1*), led to an alteration in ET signaling and resulted in enhanced susceptibility to *B. cinerea*. While, overexpression of *ethylene response factor* (*ERF1*) increases resistance to the pathogen infection (Berrocal-lobo et al., 2002; Ferrari et al., 2003). Generally, the hormone signaling pathway does not respond to pathogen invasions alone, crosstalk between different hormone signaling pathways are necessary to fine-tune plant defenses (AbuQamar et al., 2017).

1.3.3 Defense mechanisms to *B. cinerea* in strawberry

In strawberry, information about defense mechanisms against *B. cinerea* is mostly obtained from flowers and fruits, the information on leaf infection is limited, hence this part is mainly based on the results from flowers and fruits. The defense mechanism can be divided into passive (preformed) and active (induced) defenses. A schematic overview of known strawberry defense mechanisms is shown in Figure 1.5. When challenged by a *B. cinerea* infection, cuticle and cell wall as physical barriers play important roles in responding to the pathogen. A clear relationship between skin strength or fruit firmness and susceptibility to pathogen infection was reported by Barritt (1980) and Gooding (1976). For chemical barriers, Terry et al. (2004) reported that crude extracts of strawberry flowers at post-anthesis, especially the achenes, showed greater antifungal activity than the white bud and full bloom stages, and confirmed that antifungal compounds, many of which are phenolic compounds, are inhibitory to *B. cinerea*. Several studies have also found a positive correlation between the concentration of proanthocyanidins (PAs) and resistance to *B. cinerea*. Hébert et al. (2001, 2002) observed that strawberry cultivars with higher levels of PAs, which are mainly free and bound catechin and epicatechin, were more resistant to *Botrytis* infection. Induced defenses include cell wall reinforcement, accumulation of secondary metabolites, phytohormones production, oxidative burst, and so on. For example, the surroundings of

strawberry infection sites display a higher concentration of proanthocyanins, which possibly function to restrict fungal growth (Feucht et al., 1992; Jersch et al., 2018). Strawberries generally accumulate ROS around *Botrytis* infection sites, which can serve as an effective defense against the pathogen but also can lead to cell death and benefit necrotrophic fungi (Prusky & Lichter, 2007; Tomas-Grau et al., 2018). It was reported that strawberry produces proteins with antifungal activities, and antimicrobial products, such as diterpenoid phytoalexins, upon pathogen infection. For instance, overexpression of an *F.* × *ananassa* gene (*FaPE1*) that encodes pectin methyl esterase related to formation of the cell wall architecture in *Fragaria vesca*, increased strawberry resistance to *B. cinerea* (Osorio et al., 2008). The accumulation of catechin-derived procyanidins was fundamental to inhibit *B. cinerea* growth in immature strawberry fruits (Puhl & Treutter, 2008). Exogenous application of SA at appropriate concentrations to strawberry fruits increased resistance to *B. cinerea*, and effectively inhibited postharvest decay (Babalar et al., 2007).

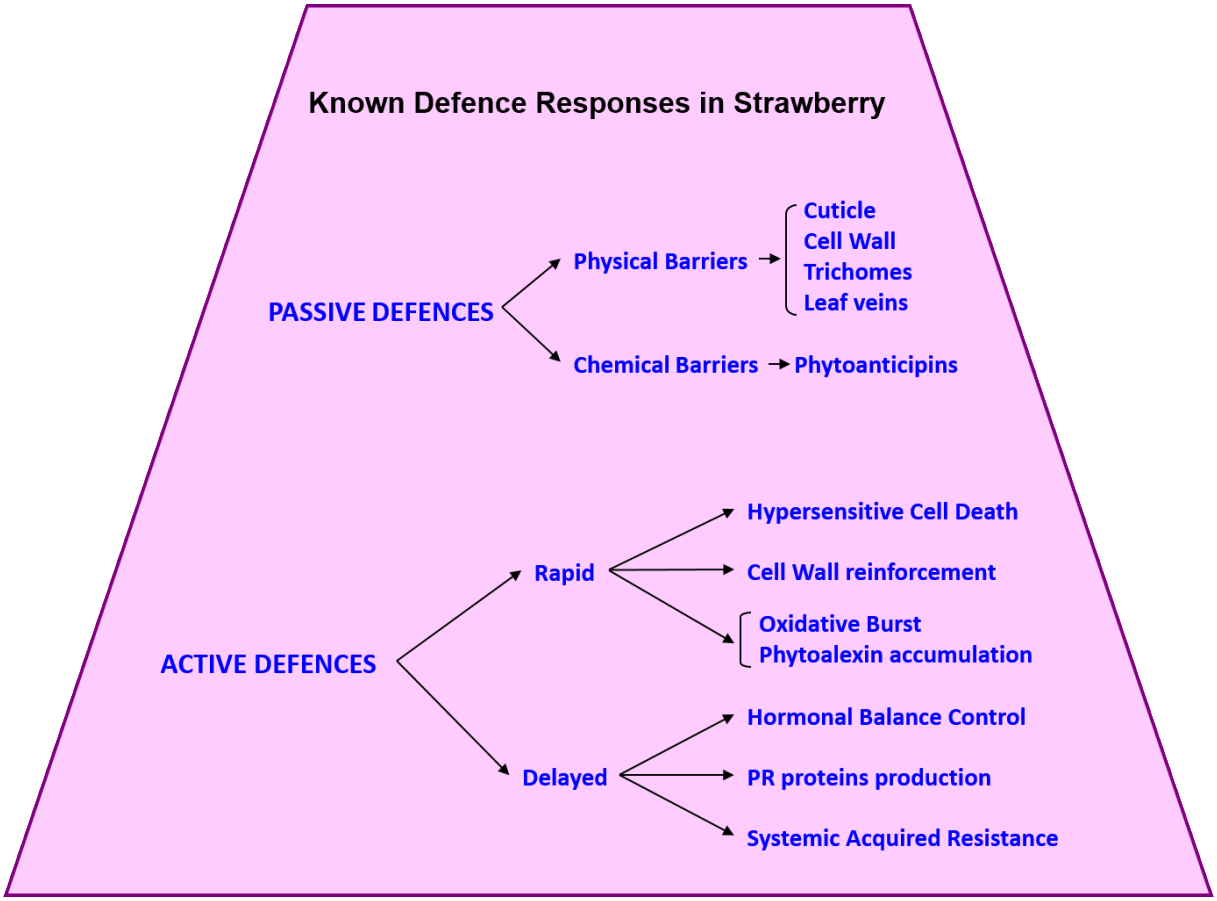


Figure 1.5 Known defense responses in strawberry (modified from Amil-Ruiz et al., 2011).

1.4 Plant responses to light

Light is one of the most crucial environmental factors for the entire life cycle of plants. It is needed not only as an energy source for photosynthesis but also for a fine-tuned regulation

of growth and development. To perceive and respond to the ever-changing light environment, plants have five families of photoreceptors: phytochromes (phy), cryptochromes (cry), phototropins, Zeitlupe family, and the UVB photoreceptor UVR8 (Figure 1.5). With these photoreceptors, plants can sense light signals from the environment to guide their development, such as germination, vegetative growth, organ orientation and transition to flowering.

1.4.1 Plant photoreceptors

1.4.1.1 Phytochromes

Red (600-700 nm) and far-red (700-800 nm) light are sensed by phytochromes in plants (Figure 1.6a). The phytochrome molecule is a 240 kDa apoprotein that exists as a dimeric chromopeptide with a N-terminal region and a C-terminal output region. The amino-terminal domain that binds a phytochromobilin tetrapyrrole chromophore is thought to define the photosensory activity of the phytochrome molecule. The carboxy-terminal region functions in dimerization and presumably contributes to relaying the light signal to downstream signaling events (Burgie & Vierstra, 2014). In the model plant *Arabidopsis thaliana*, there are five phytochromes: PhyA, PhyB, PhyC, PhyD, and PhyE, and all of them perceive red and far-red light (Clack et al., 1994; Kami et al., 2010). All phytochromes exist under two photoconvertible forms: Pr, the red light-absorbing phy and Pfr, far-red light-absorbing phy. The biologically inactive (Pr) form is transformed into an active (Pfr) form upon absorption of red light. In turn, Pfr is rapidly back-transformed to the inactive Pr following far-red light irradiation or slowly by thermal reversion (Burgie et al., 2014; Burgie & Vierstra, 2014). Thus the spectral photon flux distribution in the red and far-red wavelengths known as red:far-red ratio is strongly correlated with the equilibrium between the two forms.

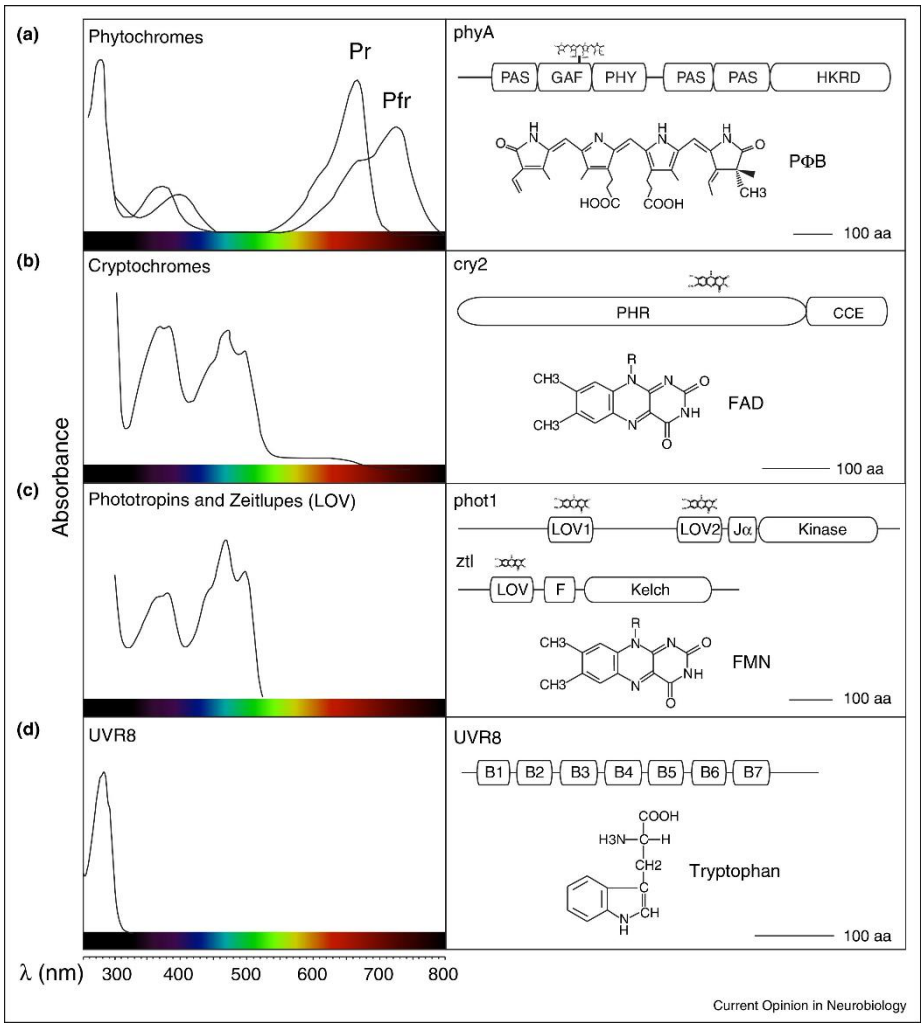


Figure 1.6 Light absorption spectrum, chromophore structure and domain organization of plant photoreceptors. A: phytochromes; B: cryptochromes; C: LOV proteins, phototropins and Zeitlupes; and D: UVR8. HKRD, histidine kinase-related domain; PAS, Per-Arndt-Sim domain; GAF, cGMP phosphodiesterase/adenyl cyclase/Fh1A domain; F, F=box domain; β-propeller domains (B1-B7) (Galvão & Fankhauser, 2015).

Phytochromes mediate different processes through the whole life cycle of plants, including induction of seed germination, seedling de-etiolation, flowering time, fruit quality, root elongation, tolerance to biotic and abiotic stresses, as well as the shade avoidance syndrome (Ballaré & Pierik, 2017; Courbier & Pierik, 2019; Demotes-Mainard et al., 2016). Low red:far-red ratio is reported to promote seed germination, stem and petiole elongation and leaf length increase. In Arabidopsis, a low red:far-red ratio causes leaves to bend upward by altering the petiole angle, and leads to reduced branching by inhibition of bud outgrowth. Through its influence on branching, leaf area and leaf orientation, the red:far-red irradiance can indirectly alter photosynthesis at the scale of the whole plant (Demotes-Mainard et al., 2016). Furthermore, far-red light or *phyB* mutation can either promote or reduce drought tolerance, depending on the species. For instance, decreased drought

tolerance by delaying stomatal closure was found in *phyB* mutants of *Arabidopsis thaliana* (González et al., 2012). Whereas in rice (*Oryza sativa*), *phyB* mutants promote its tolerance by decreasing stomatal density under drought conditions (Liu et al., 2012). Moreover, plants that are exposed to low red:far-red ratios or *phyB* mutants are more susceptible to herbivores or pathogens (Cerrudo et al., 2012; Islam et al., 1998; Kazan & Manners, 2011). This is mainly correlated to changes in leaf morphology, lower chlorophyll content, and down-regulation of the jasmonate and salicylic acid signaling pathways under low red:far-red ratios (Ballaré, 2014; Demotes-Mainard et al., 2016).

In strawberry (*Fragaria×ananassa*), two phytochrome genes *FaPHYA* and *FaPHYB* have been identified (Kadomura-Ishikawa et al., 2013). Positive effects of red mulch in fruit yield and quality possibly indicated the effective role of phytochromes in inducing physico-chemical properties in strawberry (Casierra-posada et al., 2011). End-of-day far-red light strongly promotes flowering while red light has an opposite effect, blue light results in a weak promoting effect, suggesting phytochromes are major photoreceptors in the photoperiodic control of flowering in woodland strawberry (*Fragaria vesca*) (Rantanen et al., 2014). In everbearing strawberries, blue LEDs resulted in earlier flowering compared to red light (Yoshida et al., 2012). Furthermore, end-of-day far-red light improved phytochrome levels in short-day strawberry, leading to an alteration in flowering (Zahedi & Sarikhani, 2016; 2017). Irradiation with blue light during the night phase (16:00 to 08:00) promoted flowering and fruiting by decreasing endogenous gibberellic acid levels and increasing endogenous cytokinin levels in everbearing strawberry (Magar et al., 2018).

1.4.1.2 Cryptochromes

Cryptochromes are photoreceptors that perceive UV-A (315-400 nm) and blue (400-500 nm) radiations with two wavelengths optima (370 and 450 nm) (Figure 1.6b). They are flavoproteins with structural homology to photolyases. Unlike photolyases, cryptochromes have no enzymatic activity to repair UV-damaged DNA, and display a carboxy-terminal region (Lin & Todo, 2005). This terminal region with variable extensions is essential for the photoreceptor function and is not found in photolyases. The amino-terminal domain of the cryptochrome molecule contains two types of chromophores: a light-harvesting pterin at one site and a catalytic FAD at another site (Yang et al., 2000). In the dark, Cry mainly exists as a monomer. Upon absorption of blue light, photons that convey light input information are accepted by the FAD chromophore, thus leading to Cry-active dimer formation. The photoactivated Cry interacts with downstream proteins to regulate different biological processes (Chaves et al., 2011; Shao et al., 2020). In Arabidopsis, three cryptochromes have been identified, Cry1, Cry2 and Cry3. In strawberry, *FaCry1* and *FaCry2* have been

isolated in *Fragaria×ananassa* (Kadomura-Ishikawa et al., 2013). Cry1 and Cry2 are both localized in the nucleus, and Cry1 can transfer to the cytosol upon blue light irradiance while Cry2 stays in the nucleus (Cashmore et al., 1999; Yu et al., 2007). *Arabidopsis* Cry3 is a DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, *Homo*)-type cryptochrome with photolyase activity, which is specific to single-stranded or looped duplex DNA (Pokorny et al., 2008).

It is well documented that Cry1 controls dormancy in barley, plays an important function during de-etiolation in Arabidopsis (Barrero et al., 2014; Kami et al., 2010). While Cry2 is important in photoperiodic control of Arabidopsis flowering, and mediates leaf senescence in soybean (Meng et al., 2013; Kami et al., 2010). Alteration of cry1 and cry2 sequences or expression can remarkably influence agronomic traits in crop species, such as germination, biomass development, and fruit metabolic composition (Mawphlang & Kharshiing, 2017). Additionally, these two photoreceptors regulate other aspects of plant growth and development, including the development of guard cell and stomatal opening, programmed cell death, entrainment of the circadian clock, root growth, shoot length, and shade avoidance. Moreover, they are also responsible for the responses to abiotic and biotic stresses, such as invasion by bacterial, pathogen or pests (Fantini & Facella, 2020; Huché-Thélier et al., 2016; Wang & Lin, 2020).

1.4.1.3 Phototropins and zeitlupe family

Phototropins are also flavoproteins that sense blue and UV-A lights (Figure 1.6c). They have a molecule mass of 120 kDa with a N-terminal photosensory part and a C-terminal AGC-type Ser/Thr protein kinase region. The amino-terminal part contains two flavin mononucleotide (FMN) chromophores that bind light oxygen voltage (LOV1 and LOV2) domains (Suetsugu & Wada, 2013). Phototropins are the only photoreceptors bearing two LOV domains, and these two domains are supposed to have different properties and functions, LOV1 as a dimerization domain and LOV2 as light-regulated kinase (Briggs, 2007; Christie, 2007). Two phototropins exist in Arabidopsis: phot1 and phot2. Phot1 predominates under low-fluence light and phot2 mainly responds to high-fluence light (Huché-Thélier et al., 2016). They both localize at the plasma membrane with overlapping functions in regulating several developmental processes to light. Phot1 and Phot2 both contribute to phototropism, including hypocotyl bending, stem and root growth, leaf flattening (Esmon et al., 2006; Inoue et al., 2008), as well as chloroplast movement and stomatal opening (Ma et al., 2001). However, phot2 is also responsible for the chloroplast light avoidance response (Łabuz et al., 2012). In strawberry, *FaPHOT2* was reported to be involved in blue-light induced anthocyanin accumulation (Kadomura-Ishikawa et al., 2013).

In Arabidopsis an additional family of LOV domain photoreceptors processes the UV-A/blue light signals. This family contains Zeitlupe (ZTL), Flavin-binding Kelch F-box (FKF), and LOV Kelch Protein2 (LKP2), they are collectively stated as Zeitlupes (Figure 1.6c) (Suetsugu & Wada, 2013). Unlike the phototropins, Zeitlupes harbor a single FMN-binding LOV domain at the N-terminal region followed by an F-box and six Kelch repeats at the C-terminal site (Ito et al., 2012). Despite the analogous photochemical properties to phototropin LOV domain, the LOV domains of ZTL, FKF1, and LKP2 fail to return back to their dark state (Cheng et al., 2003). The F-box is generally known to target a protein for degradation, and the Kelch domain is required for protein-protein interaction, presumably with the F-box substrate. Through these proteins, Zeitlupe family regulates the circadian clock and the photoperiodic control of the flowering pathway (Banerjee & Batschauer, 2005; Briggs, 2007; Somers, 2001).

1.4.1.4 UVR8

Ultraviolet-B radiation (UV-B; 280-315 nm) is an intrinsic part of the sunlight and is perceived by UV RESISTANCE LOCUS8 (UVR8) photoreceptor (Figure 1.5d). UVR8 is a β-propeller protein with seven blades. Rather than depending on a prosthetic chromophore, UVR8 uses a number of specific tryptophan residues (W233, W285, and W337) for light sensing, particularly Trp285, which plays an important role in UV-B-triggered signaling. UVR8 is localized in the cytoplasm and nucleus, existing as an inactive homodimer, and it is dissociated into two active monomers upon UV-B irradiation (Christie et al., 2012; Wu et al., 2012; Heijde et al., 2013; Rizzini et al., 2011). The activated monomers then travel from the cytosol into the nucleus to trigger the signal transduction pathway. Following UV-B irradiation, active monomeric UVR8 is recycled to its inactive homodimeric initial state (Jenkins, 2014). Thus, the reversible dynamic photocycle between monomers and homodimer allow plants to continuously perceive and respond to surrounding UV-B levels. Through UVR8 sensing UV-B radiation, plants can trigger large changes in gene expression leading to morphological adaptations and protectant flavonol biosynthesis to reduce UV-B damage (Rizzini et al., 2011). Moreover, UVR8 also regulates plant phototropism, stomatal movement, synchronization with the circadian clock and cross talk with hormones (Fehér et al., 2011; Tossi et al., 2014; Vandenbussche et al., 2014; Yadav et al., 2020).

1.4.2 Light quality regulates plant growth and morphology

Blue and red lights are typically used for plant growth in horticultural production owing to their great efficiency to promote photosynthesis. A wide range of wavelengths drives photosynthesis as they are absorbed by chlorophyll and additional pigments belonging to the carotenoids. Yet, the chlorophylls have two absorption peaks in the vicinity of 450 nm (blue

light region) and 660 nm (red light region) and hence the light energy absorbed by these pigments is very efficiently used in photosynthesis.

Phytochromes and cytochromes have an important role in controlling leaf morphology, internode length and branching. Thus, red and blue light are effective at controlling these responses. Generally, blue light controls cell division and represses hypocotyl internode elongation, resulting in a reduction of plant height (Huché-Thélier et al., 2016). In contrast, stem elongation and petiole extension are promoted by low red/far-red ratios (Demotes-Mainard et al., 2016; Schuerger et al., 1997). Both blue/red ratio and red/far-red ratio play important roles in these processes. In tomato, numerous studies have shown that higher blue/red ratios decrease tomato plant height (Liu et al., 2011; Nanya et al., 2012; Wollaeger & Runkle, 2015). However, conflicting results have been observed on cucumber seedlings. Plant height decreased along with the increase of blue light proportion from 0B:100R% to 75B:25R%, while 100% B light yielded much taller plants (Hernández & Kubota, 2016). In lettuce, red LEDs stimulated the elongation of hypocotyls and cotyledons of seedlings, however, red light alone induced abnormal leaf shapes (Son & Oh, 2013). This effect could be prevented by adding at least 15 mmol m^{-2} s^{-1} of blue light to the red light spectrum (Hoenecke et al., 1992). In fully-green strawberry (*Fragaria×ananassa* Duch.), elongation growth response to light was controlled by phytochromes with two distinct modes. In the period of the first leaf to emerge, end-of-day far-red light induced petiole elongation. With the development of successive leaves, a second type of response with exposure to red, far-red, mixtures of the two at low intensity was found to promote petiole elongation (Vince-Prue et al., 1976). Red LED alone was reported to induce elongation of the flowering stem and increase of whole plant dry weight in ‘Elkat’ strawberry (Samuoliene et al., 2010).

On the other hand, far-red light promotes seedling growth by increasing leaf expansion and whole-plant assimilation. As mentioned above, photosynthetic pigments strongly absorb blue and red light but reflect or transmit far-red radiation which is weakly or not effective at promoting the photosynthetic reaction (Casal, 2013). Therefore, the light environment under the plant canopy is characterized by a low red:far-red ratio resulting in the shade-avoidance syndrome (Gommers et al., 2013). The shade-avoidance response of plants is regulated by phyB and typically involves elongation of internodes, petioles, and hypocotyls (Ballaré & Pierik, 2017; Courbier & Pierik, 2019; Keuskamp et al., 2010). For instance, low red:far-red light increased stem length in *Arabidopsis* (Finlayson et al., 2010) and *Zinnia elegans*, *Cosmos bipinnatus*, *Dendranthema* × *grandiflorum*, *Anthirrhinum majus*, *Petunia* × *hybrida* (Cerny et al., 2003), and promoted petiole elongation in *Cucurbita pepo* (Holmes & Smith, 1977). In ornamental plants such as geranium, petunia, and snapdragon, plant height linearly increased as the red:far-red ratio decreased (Park & Runkle, 2017)

The effects of blue light or red light on branching are species-dependent, with stimulation or inhibition of bud outgrowth, or no effect observed. In *Alternanthera*, blue light induced the largest number of leaves per plant in comparison to white and red light (Macedo et al., 2011). In contrast, blue light reduced the leaf number of cucumber seedlings (Hernández & Kubota, 2016). Inhibition of bud outgrowth under low red:far-red light has been reported in *Solanum lycopersicon*, *Rosa hybrida*, *Trifolium repens*, *Panicum virgatum* and leads to reduced branching (Girault et al., 2008; Lötscher and Nösberger, 1997; O'Carrigan et al., 2014; Williamson et al., 2012). Red light prolonged the vegetative phase in tomato by increasing the number of leaves before flowering (Cao et al., 2016). Overall, under protected cultivation the choice of the ratio red/far-red/blue in lighting sources can steer morphology.

1.4.3 The influence of light quality on primary and secondary metabolites, and pigments

Primary metabolites are chemicals closely linked to plant productivity and quality, and loss of these compounds leads to plant death. Secondary metabolites are chemical compounds that are distinct from the intermediates and products of primary metabolism, they act to improve the fitness of an organism and help it adapt to an unpredictable environment (Lambers et al., 2008). Many of these compounds are key chemicals for plant defense against viruses, pathogens, and herbivores, as well as major donors to color, flavor, and aroma of plants (Bennett & Wallsgrove, 1994; Khalid et al., 2019; Shah & Smith, 2020). The content of primary and secondary metabolites is considerably influenced by light quality and depends on plant species and developmental stage.

The primary metabolites derived from photosynthesis are soluble carbohydrates such as sucrose, glucose and fructose. The metabolism of carbohydrates in plants or fruits is affected by the light spectrum. Red light is reported to improve the contents of fructose and glucose, and combination of red and blue light increases total carbohydrate in tomato seedling leaves (Li et al., 2017). Another study on celery showed that the soluble sugar content was highest in response to red/blue (3:1) light, and reduced with a reduction of red/blue ratio (Gao et al., 2015). Moreover, red light is effective in promotion of hexose content in green vegetables and sprouts, though a plant species dependency was noted (Samuoliene & Duchovskis, 2008). In tomato fruits, Liu et al., (2010) indicated that red/blue (2:3) resulted in the highest soluble sugar content, whereas Dong et al. (2019) found the highest tomato fruit sugar content under red/blue (3:1). So, also here genotype effects might be present.

Secondary metabolites are strongly modulated by the light spectrum (Landi et al., 2020). Phenolic acids and flavonoids are essential secondary metabolites allowing plants to adapt to biotic and abiotic environmental changes. Their concentration in plant organs is dependent

on the season in open-field production and differs at different growth stages (Lynn & Chang, 1990). Plant phenolics are not only responsible for leaf, flower and fruit colors, but also act as antioxidants and ultraviolet screen. Their synthesis upon light quality also depends on the plant species (Taulavuori et al., 2016). Red light clearly improved the total phenolic content in cranberry fruit (Zhou & Singh, 2002) as well as in the *Chrysanthemum* cultivars *Bolero* and *Tappino*, but not in the other cultivars as reported by Zheng and Van Labeke (2017). In lettuce, Li and Kubota (2009) showed the enhanced accumulation of phenolics in red leaf lettuce (*Lactuca sativa* L. 'Red Cross') by red light, while negative effects on the total phenolics were found in two green lettuce cultivars (*Lactuca sativa* L. 'Grand Rapids TBR' and 'Sunmang') (Son & Oh, 2013). Blue light irradiation improved the phenolic content in red leaf lettuce seedlings (Johkan et al., 2010) and melon seedlings (Jing et al., 2018). Both blue and red light are probably needed to modulate the accumulation of phenolics in basil (Taulavuori et al., 2016).

Plants accumulate UV-absorbing compounds such as flavonoids to protect cells and prevent damage by UV radiation (Shah & Smith, 2020). Therefore, accumulation of flavonoids by UV-B light is observed in several species, such as *Medicago* and lettuce "Revolution" (Phillips et al., 1995; Tsormpatsidis et al., 2008). Nevertheless, UV-B radiation does not increase the content of flavonoids in pepper (Hoffmann et al., 2015), and even strongly decreased the level of flavonoids in *Alnus incana* (Kotilainen et al., 2008). On the other hand, blue light was also reported to increase the flavonoids content in melon seedlings (Jing et al., 2018).

Anthocyanins, as important members of the flavonoid group, play key roles in plant defense and signaling as well act as protective agents against UV radiation and oxidative damage by scavenging reactive oxygen species (Kefeli et al., 2003; Lattanzio et al., 2006). UV-A and blue light perceived by the cryptochromes are an important signal to regulate their biosynthesis (Ninu et al., 1999). In particular, UV-A positively influenced anthocyanin content in grape (Kataoka et al., 2003) and lettuce (Tsormpatsidis et al., 2008), blue light increased anthocyanin level in lettuce (Li & Kubota, 2009), tomato (Giliberto et al., 2005) and strawberry (Kadomura-Ishikawa et al., 2013).Yet, red light resulted in enhanced anthocyanin biosynthesis in cranberry fruits (Zhou & Singh, 2002), while far-red light caused downregulation of anthocyanin accumulation in lettuce (Li & Kubota, 2009).

Chlorophyll together with carotenoids are the main pigments for plant photosynthesis and blue, red and far-red lights have been applied in modulating the synthesis of these phytochemicals. Generally, blue and red light enhance chlorophyll production, and far-red light causes a decrease in chlorophyll contents (Huq et al., 2004; Kim et al., 2004; Li et al., 2010; Miyashita et al., 1997; Moon et al., 2006; Tanaka et al., 1998; Tripathy & Brown,

1995). Carotenoids are orange and yellow pigments. They can provide protection when plants are under excessive light, thus limiting damage to proteins and membranes (Bergquist, 2006; Bergquist et al., 2007). Blue light can increase total carotenoids (Johkan et al., 2010; Kopsell & Sams, 2013; Ohashi-Kaneko et al., 2007; Wang et al., 2015), however, no difference was observed in cherry tomato seedlings under blue light compared to red, green, orange, and combinations (Liu et al., 2012). Zheng and Van Labeke (2017) also reported no differences in total carotenoids in red, blue and red+blue grown *Chrysanthemum*. So, the effect of light quality on carotenoid synthesis is dependent on the plant species.

In strawberry, carbohydrate content and pigment ratio (Chla/b) of plants increased, and fruit production and quality were enhanced when plants were grown under red and blue LEDs compared to red light (Díaz-Galián et al., 2020; Samuoliene et al., 2010). Blue light induced higher biomass accumulation, increased fruit yield as compared to red light (Nadalini et al., 2017). The flat expression of two phytochrome genes (*FaPHYA* and *FaPHYB*) in strawberry during fruit development revealed no relationship with the initiation of anthocyanin accumulation in ripening fruits (Kadomura-Ishikawa et al., 2013). Furthermore, red LED light resulted in less anthocyanin content in strawberry fruit compared to fluorescent light (Nadalini et al., 2017).. In addition, postharvest blue light treatment improved sensory quality of strawberry fruit by increasing levels of volatiles, such as acetic acid hexyl ester and methyl isovalerate, compared to darkness (Campbell et al., 2020). On the other hand, UV-C treatment at harvest reduced fruit resistance to *B. cinerea* by increasing the enzyme activities of phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO), and also the expression of several pathogenesis-related proteins (Pombo et al., 2011).

It is clear that the spectral composition of the light sources used in greenhouses or plant factories will influence the levels of these secondary metabolites in plant tissues. So choices with respect to which plant compound one wants to stimulate for a given species will impose an additional layer of complexity that should be considered when designing light recipes for crop production.

1.4.4 The effects of the circadian clock on plant behavior

As photosynthetic organisms, plants are particularly dependent on sunlight which is restricted to a limited time schedule of the day-night cycle. To time their physiology and behavior, plants have evolved an internal timekeeper, the endogenous clock. This circadian clock system that confers 24 h rhythms to biological processes includes three common modules: input, circadian oscillators, and outputs. Input pathways convert external cues (light, temperature) to circadian oscillators to reset and synchronize the clock with the local environment. Through receiving timing cues from input pathways, oscillators produce an

internal estimate of time that regulates the output processes. Output pathways under circadian clock regulation that exhibit a rhythmic response to a constant input are defined as 'gating'. This allows outputs to occur at specific times of the day or to coordinate with environmental changes such as day/night transitions (Huang & Nusinow, 2016).

The circadian clock plays an extremely important role in regulating diverse aspects of plant growth and development, such as germination, growth, leaf movements, flowering and pollination (Yakir et al., 2007). For example, the elongation of the Arabidopsis hypocotyl is under a circadian pattern immediately upon germination, with maximal growth rate in the evening and minimal in the morning (Dowson-Day & Millar, 1999). At cellular level, physiological processes such as enzyme activity, carbon dioxide fixation, hormone pathways, metabolic activities, and stomatal opening are recognized to be clock regulated (Yakir et al., 2007). Production and scavenging of hydrogen peroxide (H_2O_2) exhibit time-of-day phases in Arabidopsis. Specifically, H_2O_2 production is highest at noon, and reaches trough levels at midnight. In parallel with the peak and bottom of H_2O_2 levels, catalase activity peaks at noon, and dips at midnight (Lai et al., 2012). Moreover, dial oscillations in ethylene levels have been documented in rice (Lee et al., 1981), bean, *Kalanchoe daigremontiana* (Kapuya & Hall, 1977) and Arabidopsis (Thain et al., 2004). When plants' endogenous rhythms are well matched with the environmental cycles, the circadian clock promotes pigments, photosynthesis and growth, thus leading to improved survival and competitive advantage of the plant.

The proper matching between circadian clock and external environment not only enhances the ability of plant to sense unfavorable conditions around but also allows the clock to temporally gate the responses to environmental stresses in a timely manner. Thus, plant responsiveness to both abiotic and biotic stresses is under circadian control. For example, rhythmic changes in chilling resistance and heat resistance were reported in cotton by Rikin et al. (1993). The cotton leaves were most sensitive to cold during the light period and most resistant during the dark period, while the heat-resistant phase was developed in the light period and heat-sensitive phase was observed in the evening (Rikin et al., 1993). Similarly, defense responses of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* are regulated by the circadian clock. It was found that plants presented a time-based oscillation over the day in their resistance to *P. syringae* with a peak resistance at dawn. This temporal resistance pattern was disrupted when the clock gene, *CCA1 (CIRCADIAN CLOCK ASSOCIATED 1)* or *ELF (EARLY FLOWERING) 3*, was misexpressed, thus supporting a direct role of the circadian clock in defense regulation (Bhardwaj et al., 2011). In addition, the plant circadian clock regulates the susceptibility to the necrotrophic fungal pathogen*, Botrytis*

cinerea, which is driven by jasmonate signaling pathway in Arabidopsis. The resistance peak was present at dawn, then decreased continuously to midnight (Ingle et al., 2015).

Similarly to other stresses, the activation of defense can also regulate clock activity besides being an output of the circadian clock. Defenses activated by infection with both virulent and avirulent *P. syringae* are able to shorten the circadian period in Arabidopsis (Zhang et al., 2013). What is more, the rhythmic expression of *CCA1* is altered in plants infected with *Hyaloperonospora arabidopsis* Emwa1 (Wang et al., 2011).

Together, these feedback regulations are also important for an appropriate balance between development and resistance by reallocating the energy from costly environmental resistance responses to plant primary metabolism and growth which is definitely crucial for plant fitness and competitive advantage (Seo & Mas, 2015).

1.5 Light quality and plant defense

Light is a fundamental environment signal that acclimatizes plants to their surroundings. As introduced above, many plant developmental and physiological processes are regulated by light signals. Also, light exerts influence on plant resistance to various pathogens (Alsanius et al., 2019; Roberts & Paul, 2006). Since plants perceive light signals via various photoreceptors, specific photoreceptors are shown to be involved in plant defense responses (Cerrudo et al., 2012; Demkura & Ballaré, 2012; Jeong et al., 2010; Xie et al., 2011).

Red:far-red ratios are perceived by phytochromes in plants. Plant defense responses against insects and necrotrophic pathogens are mainly modulated by JA, and SA is a core regulatory hormone regulating defense response against biotrophic pathogens (Glazebrook, 2005; Turner et al., 2002). Red light or high red:far-red ratio was reported to increase resistance to several pathogens in different plant species. On the contrary, low red:far-red light inhibits plant immunity via JA and SA signaling pathways (Table 1.1). Several studies also have demonstrated that plants lacking phyB or exposed to low red:far-red had decreased levels of resistance against herbivores/chewing insects and/or pathogens by reducing volatile organic compounds (VOCs) and/or downregulating JA/SA signaling pathways (Cortés et al., 2016; De Wit et al., 2013; Keymer et al., 2017; Moreno et al., 2009). Moreover, low red:far-red light conditions also led to reduced numbers of nodules and arbuscular mycorrhizal fungi (AMF), which have beneficial interactions with plant roots to overcome stressful conditions (Ferguson et al., 2010; Keymer et al., 2017; Nagata et al., 2016; Suzuki et al., 2011).

Table 1.1 Effects of red and low red:far-red ratio on plant resistance to different pathogens.

Light condition	effect	Plant species	pathogens	comparison	reference
red	↑	Broad bean	*Botrytis cinerea*	White, NUV, blue, green, far-red, dark	(Islam et al., 1998); (Rahman et al., 2002); (Khanam et al., 2005b)
Low red:far-red	↓	Arabidopsis		Ambient light	(Cerrudo et al., 2012)
				White light	(De Wit et al., 2013)
red	↑	tomato		Dark, yellow, blue	(Xu et al., 2017)
Far-red	↓	Arabidopsis		White light	(Courbier et al., 2020)
red	↑	cucumber	*Corynespora cassiicola*	-red	(Rahman et al., 2010)
Red (400-700 nm)	↑	rice	*Pyricularia oryzae*	290-330nm, dark	(Arase et al., 2000)
red	↑×↓	rice	*Pyricularia oryzae*	Natural and white lights	(Shirasawa et al., 2012)
red	↑	rice	*Bipolaris oryzae*	Natural light and dark	(Parada et al., 2015)
red	↑	Broad bean	*Alternaria tenuissima*	White, dark, 400-500 nm, 450-550 nm, >780 nm	(Rahman et al., 2003)
red	↑	cucmber	*Sphaerotheca fuliginea*	White, purple, blue, green, yellow	(Wang et al., 2010)
High red:far-red	↑	cucumber	*Sphaerotheca cucurbitae*	Natural light	(Shibuya et al., 2011)
red	↑	Arabidopsis	*Pseudomonas syringae* pv. *tomato* DC 3000	White light	(Islam et al., 2008)
red	↑	tomato		Dark, purple, green	(Yang et al., 2015)
red	↑	tobacco		White light	(Moyano et al., 2020)
red	↑	watermelon	*Meloidogyne incognita*		(Yang et al., 2015; 2018)
red	↑	tomato	*Meloidogyne spp.*		(Mutar & Fattah, 2013)
red	↑	tomato	*Pseudomonas cichorii*	White light, dark	(Nagendran & Lee, 2015)

Specifically, in rice it was shown that phytochromes are required for age-related resistance to *Pyricularia oryzae* (syn: *Magnaporthe oryzea)* by studying a *phyAphyBphyC* mutant. Phytochromes indirectly up-regulate pathogenesis-related class 1 (PR1) gene expression by modifying SA- and JA-dependent defense pathways (Xie et al., 2011). In Arabidopsis, phyA and phyB are also required for the light-dependent, HR associated lesion formation and PR1 gene expression, thus directly regulating *A. thaliana* resistance (Genoud et al., 2002). Systemic acquired resistance (SAR) which acts via the SA signaling pathway was shown to be down-regulated in *phyA phyB* double mutants compared with wild-type (Griebel & Zeier, 2008). Furthermore, microarray studies showed that the expression of many plant disease resistance genes is regulated by the activity of phytochromes (Devlin et al., 2003; Tepperman et al., 2001). On the other hand, far-red light pretreatment increased

susceptibility to the necrotrophic pathogen *Botrytis cinerea* by downregulating jasmonate responses in *Arabidopsis* (Cerrudo et al., 2012).

The blue-light receptor CRY1 was positively involved in R protein-mediated resistance to avirulent *Pseudomonas syringae* with increased PR gene expression in Arabidopsis (Wu & Yang, 2010). Comparative proteomics analysis of *cry1* mutant and wild-type plants illustrates that expression of several defense-related proteins were altered in the mutant plants (Phee et al., 2007; Yang et al., 2008), moreover, the expression of *PR* genes was reported to be significantly up-regulated in the mutants of downstream components of CRY1 signaling pathway (Kim et al., 2002; Mayer et al., 1996), suggesting a correlation between CRY1 function and plant resistance. Additionally, CRY2 and PHOT2 are required for resistance (R) protein-mediated plant defense against Turnip Crinkle Virus (TCV) by maintaining the stability of the R protein in Arabidopsis (Jeong et al., 2010). The photoreceptors PHOT2, and CRY1 regulated Arabidopsis resistance to cucumber mosaic virus (CMV) infection by mediating antioxidative enzymes and protecting the integrities of chloroplast and mitochondria, and the syntheses of SA and ET are also involved in this process (Zhou et al., 2017).

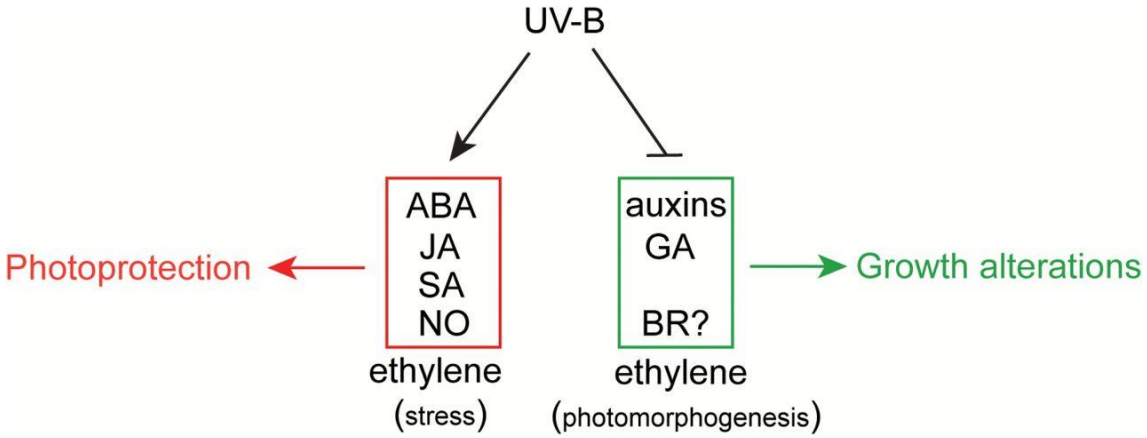


Figure 1.7 Summarizing overview of the regulation of plant hormones by UV-B. in the left-hand box, UV-B stimulates the biosynthesis or signaling of stress hormones ABA, JA, SA, and NO leading to photoprotection from UV-B light. on the right-hand box, UV-B suppresses the biosynthesis or signaling of the expansion stimulating hormones auxins, GA, and perhaps brassinosteroids (BR). This leads to growth alteration. Ethylene can be induced during UV-B stress, but its signal appears down-regulated upon photomorphogenic UV-B exposure (Vanhaelewyn et al., 2016).

UV-B radiation that is perceived by the photoreceptor UVR8 has significant effects on plant growth and defense. The positive role of UV-B in regulating plant defenses against biotic stress was reported, but it also depends on plant species as well as biotic stresses (Ballaré et al., 2012; Ballaré, 2014; Paul et al., 2012). For instance, ecologically meaningful doses of UV-B radiation were shown to increase Arabidopsis resistance to *Botrytis cinerea*, and UVR8

played an important role in this effect by controlling the expression of the sinapate biosynthetic pathway (Demkura & Ballaré, 2012). In *Lactuca sativa*, a zero UV-B environment suppressed the population growth of *Myzus persicae*, while, the severity of *B. cinerea* persistence was decreased under both environments with and without UV-B light (Paul et al., 2012). On the other hand, the effects of UV-B on plant-biotic stress interactions can be direct or indirect. For example, in Arabidopsis-herbivores interactions, UV-B light directly affected caterpillars of *Spodoptera littoralis*, while UV-B caused a great reduction in aphid population by mediating plant process, which is in an indirect way (Vandenbussche et al., 2018). Moreover, the effects of UV-B light on plant growth and defense greatly depends on the control of, and interactions with hormonal pathways (Figure 1.7). This can be dependent or independent on UVR8. Specifically, UV-B promotes the biosynthesis of ABA, JA, SA, and nitric oxide (NO) to perform photoprotection, while down-regulating auxins and gibberellins (GA) to inhibit plant growth. Ethylene can be either up-regulated for stress response, or down-regulated for plant development (Vanhaelewyn et al, 2016). In addition, a functional overlap between UVR8 and phytochromes is existing, this allows plant to deal with two great challenges: shade-avoidance syndrome (SAS) and defense syndrome (DS). Upon the interplay between UV-B and red:far-red signaling, plants fine tune growth and defense to optimize resource utilization in patchy canopy environments (Ballaré & Austin, 2019; Mazza & Ballaré, 2015; Vanhaelewyn et al., 2016).

Chapter 2

Leaf Age and Light Quality Influence the Basal Resistance against *Botrytis cinerea* in Strawberry Leaves

This chapter is based on:

Lijuan Meng, Monica Höfte, Marie-Christine Van Labeke (2019). Leaf age and light quality influence the basal resistance against *Botrytis cinerea* in strawberry leaves. *Environmental and Experimental Botany* 157: 35-45.

Abstract

In this study, the effects of both leaf age and light quality on leaf resistance against *Botrytis cinerea* were investigated. Strawberry plants were grown in a growth chamber equipped with white, blue, red or red+blue light-emitting diodes (LEDs) at a photosynthetic photon flux (PPF) of 100 µmol m^{-2} s^{-1}. The effect of leaf age and light quality on leaf morphology, resistance to *B. cinerea*, hydrogen peroxide levels, metabolites and pigments was studied. Leaf number increased under red light, while leaf petiole length was reduced by red+blue light. Leaf resistance to *B. cinerea* dramatically increased with leaf age from 1- to 4-week-old leaves but decreased again in 5-week-old leaves. Red light significantly improved leaf resistance, while white and blue-light treated leaves were the most susceptible to *B. cinerea* at all leaf ages. Hydrogen peroxide levels positively correlated with disease severity and were influenced by both leaf age and light quality. They were lowest in 4-week-old leaves and in red light-grown leaves, irrespective of leaf age. Chlorophyll and carotenoids levels negatively correlated with disease severity and increased with leaf age but were lowest in blue light-grown leaves. Total phenolics and flavonoid levels were very high in the very susceptible 1-week-old leaves and considerably lower in the older leaves. Red light stimulated total phenolics in 1- to 4-week-old leaves. Proline levels were strongly stimulated by blue light, especially in 1 and 5-week-old leaves. Overall, low hydrogen peroxide levels and high chlorophyll and carotenoids levels appear to be the best indicators for leaf resistance to *B. cinerea* in strawberry leaves. Moreover, leaf age should be taken into account when assessing the effect of light quality on disease resistance.

2.1 Introduction

Of all the environmental cues, light is probably the most important one. It is not only the energy source for photosynthesis but also acts as an environmental signal to regulate growth and development in both plants and fungi (Kami et al., 2010; Purschwitz et al., 2006). In order to cope with the fluctuating light environment, plants and fungi have various photoreceptors to sense and respond to the ambient light conditions and fine-tune their adaptation throughout their life cycle. It is therefore no surprise that new light technologies such as light emitting diodes (LEDs) which allow the application of specific wavelengths (e.g. red, blue and combinations) are increasingly studied with respect to all aspects of plant production (Choi et al., 2015; Heo et al., 2002; Liu et al., 2011; Massa et al., 2008; Nadalini et al., 2017; Nhut et al., 2003; Wu et al., 2007; Xu et al., 2017).

Bioactive compounds are important for the plant to interact with its environment for adaptation and defense. For example, phenolic compounds, flavonoids, carotenoids and anthocyanins are strongly modulated by light quality but effects are species dependent (Alokam et al., 2002; Heo et al., 2012; Huché-Thélier et al., 2016; Kim et al., 2013; Taulavuori et al., 2016). Generally, far-red up-regulates genes that are involved in the biosynthesis of anthocyanins and flavonoids in *Arabidopsis* (Izaguirre et al., 2006). In contrast the anthocyanin level is enhanced under higher red/far-red ratios in *Stellaria longipes* (Alokam et al., 2002). Blue light increases phenolics and flavonoids in tomato (Kim et al., 2013), in roses (Ouzounis et al., 2014) and in lettuce (Ouzounis et al., 2015). Phenylalanine ammonia-lyase (PAL), which is the first enzyme in the phenylpropanoids pathway, is enhanced by the combination of red and blue light in lettuce (Heo et al., 2012). Moreover, shorter wavelengths, in the range of blue and UV-light induce different genes in the flavonoid pathway and involve UV-B, UV-A/blue (cryptochrome) and phytochrome photoreceptors (Feinbaum et al., 1991; Fuglevand et al., 1996; Jenkins, 1997;Wade et al., 2001).

The phenylpropanoid metabolite pathway is also an important indicator of the basal defense response of plants. Peroxidases (POD) are involved in the cell-wall-building processes such as oxidation of phenolic compounds, suberization, and lignification of host plant cells during the defense reaction against pathogenic agents (Datta & Muthukrishman, 1999; Mohammadi & Kazemi, 2002). ROS production is also an early response in plant-pathogen interaction. However, there are several systems to scavenge H_2O_2 in plants, among which the ascorbate/phenolics/POD system has an important H_2O_2 scavenging function in the vacuoles and apoplast (Takahama & Oniki, 1997).

Leaf anatomy, developmental and physiological changes are controlled by leaf age, such as leaf thickness, leaf dry mass, carbon flow, chlorophylls, and metabolism (Pantin et al., 2012). Both primary and secondary metabolites show characteristic patterns evolving with leaf aging (Csepregi et al., 2017; Guimarães et al., 2020; Pontarin et al., 2020). In general, primary metabolites tended to be more concentrated in younger leaves to support growth. Similary, secondary metabolites such as phenolic compounds, peaked in the young leaves and decreased in relation to leaf age (Pontarin et al., 2020; Watanabe et al., 2013). Also, the development of leaf resistance (direct and indirect) changes with leaf age (Carisse & Bouchard, 2010; Farber & Mundt, 2016; Kobayashi et al., 2008; Radhika et al., 2008). Many plant species have high trichome densities on the young newly formed leaves to directly obstruct herbivore activity as they are soft and have high concentrations of nutrients (Yamawo et al., 2012). The indirect defense traits such as the quantity and/or quality of volatile emissions, usually function on young to middle-aged leaves. Thus, leaf aging plays an effective role in promoting the shift from direct to indirect defense (Kobayashi et al., 2008; Radhika et al., 2008; Yamawo et al., 2012).

Strawberry is a very popular economical fruit crop worldwide. In northern latitudes strawberry is mainly grown in greenhouses in which supplementary light is needed for the winter production. LED light has thus also potential for this crop. LED light, compared to fluorescent lamps, increased leaf photosynthetic rates, promoted plant growth and produced higher yields in strawberry (Hidaka et al., 2013). Furthermore, light quality has long been known as an important environmental factor in pathogen defense. An increasing number of studies showed that red light is able to improve plant defenses to biotic stresses by multiple regulatory pathways, such as H_2O_2 generation, salicylic acid accumulation, expression of defense genes and suppression of leaf permeability and lesion formation (Islam et al., 2003; Wang et al., 2010). In broad bean, red light improved leaf resistance to *B. cinerea*, this could be explained by the production of antifungal compounds in living host cells, and enhanced host photosynthesis and protein synthesis (Khanam et al., 2005a; Rahman et al., 2002). Proline and polyphenol levels increased in blue LED light grown tomato leaves. Moreover, tomato leaves inoculated with *B. cinerea* were more resistant when incubated under blue LED light than under white light (Kim et al., 2013). Xu et al. (2017) showed that lesion development after inoculation with *B. cinerea* was significantly lower when tomato leaves were irradiated with red or purple light in comparison with blue light. However, a low red:far-red ratio, which is indicative of shade, triggers the shade avoidance response in sun-responsive plants. This leads to a stimulation of elongation growth and suppression of salicylic acid- and jasmonate-dependent defense responses (Cerrudo et al., 2012; Moreno et al., 2009; Ballaré, 2014).

The broad host-range necrotrophic fungus, *Botrytis cinerea*, is one of the most destructive pathogens in strawberry production (Xu et al., 2000). *B. cinerea* can develop microscopic infections in epidermal cells of young strawberry leaves. These infections remain latent for a certain period but after sporulation they becomes a source of primary inoculum for fruit infections. Fungicides are the primary means to control grey mold, however, increased insensitivity of *B. cinerea* to a combination of chemical agents threatens the effective control (Hunter et al., 1987; Katan et al., 1989). Moreover, application of conventional fungicides is increasingly restricted because of residue risks up to the post-harvest phase of the strawberries (Williamson et al., 2007). Thus, specific light quality applications open a promising way for disease control due to changes in the plant defense system though no information on strawberry is available.

Light quality might have also a direct effect on the pathogen. In *B. cinerea*, at least eleven potential photoreceptors were found to perceive different light qualities, influencing tropism of direct growth, sexual development, protective mechanisms against stresses and timing for infection and survival (Schumacher, 2017). In detail, continuous black light (300-420 nm) retarded linear hyphal growth of *B. cinerea* while sporulation was stimulated by near ultraviolet light (NUV), slightly inhibited by red and yellow light but inhibited by blue and green light (Tan & Epton, 1973). Moreover, red light was reported to inhibit infected hypha formation and lesion development of *B. cinerea* on plant leaves, such as broad beans, grapevine, and tomato (Ahn et al., 2015; Islam et al., 1998; Xu et al., 2017).

In this study we hypothesized that both light quality and leaf age affect the basal resistance to *Botrytis cinerea* in strawberry leaves. Experiments were set-up to interpret effects of leaf age and effects of monochromatic red and blue and dichromatic red+blue on changes in resistance to *Botrytis.* Therefore, we measured levels of H_2O_2, selected antioxidants (phenolics and flavonoids) and anti-oxidative enzymes (APX and POD), as well as pigments to study their roles in the resistance of strawberry leaves to *Botrytis*.

2.2 Materials and Methods

2.2.1 Plant material and experimental setup

Three successive experiments were set-up with strawberry (*Fragaria* × *ananassa* ‘Elsanta’). For each experiment, 80 cold-stored young plants were transplanted in 0.3 L pots using a peat-based potting soil (Van Israel nv, Belgium). The plants were randomly subjected to four light quality treatments (20 plants per treatment) in a growth chamber where the air temperature was maintained at 20°C and RH averaged 70%. Fertigation (Soluplant, Haifa, The Netherlands, EC=1.5 dS/m, pH=5.7) was applied three times per week.

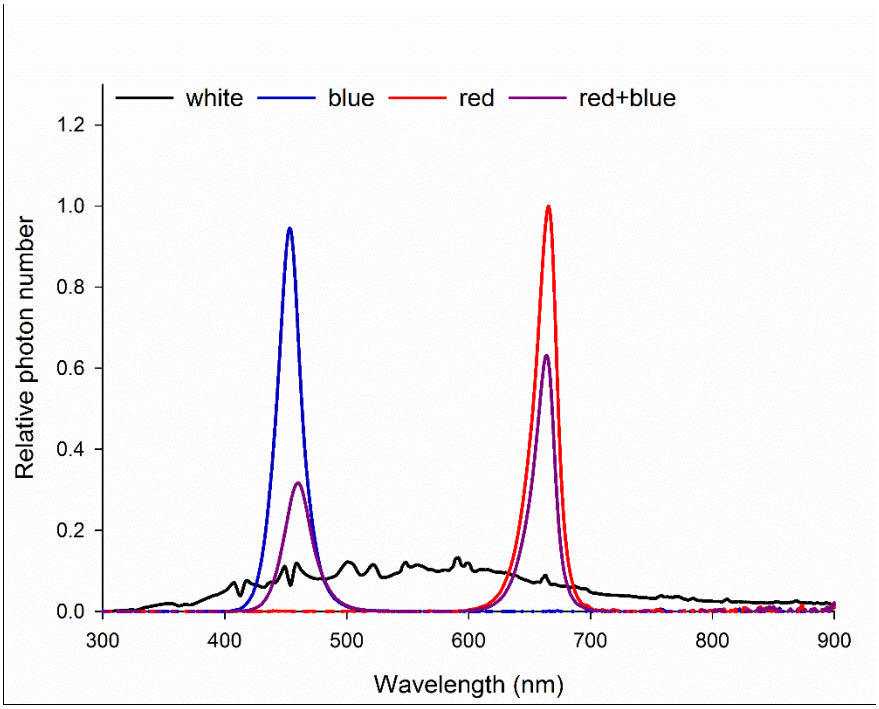


Figure 2.1 Spectral distribution of LEDs used in the experiment: White LEDs (white), Blue LEDs (blue), Red LEDs (red), Red plus Blue (red+blue). Spectrum was measured with a JAZ spectroradiometer (Ocean optics, USA).

For the control treatment white LEDs (300-800 nm, Philips, The Netherlands) were used in experiment 1 and 2 while plasma lamps (Gavita, The Netherlands), were used as white light in experiment 3. The other treatments were blue (B, peak at 460 nm), red (R, peak at 660 nm), red+blue (RB, 50%/50%, peak at 460 nm and 660 nm). A photoperiod of 16 h and a photon flux density of ± 100 µmol $m^{-2}s^{-1}$ at plant level was provided. Light quality and light quantity were measured using a spectroradiometer (JAZ-ULM-200, Ocean Optics, US), and the spectral distribution of the light sources are shown in Figure 2.1. Light quality treatments were inside the same growth chamber and separated with non-reflective black screens.

New leaves were labeled on the date of emergence and sampled after respectively one week, two weeks, three weeks, four weeks and five weeks. Depending on the analysis leaves were immediately used (inoculation assays) or immediately frozen in liquid nitrogen and stored at -80°C until analysis (metabolites).

2.2.2 Plant morphology (Exp 1)

Leaves initiated and fully developed from plants in the first four weeks under light treatments were noted. Petiole lengths from sampled leaves with different ages were measured with a ruler. Leaf anatomy under different light treatments from week-1 to week-5 was studied by paraffin sectioning as described by Zheng (2017). The sections were stained with staining

solution (1% astrablue, 0.5% chrysoidine, and 0.5% acridinere) and imaged with a ColorView III camera and edited with the software package CELL-F (Olympus Soft Imaging Solutions).

2.2.3 *Botrytis cinerea* inoculation and disease rating (Exp 2 and 3)

B. cinerea strain R16 (Faretra & Pollastro, 1991) was used for leaf inoculation and was cultured on potato dextrose agar (PDA, Becton, Dickinson, and Company) medium in Petri dishes. After 10 days, conidia were washed from the plates with ¼ potato dextrose broth (PDB, Becton, Dickinson, and Company) solution containing 0.01% (v/v) Tween 20 (MERCK, Germany). After removing the mycelium fragments, spore titers were determined microscopically using a Thoma counting chamber. A final conidia concentration of 5×10^5 spores mL^{-1} was used for the inoculation.

Twelve one-cm-diameter leaf discs per leaf with four replicates (each replicate was one plant from which one leaf was taken) per treatment were cut the day before inoculation and placed in disposable plates with water. The next day, each disc was inoculated with 10 µL droplets of conidial suspension on the adaxial leaf surface. Incubation was at 24 °C under dark conditions. Disease symptoms were scored after 4 to 5 days, and disease ratings or disease index were expressed according to Curvers et al. (2010) and De Vleesschauwer et al. (2010). All inoculation experiments were repeated twice with similar results.

2.2.4 Measurements of pigments (Exp 1)

Pigments were measured according to Chen et al. (2004) with slight modifications. Twenty leaf discs per leaf (0.8 cm in diameter) were punched and then immersed in 80% ethanol (VWR Chemicals) in 15-mL tubes with cap. Total chlorophyll was extracted in the dark at room temperature on a shaker platform for 24h. The extracted chlorophyll was measured using a spectrophotometer (Infinite M200, TECAN Group Ltd., Switzerland) at λ = 470nm, 647nm and 664 nm. The total chlorophyll and carotenoids content was calculated according to Lichtenthaler and Buschmann (2001).

2.2.5 Hydrogen peroxide and metabolites (Exp 3)

Determination of hydrogen peroxide was performed as described by Junglee et al. (2014). Homogenized leaf material (30-40 mg) was extracted in 10 mM potassium phosphate buffer (pH 5.8) with 1% TCA (Trichloroacetic acid, Carl Roth) and 1 M KI (Potassium iodure, Ucb, Belgium) at 4°C. After centrifugation at 15,000 *g* for 15 min at 4°C, the supernatant was measured spectrophotometrically at λ = 350 nm (Infinite M200 TECAN), leaf hydrogen peroxide concentration was determined based on a standard curve obtained with H_2O_2 (VWR Chemicals) and was calculated as µmol H_2O_2 mg^{-1} FW.

Leaf proline content was measured according to Bates et al. (1973). Plant leaves (1 g) were extracted with 10 mL 3% (w/v) sulfosalicylic acid (ACROS ORGANICS, USA). After filtration, 1 mL acid ninhydrin (SERVA Electrophoresis GmbH, USA) and 1 mL glacial acetic acid (Carl Roth) were added to the extracts (1 mL) and this mixture was kept at 95°C for 1 h in a water bath, then the reaction was stopped in an ice-bath. The formed chromophore was extracted from the acid aqueous solution by cold toluene (2 mL) and the absorbance was measured at λ = 520 nm (Infinite M200 TECAN). The proline content was expressed as µmol proline g^{-1} FW.

Total phenolics and flavonoids were analyzed according to Hong et al. (2008). Homogenized leaf material (50 mg) was stirred in 10 mL of 80 % methanol and sonicated for 30 min. The suspension was centrifuged for 15 min and the supernatant was collected for the determination. To determine the total phenolics an aliquot of 200 µL reacted with Folin-Ciocalteu (SIGMA-ALDRICH) (1:10) reagent and 7.5% Na_2CO_3 (Sodium carbonate, MERCK, Germany) for 2 h in the dark at room temperature. The reaction product was measured at λ =765 nm (Infinite M200, TECAN Group Ltd., Switzerland) and the total phenolic concentration was expressed as mg gallic acid g^{-1} FW. To determine the flavonoids an aliquot of 200 µL reacted with 60 µL of 5% $NaNO_2$ (Sodium nitrite, Carl Roth) in 800 µL distilled water for 5 min, then 120 µL of 10% $AlCl_3$ (Aluminum chloride, MERCK, Germany)was added to react for 6 min. At last, 1 M NaOH (Sodium hydroxide, Carl Roth) (400 µL) and distilled water (400 µL) was added to finish the reaction. Absorption of the reaction at λ = 510 nm was measured (Infinite M200, TECAN Group Ltd., Switzerland) and total flavonoid content was calculated as rutin equivalents g^{-1} FW.

The determination of anthocyanins was performed according to Pirie and Mullins (1976) with minor modifications. Leaf material (50 mg) was extracted in 2 mL of 1% (v/v) HCl-methanol. Homogenate was centrifuged at 19,000 g for 15 min and diluted with 1% (v/v) HCl-methanol to 4 mL. Absorbance was measured at λ = 530 nm and 600 nm and anthocyanin content was expressed as the changes of 0.1 unit of difference between OD 530 nm and OD 600 nm.

2.2.6 Antioxidant enzymes (Exp 2)

For the ascorbate peroxidase (APX) and guaiacol peroxidase assays, leaves were homogenized with 2 mL ice-cold 50 mM potassium phosphate buffer (pH 6.1) including 0.1 mM EDTA (Ethylenediaminetetraacetic acid, Carl Roth), 10% PVPP (Polyvinylpolypyrrolidone, AppliChem, Germany) and 1 mM L-ascorbic acid. After cold extraction for 15 min, the homogenates were centrifuged at 4 °C for 20 min. The supernatant was used for the determination of antioxidant enzymes. The activity of APX was assayed using the method of Nakano and Asada (1981) by recording the rate of ascorbate oxidation

at λ = 290 nm. POD was analyzed according to Chance and Maehly (1955) by estimating the increase in absorbance at λ = 436 nm.

2.2.7 Statistical analysis

Data were analyzed by 1-way ANOVA, and comparisons of the relationship between light quality, leaf age and light quality × leaf age were carried out by 2-way ANOVA. Post-hoc comparisons for statistical analysis were calculated using Tukey HSD test ($p \leq 0.05$). Disease rating was compared by Kruskall-Wallis test ($p \leq 0.05$). Correlation was conducted by Pearson's correlation coefficients ($p \leq 0.05$). Different letters indicate significant different values at $p \leq 0.05$. All analysis was performed using the SPSS statistical software version 25 (SPSS Inc., Chicago, USA). To highlight the relevance of physiological parameters with respect to the disease index, we applied the conditional formatting tool in Excel resulting in a visual representation comparable to heatmaps.

2.3 Results

2.3.1 Plant morphology

Light quality affected plant morphology (Figure 2.2). Red light significantly increased the number of leaves per plant, while a combination of red and blue LED light decreased leaf initiation after a 4-week light treatment compared to white light (Figure 2.2A). In addition, the petiole length was affected by the light quality, irrespective of leaf age (Figure 2.2B). Leaf petiole length was repressed by red+blue LEDs ranging from 24% for 3-week-old leaves to 65% in 5-week-old leaves when compared to white light. Monochromatic blue and red light alone only reduced petiole length in 5-week old leaves. Leaf thickness and anatomy was not affected by the light treatments, this for the studied leaf ages (1-week-old to 5week-old leaves, Figure S2.1).

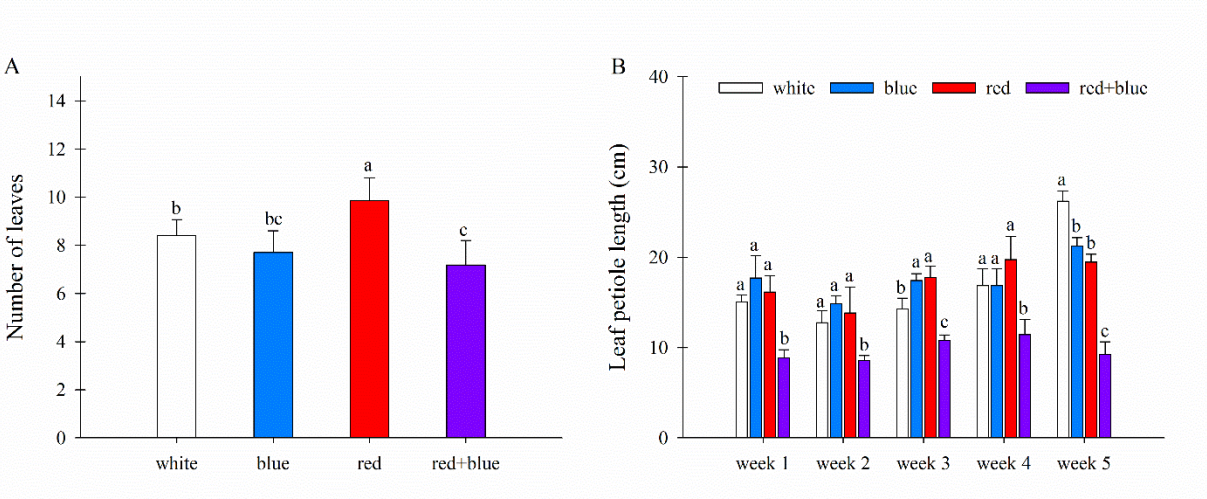


Figure 2.2 Effect of light quality on leaf initiation for the first four weeks (A) and petiole length (B) from 1-week-old to 5-week-old leaves. Week 1, week 2, week 3, week 4, and week 5 in figures represent leaf age from 1-week-old to 5-week-old, respectively. Different letters indicate significant differences between values at $p \leq 0.05$. Values are presented as means ± S.D. (A: n = 20; B: n = 5).

2.3.2 Resistance against *B. cinerea*

Leaf age analysis showed that leaf resistance against *B. cinerea* increased gradually and significantly with increasing leaf age until 4-week-old leaves, while it notably decreased in 5-week-old leaves (Table 2.1).

The effect of light quality during leaf development on resistance to *B. cinerea* was explored by assessing lesion development on leaf discs after 4 or 5 dpi under dark condition. Red and red+blue light significantly enhanced the resistance against *B. cinerea* in leaves up to 3-week-old when compared to white light (Figure 2.3A). The lesions of 4-week-old leaves developed slowly and red light grown leaves hardly showed symptoms at 4 dpi (Figure 2.3A). At 5 dpi, lesion development on 4-week-old blue light grown leaves increased while lesions hardly developed for red light grown leaves when compared to white light (Figure 2.3B). For 5-week-old leaves susceptibility increased compared to 4-week-old leaves. Here, the disease rating was the highest in leaves grown under red+blue and lowest under red light (Figure 2.3A). Overall, red light enhanced leaf resistance against *B. cinerea*.

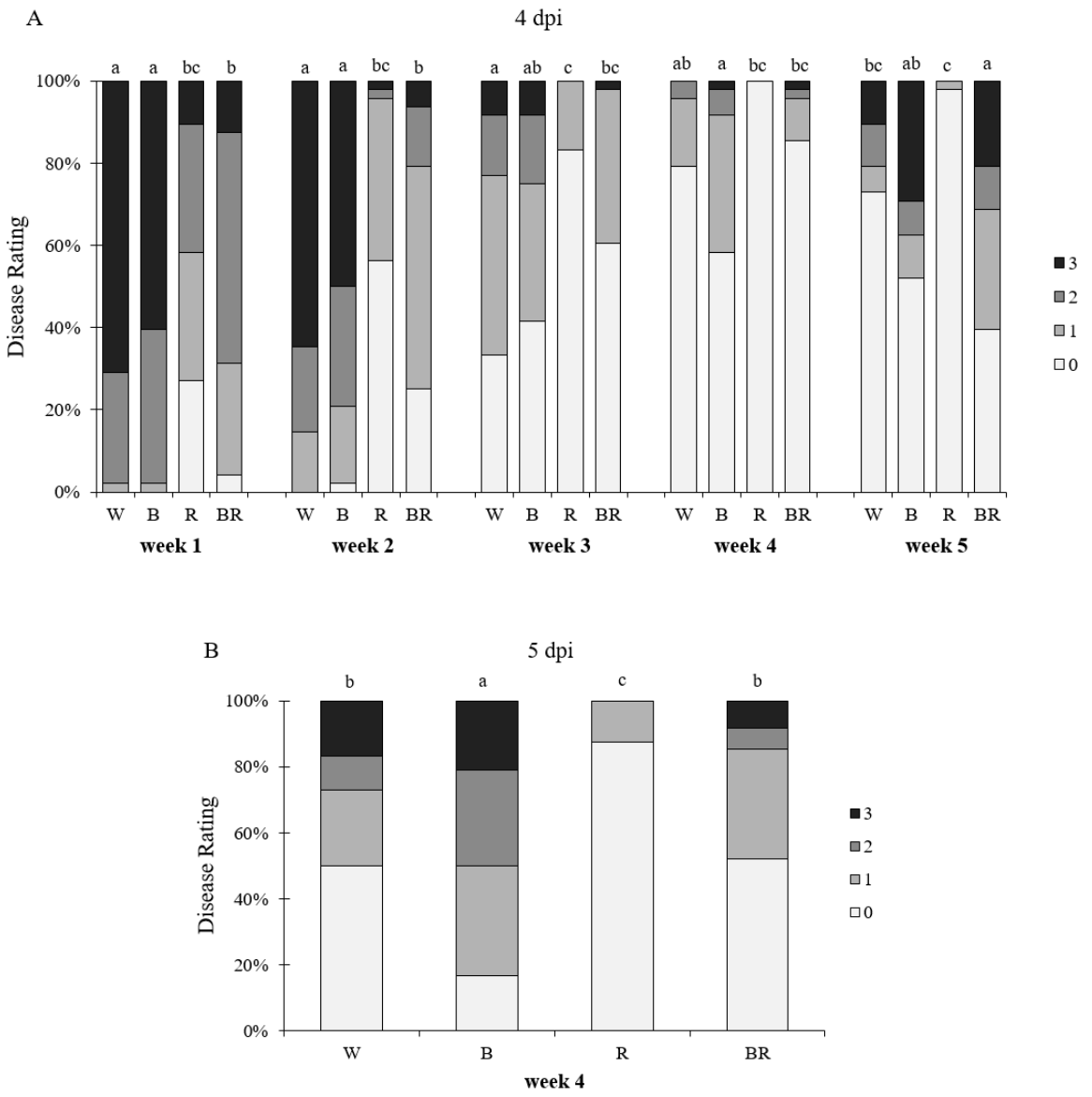


Figure 2.3 Light quality affects resistance against *B. cinerea* in strawberry. Leaves were punched and inoculated with *B. cinerea* spores and then kept in the dark for 4 or 5 d. A, Effects of light quality on strawberry leaves aged from week 1 to week 5. Disease evaluation was conducted 4 d post inoculation. B, Effects of light quality on 4-week-old leaves assessed at 5 d post inoculation. Disease rating was evaluated using four scoring categories (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). W, white; B, blue; R, red; BR, red+blue. Different letters indicate statistically significant differences (Kruskall-Wallis test, $p \leq 0.05$) between light quality treatments.

2.3.3 Hydrogen peroxide

Leaf age considerably affected H_2O_2 levels in strawberry leaves. The youngest, 1-week-old leaves had the highest level of H_2O_2, while the level decreased with increasing leaf age till 4-week-old leaves. The lowest H_2O_2 level was found in 4-week-old leaves, while it increased

from 4-week-old to 5-week-old (Table 2.1). Under red light H_2O_2 was significantly lower for all leaf ages, a reduction of respectively 34.9%, 26.3%, 24.6%, 14.3% and 44.2% was found in 1-week-old to 5-week-old leaves, when compared to white light (Figure 2.4A). In 1-week-old and 4-week-old leaves, the highest H_2O_2 level was observed under red+blue, while for 5-week-old plants, the greatest H_2O_2 content was found in blue light grown leaves. Overall, blue and red+blue light increased H_2O_2 level, while red light decreased H_2O_2 remarkably (Table 2.1).

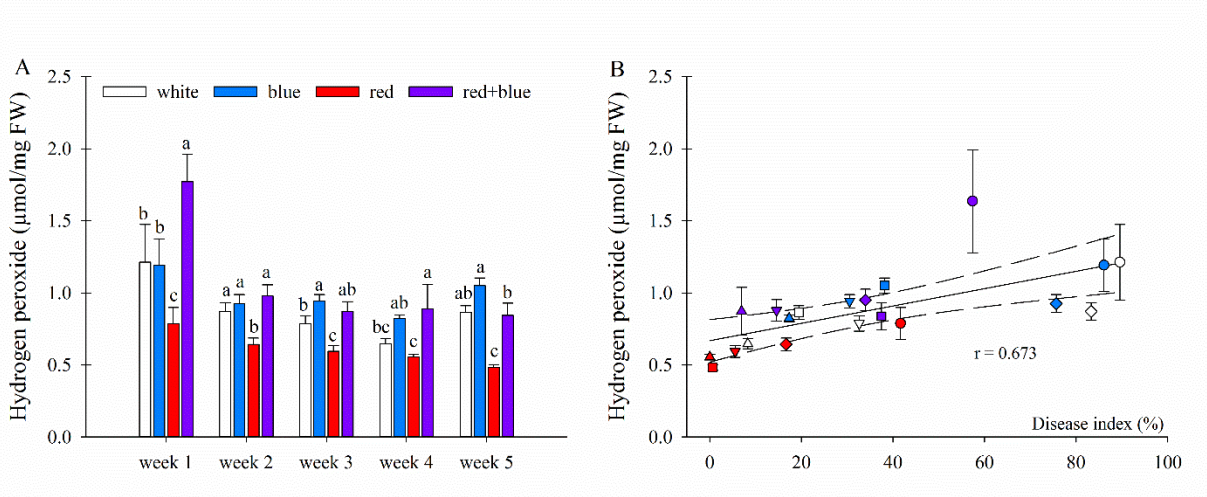


Figure 2.4 Effects of light quality on hydrogen peroxide (A) content in strawberry leaves from 1-week-old to 5-week-old, and correlation between disease index and hydrogen peroxide (B). Different symbols mean different leaf ages: ○, 1-week-old; ◊, 2-week-old; ▽, 3-week-old; △, 4-week-old; □, 5-week-old; colors show the light quality treatment: white (white color); blue (blue color); red (red color) and red+blue (purple color). Values are presented as means of four replicates with standard deviation shown by vertical bars. Different letters indicate statistical differences between the light quality treatments based on Tukey's test ($p \leq 0.05$).

The effects of leaf age and light quality on the innate H_2O_2 content were correlated with *Botrytis* resistance in strawberry (Figure 2.4B). Disease index (%) had a high positive correlation with H_2O_2 ($r = 0.673$) in strawberry leaves. One-week-old leaves had the greatest disease index and highest H_2O_2 level, while 4-week-old leaves had the lowest disease index with the lowest H_2O_2 level.

2.3.4 Metabolites and antioxidant enzymes

A higher concentration of total phenolics was found in 1-week-old leaves, while it decreased by 59.7% for 2-week-old leaves and then remained at the same level for older leaves (Table 2.1). Independent of leaf age, higher concentrations of total phenolics were found in 1-week to 4-week-old leaves grown under red light, while no significant differences for the other light

treatments were observed (Figure 2.5A). No effects of light quality on total phenolics were found in 5-week-old leaves.

Disease index moderately correlated with total phenolics (r = 0.581) (Figure 2.5B). This positive correlation is mainly due to the high levels of phenolics in the most susceptible 1-week-old leaves.

Flavonoids were highest in 1-week-old leaves, and then decreased by 69.6% for 2-week-old leaves. The lowest flavonoid level was in 4-week-old leaves while it increased again for 5-week-old leaves (Table 2.1). With respect to light quality, red+blue light grown leaves had the highest flavonoid content from 1-week-old to 3-week-old (Figure 2.5C). Irrespective of leaf age, red+blue light resulted in the highest flavonoid content, while blue and red light had intermediate levels and a significant lower content was found for leaves that developed under white light (Table 2.1). A moderate correlation was found between disease index and total flavonoid (r = 0.581) (Figure 2.5D). Again, this positive correlation was mainly due to the high flavonoid levels in the susceptible 1-week-old leaves.

Blue light significantly increased proline content for all leaf ages (Figure S2.2A). No differences in proline content were found for leaves that developed under red, red+blue and white light with exception for 1-week-old leaves where proline content was significant lowest under red+blue and 3-week-old leaves where proline was lowest under red light (Figure S2.2A). Overall, blue light increased proline content, while red+blue light decreased it significantly (Table 2.1). Leaf age also affected the proline content with higher proline levels in 1-week-old and 5-week-old leaves (Table 1). There is a weak positive correlation between disease index and proline content with r = 0.390 (Figure S2.2B).

Table 2.1 Effects of light quality and leaf age on disease index (%), H_2O_2, total phenolic, total flavonoid, proline, APX, POD, total chlorophyll, carotenoid, chl a/b and anthocyanin. Data are analyzed in a 2-factorial way to analyze the overall effect of light quality and of leaf age. LQ: light quality; LA: leaf age.

Light quality	**Disease index** %	**H_2O_2** (µmol/mg FW)	**Total phenolic** (mg g^{-1} FW)	**Total flavonoid** (mg g^{-1} FW)	**Proline** (µmol/mg FW)	**APX** (µmol/min·mg Protein)	**POD** (µmol/min·mg Protein)	**Total chlorophyll** (µg ml^{-1})	**Carotenoid** (µg ml^{-1})	**Chl a/b**	**Anthocyanin** (unit×10^3/g FW)
white	46.7a	0.884b	8.0b	9.3c	0.106b	1.430a	0.330a	38.0b	5.87a	1.93b	11.3b
blue	49.6a	0.993a	8.5b	10.4b	0.192a	1.027b	0.276a	30.9c	4.95b	2.25a	10.9b
red	12.9c	0.627c	10.9a	10.4b	0.099bc	1.498a	0.339a	41.8a	5.88a	1.86b	12.8a
red+blue	30.4b	1.074a	8.8b	11.8a	0.077c	1.673a	0.344a	38.7b	5.69a	1.95b	13.9a
LQ	***	***	***	***	***	***	ns	***	***	***	***
Leaf age											
1-week-old	69.1a	1.241a	20.4a	24.7a	0.164a	2.148a	0.195cd	23.1d	3.64c	2.17a	10.8c
2-week-old	52.4b	0.848b	8.2b	7.5bc	0.074b	1.076cd	0.260c	35.9c	5.40b	2.26a	14.5a
3-week-old	20.8cd	0.800bc	5.9b	6.6cd	0.099b	1.570b	0.612a	33.5c	4.94b	2.13a	11.6bc
4-week-old	8.2d	0.733c	5.6b	6.4d	0.095b	0.771d	0.133d	43.6b	7.03a	1.95b	11.9bc
5-week-old	23.9c	0.847b	6.1b	7.6b	0.152a	1.440bc	0.417b	50.8a	6.97a	1.49c	13.2ab
LA	***	***	***	***	***	***	***	***	***	***	***
LQ×LA	**	***	**	**	***	***	ns	ns	***	***	***

Different letters indicate statistical differences based on 2-way ANOVA using Tukey's test ($p \leq 0.05$).
ns means no statistical differences.
** indicates significant difference at $p < 0.01$.
*** indicates significant difference at $p < 0.001$.

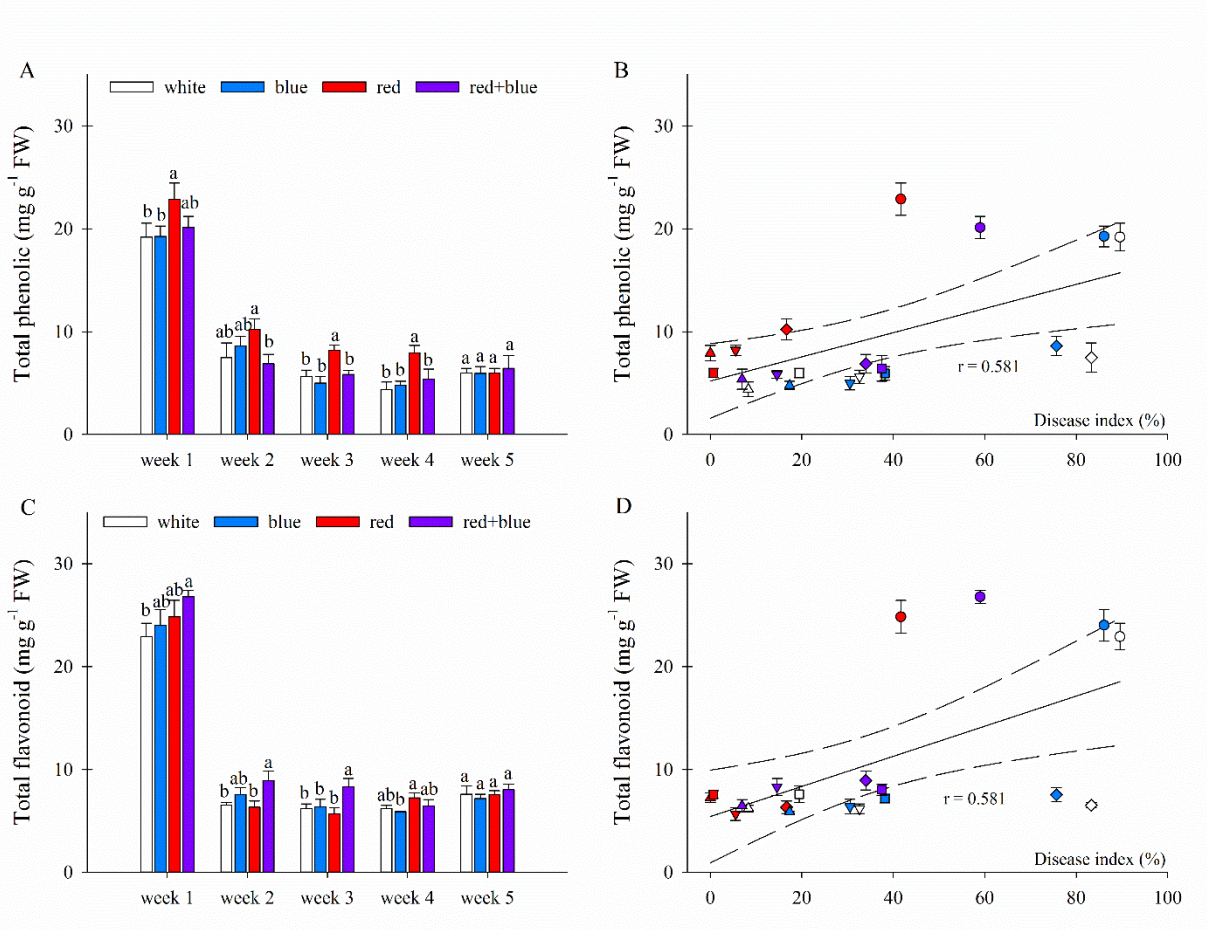


Figure 2.5 Effects of light quality on total phenolic (A), and total flavonoid (C) content in strawberry leaves from 1-week-old to 5-week-old, and correlations between disease index and total phenolic (B) and total flavonoid content (D). Different symbols mean different leaf ages: ○, 1-week-old; ◊, 2-week-old; ▽, 3-week-old; △, 4-week-old; □, 5-week-old; colors show the light quality treatment: white (white color); blue (blue color); red (red color) and red+blue (purple color). Values are presented as means of four replicates with standard deviation shown by vertical bars. Different letters indicate statistical differences between the light quality treatments based on Tukey's test ($p \leq 0.05$).

Additionally, antioxidative enzymes ascorbate peroxidase (APX) and guaiacol peroxidase (POD) were studied (Figure S2.3). As a whole, lower APX activity was observed in leaves grown under blue light, while no significant changes were found under red and red+blue light compared to white (Table 2.1). APX activity also fluctuated with leaf age with the highest activity found for 1-week-old leaves, and the lowest for 4-week old-leaves (Table 2.1). POD activity was not affected by light quality, but was significant influenced by leaf age. The highest POD activity was found in 3-week-old leaves, while the lowest was found in 4-week-old leaves (Table 2.1).

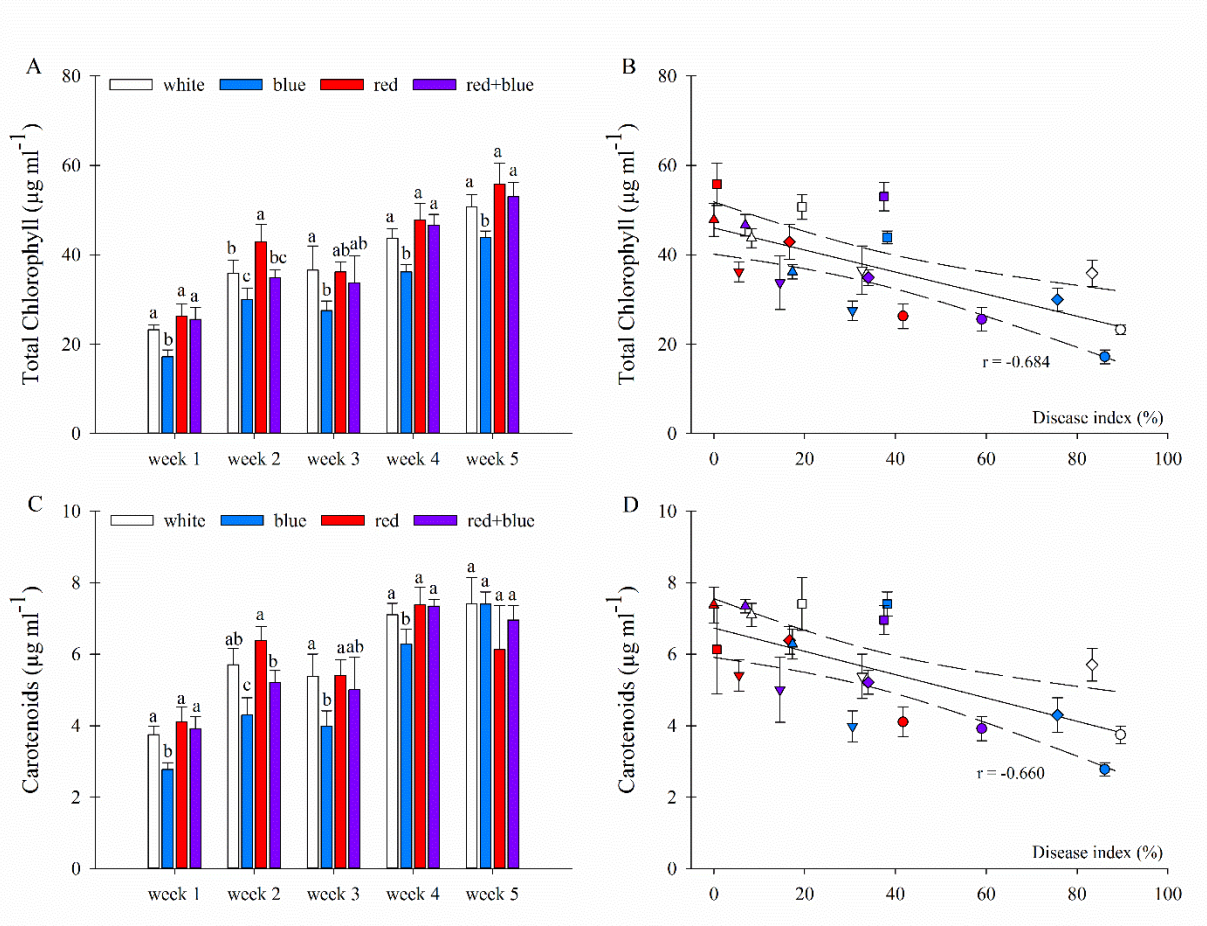


Figure 2.6 Effects of light quality on total chlorophyll (A) and carotenoids (C) content and their correlations with disease index from 1-week-old to 5-week-old strawberry leaves. Data shown are mean values (± S.D.) from four replicates. Different symbols mean different leaf ages: ○, 1-week-old; ◊, 2-week-old; ▽, 3-week-old; △, 4-week-old; □, 5-week-old; colors show the light quality treatment: white (white color); blue (blue color); red (red color) and red+blue (purple color). Different letters indicate significant differences between values (Tukey test, $p \leq 0.05$).

2.3.5 Leaf pigments and anthocyanin

Total chlorophyll was mediated by leaf age with lowest chlorophyll in 1-week-old leaves and a gradual increase with increasing leaf age from 1 week to 5 weeks (Table 2.1). Blue light-grown leaves had a significantly lower total chlorophyll content at all leaf ages. The other light qualities did not cause marked changes in total chlorophyll content, except for 2-week-old leaves where the highest chlorophyll content was found under red (Figure 2.6A). Overall, the highest content of total chlorophyll was found in leaves grown under red light, followed by red+blue and white and lowest under blue (Table 2.1). However, there was no interaction between leaf age and light quality (Table 2.1). The Chl a/b ratio decreased with leaf aging, leaves up to 3-week-old had the highest ratio, then it fell from 3-week-old to 5-week-old leaves (Table 2.1). The Chl a/b ratio was highest under blue light, while no difference was

found between the other treatments. A negative correlation between total chlorophyll and disease index was found (r = -0.684) (Figure 2.6B).

Carotenoids increased with leaf age, with the lowest level in 1-week-old leaves and the highest level in 4 and 5-week-old leaves (Table 2.1). Blue light resulted in a lower carotenoids content in 1-week-old to 4-week-old strawberry leaves, while no marked differences were present between the other treatments relative to white (Figure 2.6C). No significant changes were observed in 5-week-old leaves. As a whole, blue light lowered the level of carotenoids (Table 1). There was a negative correlation between disease index and carotenoids with r = -0.660 (Figure 2.6D).

Anthocyanin content differed according to leaf age and light quality treatment (Figure S2.2C). No clear effect of light quality on anthocyanin level with respect to leaf age could be observed. Irrespective of the light quality, the highest content of anthocyanin was present in 2-week-old leaves followed by 5-week-old and the lowest level was found for 1-week-old leaves (Table 2.1). Overall, red and red+blue light enhanced the anthocyanin content (Table 2.1). There is no significant correlation between disease index and anthocyanin (Figure S2.2D).

2.4 Discussion

2.4.1 Effects of light quality on leaf initiation and petiole elongation in strawberry

Light quality notably influenced both leaf initiation and petiole elongation in strawberry. Leaf initiation rate as determined by the number of leaves is a major parameter for plant growth. In strawberry, this parameter was significantly enhanced in plants grown under red light, while the combined red+blue treatment resulted in the lowest leaf initiation rate (Figure 2.2A). Leaf initiation is mainly driven by light irradiance through the availability of photosynthates, while effects of light quality on leaf initiation are unclear and seem to be species-dependent. Macedo et al. (2011) found that blue light induced the largest number of leaves/plant in *Alternanthera*, when compared to white, red and green light. In contrast, Hernández and Kubota (2016) showed that blue light reduced the number of leaves. Also, red light prolonged the vegetative phase in tomato by increasing the number of leaves before flowering (Cao et al., 2016).

In strawberry, red+blue light reduced petiole length, while petiole length was not suppressed by blue or red light (Figure 2.2B). Internode and petiole length are mediated by cryptochromes and phytochromes. Both red/far-red ratio and blue/red ratio impact these traits (Casal, 2000; Hernández & Kubota, 2016). Van Ieperen et al. (2012) showed that

petiole lengths of cucumber plants under monochromatic red light were 34% longer than under the combination of 30B:70R. In cherry tomato, plants grown under red light almost doubled their plant height compared to the 50B:50R treatment (Liu et al., 2011). Also strawberry petioles were shorter under the combined 50B:50R treatment (Figure 2.2A). This effect is probably due to an antagonistic interaction between phytochromes and cryptochromes (Brown et al., 1995; Nhut et al., 2003).

2.4.2 The effects of leaf age and light quality on *B. cinerea* resistance

Basal resistance against *B. cinerea* in strawberry is considerably influenced by both leaf age and light quality. Leaf resistance varied along with leaf ages with the highest resistance in 4-week-old leaves (Table 2.1), while red light dramatically improved leaf resistance independent of leaf age (Figure 2.3).

Plant and leaf developmental age are important determining factors of stress responses. Plants exhibit complex temporal and spatial variability of leaf development. The developmental phase can be linked to stress susceptibility as during leaf development a range of physiological, biochemical and structural characteristics change (Coleman, 1986). Here, we defined leaf age by recording the time from visible initiation. Strawberry leaves of different developmental age were studied for their influence on resistance against *B. cinerea*. Strawberry leaf resistance to *B. cinerea* gradually increased with developmental stage, and peaked at week 4, then decreased significantly from week 4 to week 5 (Table 2.1). Likewise, Sirjusingh et al. (1996) observed that the resistance of geranium leaves to *B. cinerea* continuously increased as leaf age increased from 1-week-old to 4 weeks, and declined from 4-week-old to 10-week-old. Although *B. cinerea* and *Podosphaera aphanis* that causes powdery mildew on strawberry are different in lifestyle and infection strategy, Amsalem et al. (2006) as well as Carisse and Bouchard (2010) also reported that young strawberry leaves were more susceptible to infection by *P. aphanis* than older ones and this susceptibility of plants was reported to depend on the thickness of the cuticle (Jhooty & McKeen, 1962). These studies indicate the important role of leaf developmental stage in plant defense responses.

Several studies have focused on the effects of different light qualities on the resistance of plant leaves after inoculation with *B. cinerea* (Ahn et al., 2015; Islam et al., 1998; Kim et al., 2013, Xu et al., 2017). However, the influence of light quality on basal host resistance of plant leaves against *B. cinerea* has hardly been studied. Here, we investigated the effect of pre-treatment under different light qualities on the establishment of resistance in strawberry leaves with different leaf ages to *B. cinerea*. Our results demonstrated that red light pre-treatment has a major effect on up-regulating leaf resistance to the necrotrophic pathogen *B.*

cinerea (Figure 2.3), while blue light resulted in a reduced resistance to *B. cinerea*. Strawberry leaves under red+blue light showed an intermediate response (Table 2.1). Previously, Islam et al. (1998) found that a pretreatment with red light for 24 h greatly enhanced the resistance of broad bean to *B. cinerea*, while no significant difference was observed under blue light compared to white. Moreover, this pretreatment under red light notably inhibited infection hypha formation of *B. cinerea* on broad bean leaflets 48 h after inoculation. A low red/far-red ratio which leads to inhibition of phytochrome B severely depressed *Arabidopsis* resistance to *B. cinerea* (Cerrudo et al., 2012). Xu et al. (2017) irradiated detached tomato leaves inoculated with *B. cinerea* with different light qualities and found that red and purple light inhibited lesion development.

2.4.3 Hydrogen peroxide

Hydrogen peroxide levels were affected by both leaf age and light quality and inversely correlated with *B. cinerea* resistance.

Leaf age played an important role in defining hydrogen peroxide level in strawberry leaves, with a decrease from 1-week-old to 4-week-old leaves followed by an increase in 5-week-old leaves. Hydrogen peroxide, as the most stable ROS, regulates many plant developmental processes, such as seed germination, root hair growth and senescence (Singh et al., 2016). In relation to leaf development, Bieker et al. (2012) showed that H_2O_2 levels were higher in young oilseed rape leaves than in old leaves, which is in accordance with our results. In *Arabidopsis*, overall H_2O_2 levels increased when plants started to bolt and flowering indicating that the increase in H_2O_2 appears to be a systemic signal (Bieker et al., 2012; Zimmermann et al., 2006). The increase in H_2O_2 levels from 4-week-old to 5-week-old leaves may also serve a signaling role in strawberry.

In this study, a significant accumulation of H_2O_2 was observed in blue light grown leaves with higher susceptibility to *Botrytis*, while decreased H_2O_2 was found in leaves grown under red light with higher resistance (Table 2.1). In plants, H_2O_2 has two sides in regulating defense reaction (Chamnongpol et al., 1998; Govrin & Levine, 2000). Generally, H_2O_2 is involved in the induction of several defense reactions, such as induction of defense genes, phytoalexin synthesis, and hypersensitive response (Chen et al., 1993). On the other hand, H_2O_2 accumulation is also involved in successful infection by necrotrophic pathogens such as *Botrytis*. Upon *Botrytis* infection, H_2O_2 was generated in and around the penetrated cell wall as well as in the plant plasma membrane, leading to an oxidative burst and hypersensitive response. These oxidative processes resulted in host cell death and contributed to the disease progress (Temme & Tudzynski, 2009). Govrin and Levine (2000) reported that H_2O_2 levels positively correlated with necrosis produced by *B. cinerea* and its growth in

Arabidopsis. In broad beans, salicylic acid-pretreatment enhanced H_2O_2 generation and its accumulation increased leaf susceptibility to *Botrytis*. Moreover, H_2O_2 pretreatment also enhanced lesion formation by *B. cinerea* in broad bean (Khanam et al., 2005a). In strawberry, it is therefore possible that the higher H_2O_2 level in blue-light-leaves reinforced programmed cell death caused by *Botrytis* infection, thus contributing to the disease progress. In contrast, the lower level of H_2O_2 in leaves grown under red light inhibits lesion formation, thus increasing leaf resistance to *B. cinerea*. Interestingly, the study of Xu et al. (2017) with detached tomato leaves inoculated with *B. cinerea* revealed that red light inhibited the oxidative stress caused by disease stress when compared to darkness. Red light rapidly decreased the hydrogen peroxide content to a lower level and inhibited the increase of the superoxide production rate.

2.4.4 Metabolites

In strawberry, it is noteworthy that much higher phenolic and flavonoid levels were observed in 1-week-old leaves compared to older leaves (Table 2.1). With respect to light quality, red light increased phenolics significantly, while red+blue light increased flavonoid levels in 1 to 3-week-old leaves (Figure 2.5A and 2.5C). Proline levels were mainly influenced by light quality. Blue light significantly increased proline content at all leaf ages.

Phenolic compounds are essential secondary metabolites in plant disease resistance (Ashry & Mohamed, 2011), and their synthesis upon light spectrum is species-specific (Taulavuori et al., 2016). In strawberry, red light clearly boosted the synthesis of total phenolics, which is in agreement with the influence of red light on the *Chrysanthemum* cultivars *Bolero* and *Tappino*, but different with the other *Chrysanthemum* cultivars reported by Zheng and Van Labeke (2017). Moreover, both blue and red light are probably needed to regulate the accumulation of phenolics in basil (Taulavuori et al., 2016). In addition, increased accumulation of phenolic compounds is one of the best characterized responses to different stresses (Chérif et al., 2007; Demkura et al., 2010). Phenolics that occur constitutively and function as preformed antimicrobial compounds can directly inhibit spore germination and mycelial growth of pathogenic fungi but also contribute to cell wall thickening to physically protect the plant against pathogen attack (Chérif et al., 2007). Wurms et al. (2003) reported that higher constitutive concentrations of phenolics contributed to the higher constitutive resistance against *B. cinerea* in kiwifruit cultivar *Actinidia chinensis* “Hort 16A”. Accordingly, the highest resistance in red light strawberry leaves in this study might be attributable, in part, to higher total phenolic levels. However, the correlation analysis showed that total phenolic levels had a moderately positive relationship with the disease index. This is due to the fact that 1-week-old leaves, which are very susceptible to *B. cinerea*, had very high total

phenolic levels, indicating that also leaf age played an important role in phenolic content. Consequently, total phenolic levels in strawberry leaves are not a dominant factor that decides basal resistance against *Botrytis*.

Flavonoids are low molecular weight polyphenolic substances produced by plants to protect against pathogens, herbivores, and oxidative cell injury (Agati et al., 2012; Cook & Samman, 1996). The flavonoid content in strawberry leaves varied along with leaf developmental stages, consistently decreasing from 1-week-old to 4-week-old leaves followed by an increase in 5-week-old leaves. Most strikingly, total flavonoid content was much higher in young (1-week-old) than in older leaves (Table 2.1). Likewise, it was observed that flavonol concentrations were higher in young, compared to old leaves in *Sinapis alba* (Reifenrath & Müller, 2007) and more mature plants of *Spinacia oleracea* (Bergqust et al., 2005). There was a moderate positive correlation (r = 0.581) between disease index and flavonoids, which is mainly explained by the high flavonoids content in the susceptible one-week-old leaves.

Blue light significantly increased proline levels, especially in one week and five-week-old strawberry leaves (Figure S2.2A). Blue light also increased proline levels in tomato leaves and stems (Kim et al., 2013) and in *Chrysanthemum* leaves (Zheng & Van Labeke, 2017a). The positive effects of blue light on tomato disease resistance to *B. cinerea* observed by Kim et al. (2013) are probably due to a direct inhibitory effect of blue light on mycelium growth, as shown by Xu et al. (2017) rather than by plant-mediated defense mechanisms.

2.4.5 Leaf pigments

Total chlorophyll and carotenoid levels were positively influenced by leaf age and negatively influenced by blue light (Table 2.1 and Figure 2.6A and 2.6C).

Decreased chlorophyll was one of the most striking phenomena in strawberry leaves grown under blue light. It is reported that chlorophyll involved in photosynthesis is regulated by phyB or red and far-red lights, and low red/far-red ratio or reduced *phyB* expression decreases chlorophyll concentration in many species (Distefano et al., 2013; Heraut-Born et al., 1999; Tucker, 1981). Therefore, the lower chlorophyll content under blue may be due to lack of red light. Furthermore, *phyB* mutants or plants exposed to low red:far-red light with decreased *phyB* expression exhibited increased susceptibility to insects or pathogens (Cerrudo et al., 2012; Islam et al., 1998; Kazan & Manners, 2011b). Moreover, the pale color of leaves due to a lower chlorophyll content contributes to the enhanced sensitivity of plants to pathogen and herbivores (Demotes-Mainard et al., 2016). As a consequence, lower chlorophyll content in young leaves or under blue light, to some extent, led to the increased leaf susceptibility to *B. cinerea* in strawberry. In addition, the chl a/b ratio is modified by

environmental factors and varies according to light conditions (Lichtenthaler, 1987). Many studies have reported that both monochromatic blue and higher proportion of blue light in plant lighting tend to increase the chl a/b ratio (Hogewoning et al., 2010; Wang et al., 2015; Huché-Thélier et al., 2016), which is in line with our results. Plants with less chlorophyll are reported to have higher absorbance of blue light (Abadía et al., 1999), indicating plants highly adapt to light environment. Comprehensively, the lower chlorophyll content in young leaves or in blue light-grown strawberry leaves leads to the higher sensitivity to *B. cinerea*. Since there was a good negative correlation (r = -0.684) between disease index and total chlorophyll, its concentration in strawberry leaves plays a significant role in determining the susceptibility to grey mold depending on both leaf age and light quality.

Carotenoids are not only essential pigments for photosynthesis but also efficient antioxidants protecting plants against oxidative stresses (Stahl & Sies, 2003). A significant decrease was found in carotenoids in blue light grown-strawberry leaves compared to the other treatments, while no difference was observed in cherry tomato seedlings under blue light compared to red, green, orange, and combinations (Liu et al., 2012). Zheng and Van Labeke (2017) also reported no total carotenoids differences in red, blue and red+blue growth *Chrysanthemum*. It can be concluded that carotenoids synthesis regulated by light quality is dependent on plant species. Carotenoids are involved in ROS scavenging and also in reducing the generation of radicals and singlet oxygen (Stahl & Sies, 2003). The ROS generated in blue light-grown leaves is probably not efficiently scavenged due to lower carotenoids levels in these leaves. This may be one of the reasons why leaves grown under blue light have a higher susceptibility to *Botrytis,* which also can be confirmed by the negative correlation between disease index and carotenoids (r = -0.660).

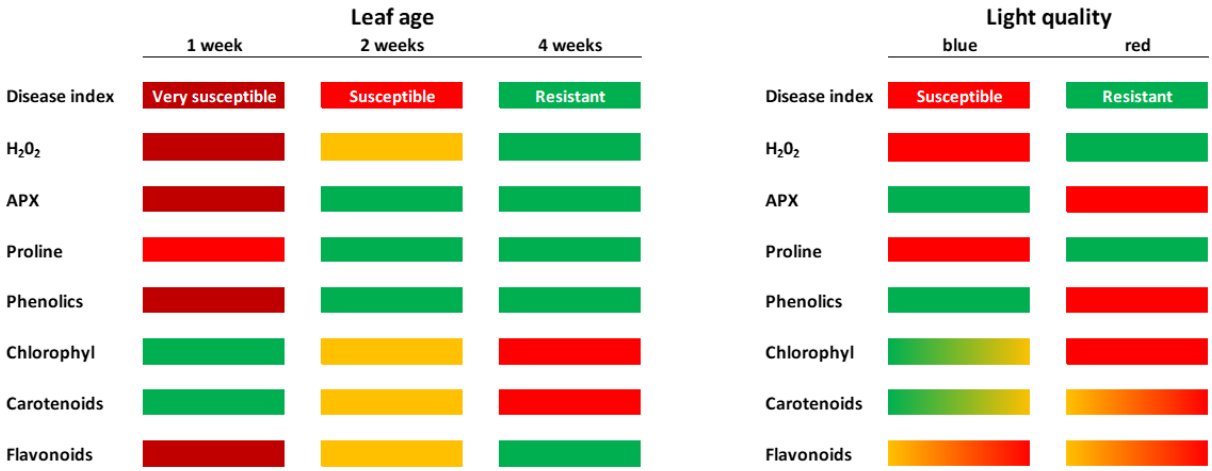


Figure 2.7 Effect of leaf age and light quality on various plant parameters. Color codes are given based on the values and corresponding statistical grouping given in Table 1 using the conditional formatting tool in Excel. Green: low levels; Orange: intermediate levels; Red: high levels; Dark red: very high levels.

2.5 Conclusion

Leaf developmental stage is an intrinsic property of plants, while light quality is an environmental factor. In this study, it is clearly shown that leaf age and light quality are two forces that considerably influence leaf resistance to *B. cinerea*. Leaf resistance consistently increased from 1-week-old to 4-week-old leaves but decreased in 5-week-old leaves independent of light quality, while red light significantly improved leaf resistance in each leaf developmental stage. The three parameters that best correlated with basal leaf resistance to *B. cinerea* are low hydrogen peroxide levels and high total chlorophyll and carotenoids levels (Figure 2.7). However, Figure 2.7 also shows that contrasting effects of leaf age and light quality on parameters such as total phenolics and APX levels, may mask possible effects of these parameters on *B. cinerea* leaf resistance. In general, our study reveals that leaf age should be taken into account when assessing the effect of light quality on disease resistance.

2.6 Supplementary material

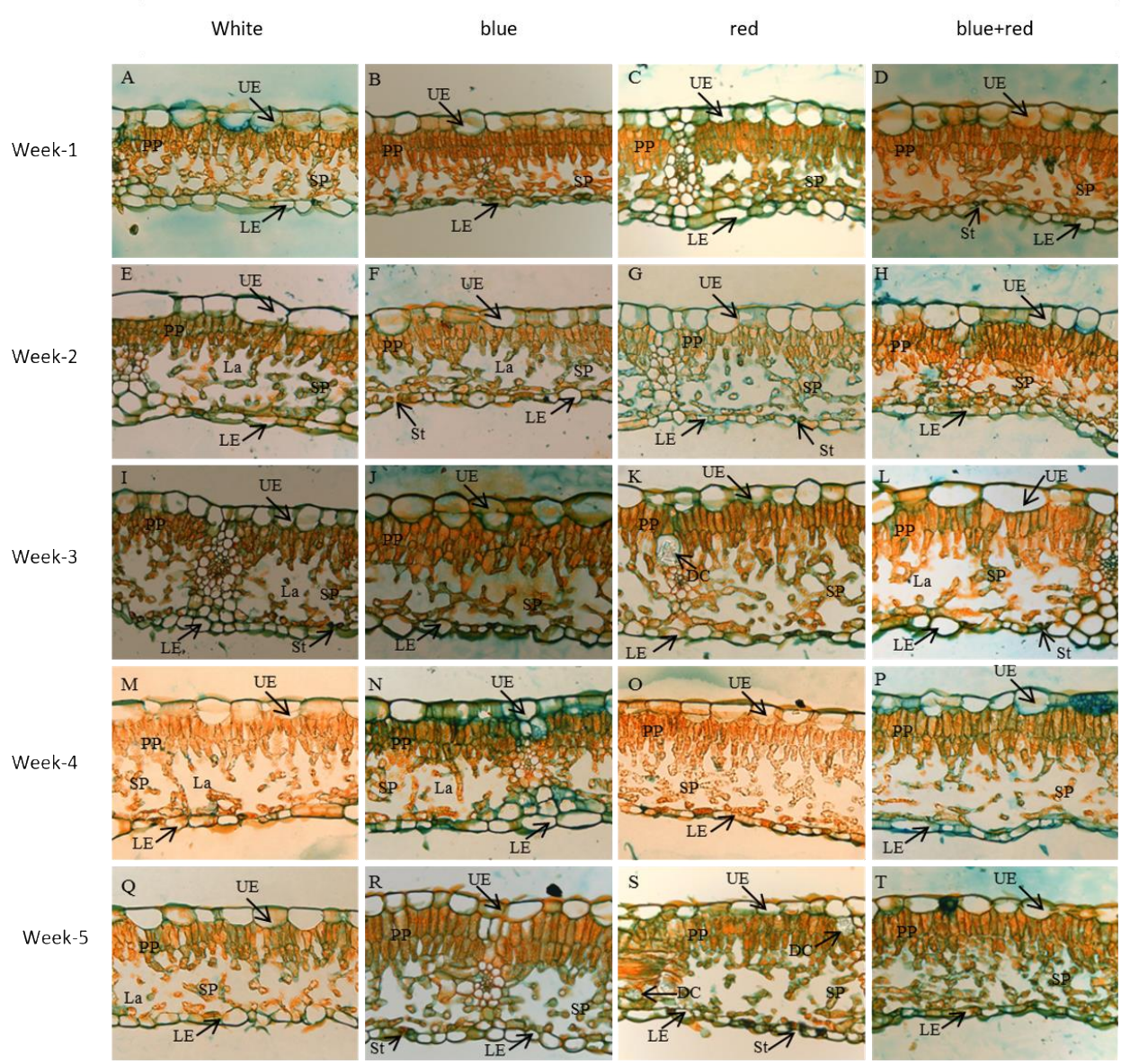


Figure S2.1 Cross-sections of strawberry leaves grown under white (A, E, I, M,Q), blue (B, F, J, N, R), red (C, G, K, O, S), red+blue (D, H, L, P, T) LED lights and aged from 1-week-old to 5-week-old. Week 1: A, B, C, D; week 2: E, F, G, H; week 3: I, J, K, L; week 4: M, N, O, P; week 5: Q, R, S, T. UE: upper epidermis; LE: lower epidermis; PP: palisade parenchyma; SP: spongy parenchyma; DC: druse crystals; La: lacunae; St: stomata.

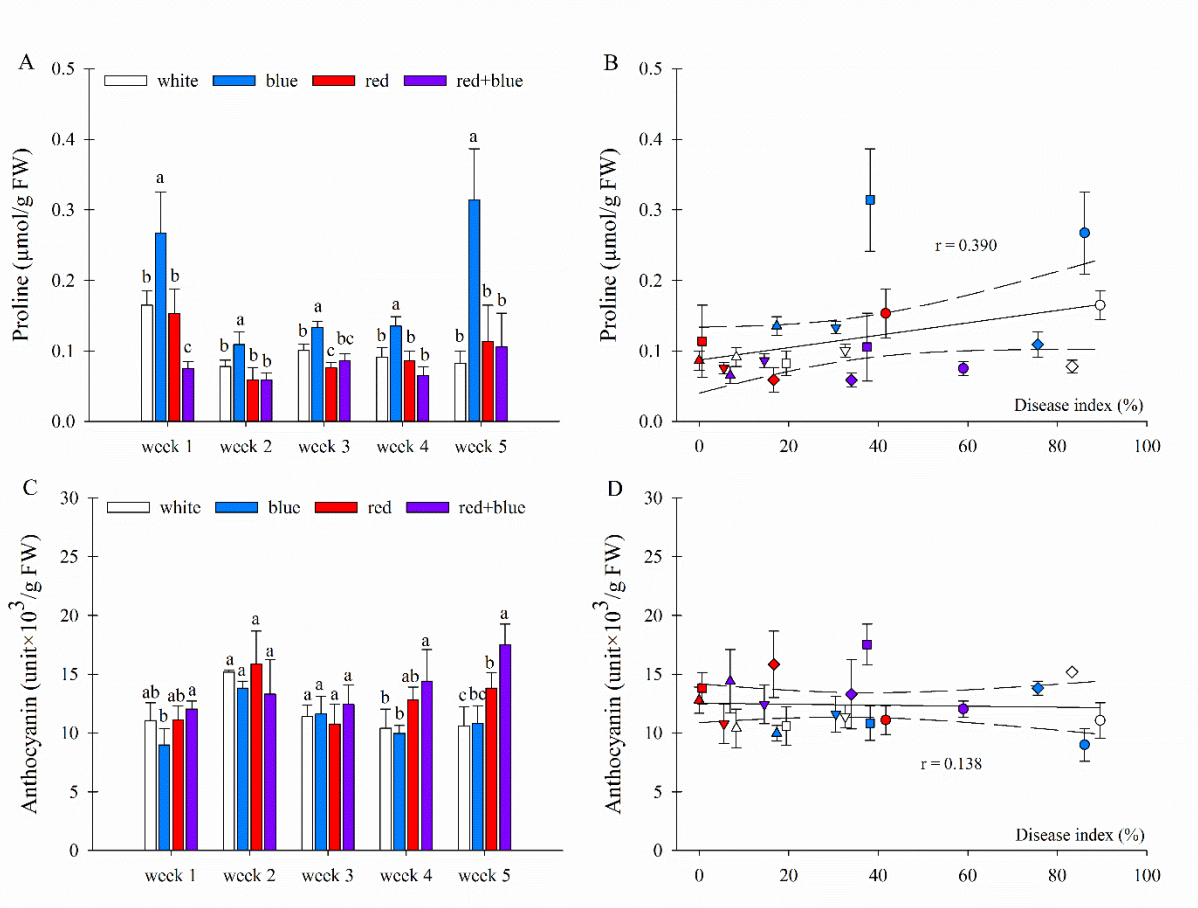


Figure S2.2 Effects of light quality on proline (A), anthocyanin (C) and their correlations with disease index in 1-week old to 5-week old strawberry leaves. Data shown are mean values (± S.D.) from four replicates. Different symbols mean different leaf ages: ○, 1-week-old; ◊, 2-week-old; ▽, 3-week-old; △, 4-week-old; □, 5-week-old; colors show the light quality treatment: white (white color); blue (blue color); red (red color) and red+blue (purple color). Different letters indicate significant differences between values (Tukey test, $p \leq 0.05$).

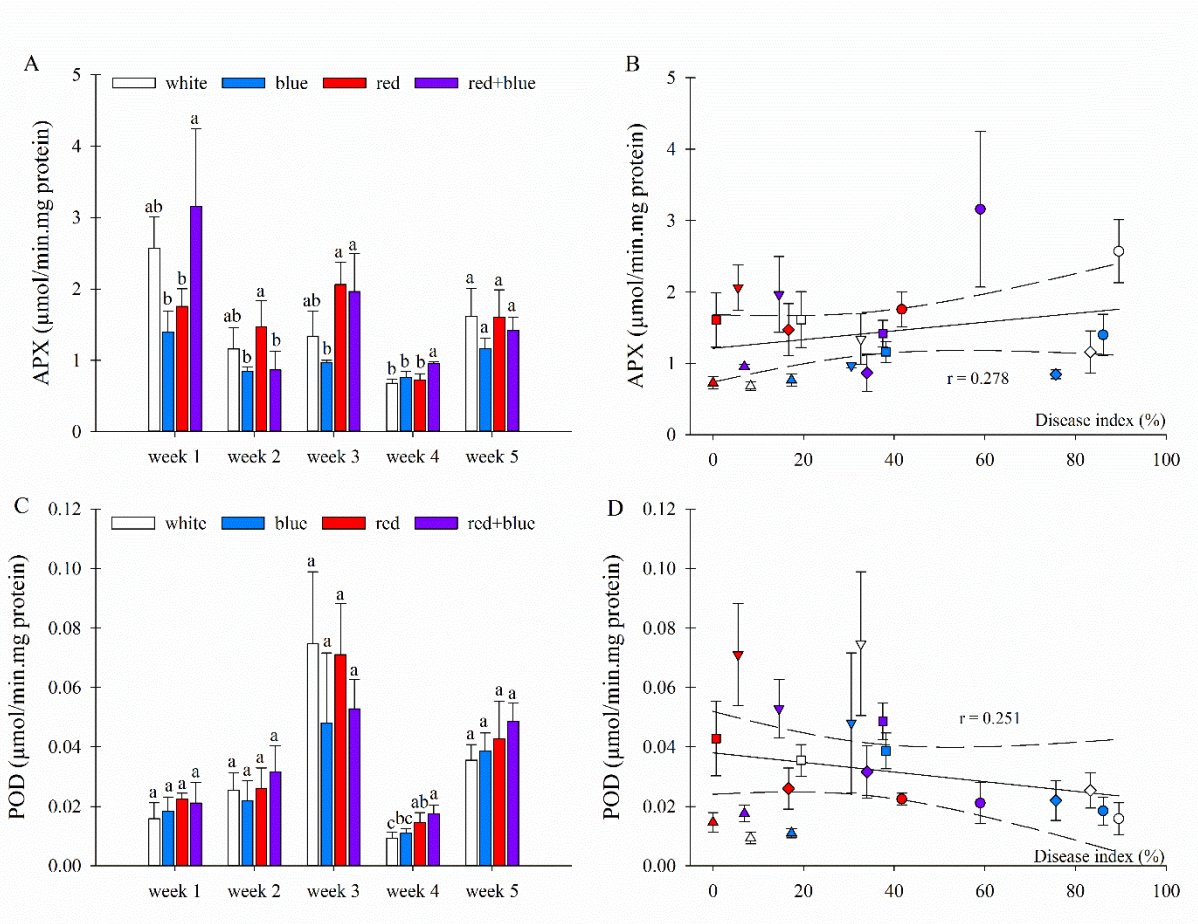


Figure S2.3 Effects of light quality on APX (A), POD (C) and their correlations with disease index in 1-week old to 5-week old strawberry leaves. The mean and SD of the activities from four replicates are presented. Different symbols mean different leaf ages: ○, 1-week-old; ◊, 2-week-old; ▽, 3-week-old; △, 4-week-old; □, 5-week-old; colors show the light quality treatment: white (white color); blue (blue color); red (red color) and red+blue (purple color). Different letters indicate significant differences between values (Tukey test, $p \leq 0.05$).

Chapter 3

Timing of Light Quality Affects Susceptibility to *Botrytis cinerea* in Strawberry Leaves

This chapter is based on:

Lijuan Meng, Marie-Christine Van Labeke, Monica Höfte (2020). Timing of light quality affects susceptibility to *Botrytis cinerea* in strawberry leaves. *Journal of Photochemistry and Photobiology B: Biology* 211: 111988.

Abstract

Light plays an essential role in the outcome of plant-pathogen interactions. In this study, the effect of pre- and post-inoculation light quality on *Botrytis cinerea* disease development in strawberry leaves was investigated. Circadian variations in leaf susceptibility to *Botrytis cinerea* were tested by inoculating at 5 h, 12 h, or 16 h after the start of photoperiod. The effects of 4 h and/or 12 h of red or blue light combined with white light in a 16 h photoperiod on leaf susceptibility and metabolites were studied. Light presence significantly decreased leaf susceptibility to *Botrytis* irrespective of light quality. Leaf susceptibility to *B. cinerea* showed significant circadian variations. Susceptibility was highest in leaves inoculated 12 h after the start of photoperiod followed by incubation in the dark. When disease development took place under the light regime, the shorter the light period after inoculation the stronger the disease symptoms. Twelve hours of red light radiation in a 16 h photoperiod increased, while 12 h blue light radiation decreased leaf resistance, resulting in alterations in the circadian variation of leaf susceptibility. These alterations correlated with changes in leaf metabolites, such as hydrogen peroxide, total phenolics and hexoses. Overall, we conclude that twelve hours of red light followed by white light in a 16 h photoperiod efficiently increased leaf resistance to *B. cinerea*.

3.1 Introduction

Light is an essential environmental factor in almost all ecosystems by being a source of energy, information as well as stress. Light is crucial for successful competition and survival in nature. Plants perceive information about light via photoreceptors that regulate growth and development, defense responses, and the circadian clock (Ballaré, 2014; Kami et al., 2010). Arabidopsis presents a diurnal variation in susceptibility to *B. cinerea*, and inactivation of phytochrome B by light with a low red/far-red ratio reduced its resistance by down-regulation of defense marker gene expression (Cerrudo et al., 2012; Ingle et al., 2015). By contrast, red light irradiation enhances disease resistance in plants. Red light irradiance induced resistance to *Botrytis cinerea* in broad bean (Islam et al., 1998). Khanam et al., (2005a) further confirmed this induction by red light, and hypothesized that induction of antioxidant enzyme catalase activity contributes to elicitor-dependent production of antifungal compounds by living host cells. A study on the development of broad bean leaves and red light-induced resistance against *B. cinerea*, concluded that the induced resistance by red light possibly depends on host photosynthesis and protein synthesis (Rahman et al., 2002). In strawberry, induced resistance against *B. cinerea* by red light correlated with decreased hydrogen peroxide levels (Meng et al., 2019).

Likewise, filamentous fungi maintain a multifaceted regulatory network to sense light as a signal to induce adaptive responses, to guide growth and to control development (Schumacher, 2017). In *B. cinerea*, light, or its absence, triggers absolute responses with exclusive production of conidia in the light and exclusive sclerotia formation in constant darkness. Interestingly, more conidia are formed by cultures that are incubated in light-dark cycles than in constant light. Tan & co-workers performed extensive experiments with different light qualities and showed that both conidial and sclerotial development were reduced by blue light, whereas near-UV alone led to the conidiation (Tan & Epton, 1973, 1974). Moreover, Islam et al. (1998) found that near-UV, blue and far-red light marginally inhibited germ tube growth and induced negative phototropism with increasing numbers of penetrating germ tubes. Red light, however, increased germ tube elongation and induced positive phototropism, which resulted in a high proportion of germ tubes merely growing across the surface of the host tissue. Taken together, the importance of light with respect to the outcome of plant-pathogen interaction involves its effects on both the host response and virulence of the attacking pathogen (Roden & Ingle, 2009).

In nature, day and night are cycled in 24 hours. To adapt to this day/night cycle, plants have accordingly evolved a time-keeper, the circadian clock, that regulates their physiology, behavior, and response. The proper matching between the plant circadian clock and the

external environment not only enhances the ability of plants to sense harsh conditions but also allows to temporally gate the responses to environmental stresses in a timely manner. Thus, plant responsiveness to both abiotic and biotic stresses is under circadian control. For example, rhythmic changes in chilling and heat resistance were reported in cotton by Rikin et al. (1993). Cotton leaves were most sensitive to cold during the light period and most resistant during the dark period, while the heat-resistant phase developed in the light period and the heat-sensitive phase was observed in the evening. Similarly, defense responses of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* are regulated by the circadian clock. Plants show a time-based oscillation over the day in their resistance to *P. syringae* with a peak resistance at dawn. This temporal resistance pattern was disrupted when the clock gene, *CCA1 (CIRCADIAN CLOCK ASSOCIATED 1)* or *ELF 3*, was miss-expressed, thus supporting a direct role of the circadian clock in defense regulation (Bhardwaj et al., 2011). In addition, the plant circadian clock regulates the susceptibility to *B. cinerea*, which is driven by the jasmonate signaling pathway in Arabidopsis. A resistance peak occurred at dawn, then decreased continuously to midnight (Ingle et al., 2015). In turn, activation of defense responses by pathogens can regulate the plant clock activity. Zhang et al. (2013) firstly showed that defenses activated by infection with both virulent and avirulent *Pseudomonas syringae* were able to shorten the circadian period in Arabidopsis. The core-clock regulator, CCA1 was altered in its expression in plants when infected with the downy mildew pathogen *Hyaloperonospora arabidopsis* Emwa1 (Wang et al., 2011). Therefore, the circadian regulation is of importance in the outcome of plant-pathogen interactions.

Light qualities with different spectra influence plant defense responses. In strawberry, monochromatic red light predominantly inhibited disease symptoms caused by *Botrytis cinerea* (Meng et al., 2019). However, for several crops it has been reported that leaves that develop under monochromatic red light display a dysfunctional photosynthesis with a lower CO_2 fixation (Hogewoning et al., 2010). Therefore, it is important to know if a shorter red light treatment during the photoperiod could still induce this resistance to *Botrytis*, while reducing the potential negative effects on primary metabolism.

In our previous research (Meng et al., 2019), we only tested the effects of pretreatment with different light qualities on leaf resistance to *B. cinerea* since inoculated leaves were incubated in constant darkness. In addition, leaves were always inoculated 5 h after the start of the photoperiod, so the influence of time of inoculation on disease resistance was not assessed. Based on the information given above and our previous research, three research questions were formulated. First, how does the disease develop if inoculated leaves are incubated under light/dark cycles? Second, does the circadian clock have an effect on leaf susceptibility depending on the time of inoculation? Third, how many hours of red or blue

light are needed in the photoperiod to have an effect on disease resistance? According to these questions, we explored the effects of light/dark incubation on the infection with *Botrytis cinerea* in this study. Moreover, circadian variation in leaf susceptibility and leaf metabolism was investigated, and the effects on leaf resistance of 4 h or 12 h light quality treatments in a 16 h photoperiod were tested.

3.2 Materials and Methods

3.2.1 Plant material and light experiments

Strawberry (*Fragaria* × *ananassa* 'Elsanta') plants were used throughout this study. Cold-stored young plants were potted in 0.3 L pots using a peat-based potting soil (Van Israel nv, Belgium) and grown in a climate room under a photoperiod of 16 h at 20°C and an average relative humidity of 60%. The photosynthetic photon flux density of the light treatments was 100 µmol m^{-2} s^{-1}. The plants were fertilized with Soluplant nutrition (Haifa, The Netherlands, EC=1.5 dS/m, pH=5.7) three times a week. Light treatments were separated by non-reflective dark screens.

In experiment 1, four light treatments were applied: a control with sun-like white light (300-900 nm, Gavita light-emitting plasma lamps, The Netherlands) and three LEDs treatments: blue light (B, peak at 460 nm, Philips, the Netherlands), red light (R, peak at 660 nm, Philips, The Netherlands), and red+blue light (RB, 60%/40%, peak at 460 nm and 660 nm, CID -800 programmable LED lighting system, CID Bio-Science USA) (Figure 3.1A, Figure S3.1).

In experiment 2, three light treatments were applied: white light (16 h), white (12h) followed by 4 h blue light and white (12 h) followed by 4 h red light. White light was provided as sun-like wide spectrum light LED growth light (NS1, Valoya, Finland) (Figure 3.1B, Figure S3.1).

In experiment 3, three light treatments were applied: white light (16 h), blue light (12h) followed by 4 h white light, and red light (12h) followed by 4h white light. White light was provided as sun-like wide spectrum light LED growth light (NS1, Valoya, Finland) (Figure 3.1C, Figure S3.1).

In the three experiments, strawberry plants were exposed to the indicated light treatments for 2 weeks until sampling.

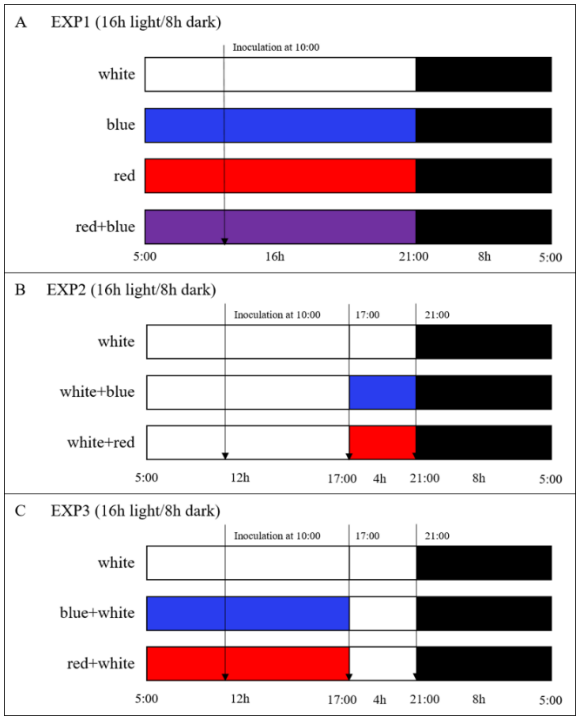


Figure 3.1 Experimental set-up used in this study. Light treatments and *B. cinerea* inoculation times are shown. In the three experiments, the photoperiod was 16 h and the dark period 8h. A. Experiment 1: four light qualities were tested (16 h W, 16 h B, 16 h R, and 16 h R+B) and *B. cinerea* was inoculated 5 h after the start of the photoperiod. B. Experiment 2: plants were exposed to three different light regimes at the end of the photoperiod (12 h W + 4 h W, 12 h W + 4 h B, 12 h W + 4 h R) and inoculated with *B. cinerea* 5 h, 12 h or 16 h after the start of the photoperiod. C. Experiment 3: plants were exposed to three light regimes at the start of the photoperiod (12 h W + 4 h W, 12 h B + 4 h W, 12 h R + 4 h W) and B. cinerea was inoculated 5 h, 12 h or 16 h after the start of the photoperiod. In all experiments *B. cinerea* was inoculated on leaf discs prepared from 2-week-old leaves. Inoculated leaf discs were incubated under the same light regime or in the dark.

3.2.2 Disease assays

Conidia were collected from 10-day-old *B. cinerea* strain R16 (Faretra & Pollastro, 1991) cultures grown on potato dextrose agar medium (PDA, Becton, Dickinson, and Company) at 24 °C. A suspension was prepared by flooding culture plates with sterilized ¼ potato dextrose broth (PDB, Becton, Dickinson, and Company) containing 0.01% (v/v) Tween 20 (MERCK, Germamy). After filtering the mycelium fragments over cheesecloth, the spore concentration was adjusted to 5×10^5 spores/ml with a haemocytometer. Four individual plants were used for each inoculation assay, this was repeated three times (n = 12). Of each plant, two-week-old fully developed strawberry leaves were used for the inoculation. Twenty-four leaf discs (1-cm-diameter) from each leaf were randomly divided into two incubation treatments, incubation in the light cycle or incubation in the dark. Leaf discs were inoculated with 10 µL droplets of the *B. cinerea* spore suspension on the upper leaf surface. For

incubation in the light cycle microplates were placed back to the respective light treatments after inoculation.

Four individual plants were used for each inoculation assay, this was repeated three times with different plants (n = 12). Of each plant from each light treatment (Figure 3.1), two-week-old fully developed strawberry leaves were sampled for the inoculation. Twenty-four leaf discs (1-cm-diameter) from each leaf were randomly divided into two identical groups and inoculated with 10 µL droplets of the *B. cinerea* spore suspension on the upper leaf surface. After inoculation, one group of leaf discs was immediately placed back to the respective light treatments in Figure 3.1, the other group was immediately placed in the dark.

Depending on the experiment one or three time sampling points during the light cycle were taken. In experiment 1, the inoculation was conducted 5 hours after the start of the photoperiod (10:00). In experiment 2 and 3, leaves were inoculated 5 h, 12 h and 16 h after the start of the photoperiod (10:00, 17:00 and 21:00).

Disease severity and lesion spreading were recorded four days post inoculation. Disease ratings were expressed using four scoring categories for the lesion developments: 0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion.

3.2.3 Determination of leaf metabolism

Leaf material (2-week-old) was collected in Experiment 3 every four hours over a 24 h light/dark period at 0 h, 4 h, 8 h, 12 h, 16 h, and 20 h after the start of photoperiod. Leaf material was ground in liquid nitrogen and stored at -80°C until analysis.

Leaf H_2O_2 levels were determined with 1% TCA and 1 M KI in potassium phosphate buffer (pH 5.8) (Junglee et al., 2014). Leaf material was extracted in 80% methanol for total phenolic and total flavonoid analysis. Phenolics were measured according to the Folin-Ciocalteu method and flavonoids were determined using the aluminum chloride method (Hong et al., 2008). The leaf chlorophyll and carotenoid content were extracted in 80% acetone for 24 h. The supernatant was measured with a spectrophotometer (Infinite 200, Tecan Group Ltd. Switzerland) (Chen et al., 2004) and leaf chlorophylls and carotenoids were calculated according to Lichtenthaler & Buschmann (2001). Leaf soluble sugars were extracted in 80% ethanol for 3 h at 45˚C and the supernatant was purified with PVPP (Polyvinylpolypyrrolidone, AppliChem, Germany). The concentration of glucose, fructose and sucrose in filtered (0.45 µm, Millipore) diluted samples were quantified using Ultra-Performance Liquid Chromatography (UPLC) with a PA20 column (Christiaens et al., 2016).

3.2.4 Trypan blue staining

B. cinerea hyphae in infected plant tissues were visualized with trypan blue staining according to Canessa et al. (2013) with small modifications. Briefly, leaf discs were washed in absolute ethanol with gentle agitation to remove chlorophyll. Thereafter, leaves were incubated in trypan blue (ACROS, Organics) staining solution for 30 min, destained and transferred into a 50% glycerol solution. Bright-field microscopy was performed with an Olympus BX-51 microscope and images were captured with a ColorView Ⅲcamera and edited with the software package CELL-F (Olympus Soft Imaging Solutions).

3.2.5 Statistical analysis

Statistical analysis was performed using the SPSS System (SPSS 25.0, SPSS Inc, Chicago, USA). Disease rating data were analyzed by Kruskall-Wallis test followed by a post-hoc Dunn's test ($p \leq 0.05$, n=12). Friedman two-way ANOVA was used to compare dark-incubated treatments with light-incubated treatments. Significant differences were calculated at $p < 0.001$. For leaf metabolism assays post-hoc comparisons were conducted using Tukey HSD test ($p \leq 0.05$, n=5).

3.3 Results

3.3.1 Influence of light quality before and during infection on disease symptom development (Experiment 1)

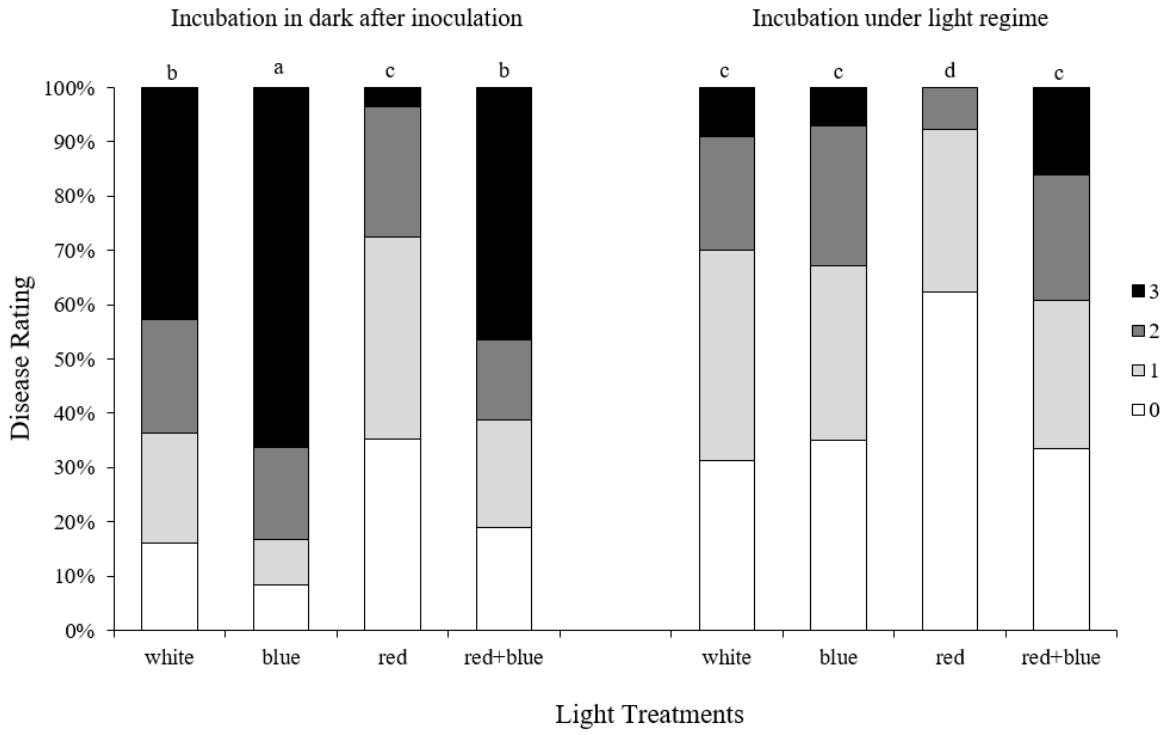


Figure 3.2 Effect of light quality (white, blue, red, red+blue) on disease symptoms of strawberry leaf discs inoculated with *Botrytis cinerea* 5 h after the start of the light period (at 10:00). Strawberry leaves were grown under white, blue, red and red+blue light conditions. After inoculation, leaf discs were incubated in the dark or under the original growth light conditions (incubation under light regime). Disease severity was assessed four days post inoculation using a 0-3 scoring system (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). Different letters indicate statistically significant differences among the treatments (n=12, Kruskall-Wallis followed by a post-hoc Dunn's test, $p \leq 0.05$).

Leaf discs inoculated with *B. cinerea* and incubated under dark conditions showed significantly more disease symptoms than leaf discs incubated under light, irrespective of the light quality (Figure 3.2). Red light-grown leaves were the least susceptible to *B. cinerea*, while blue light-grown leaves were the most susceptible. Leaves that had developed under white or red+blue light showed an intermediate susceptibility. Disease development on inoculated leaf discs exposed to white, blue or red+blue light was similar, but clearly lower on red light exposed leaf discs.

3.3.2 Influence of 4 h end-of-day light quality treatment and time of inoculation on disease development (Experiment 2)

Time-of-day differences in susceptibility to *B. cinerea* were observed for all treatments (Figure 3.3A-C) while disease severity was influenced by the presence/absence of light during incubation (Figure 3.3). As in Exp 1, disease development was stronger if post-inoculation conditions were in the dark compared to leaves that remained under their respective light regimes.

The highest susceptibility to *Botrytis* was found for dark-incubated leaves that were inoculated 12 h after the start of the photoperiod. However, when inoculated leaves remained under their respective light regimes, the highest susceptibility to *Botrytis* was observed 16 h after the start of the photoperiod (start of the night phase), followed by 12 h white light after the start of photoperiod, and was lowest when leaves were inoculated after 5 h of white light (Figure 3.3A-C).

Overall, susceptibility of the strawberry leaves to *B. cinerea* was highest 12 h after the start of the photoperiod followed by post-inoculation in the dark. If disease development took place under the light regime, the shorter the light period after inoculation the stronger the disease symptoms (Figure 3.3D).

A four hour end-of-day light quality treatment had multiple effects on leaf resistance to *B. cinerea* (Figure S3.2). In general, four hours of blue light had no significant positive or negative influence on leaf resistance, but it reduced disease symptoms when inoculation took place 16 h after the start of photoperiod and leaves were incubated under the same light regime (Figure S3.2C). Four hours end-of-day radiation of red light had variable effects depending on the time of inoculation and dark or light incubation (Figure S3.2). Overall, no significant differences were observed on leaf resistance by 4 h blue or red light at the end of photoperiod irrespective of inoculation time (Figure S3.2D).

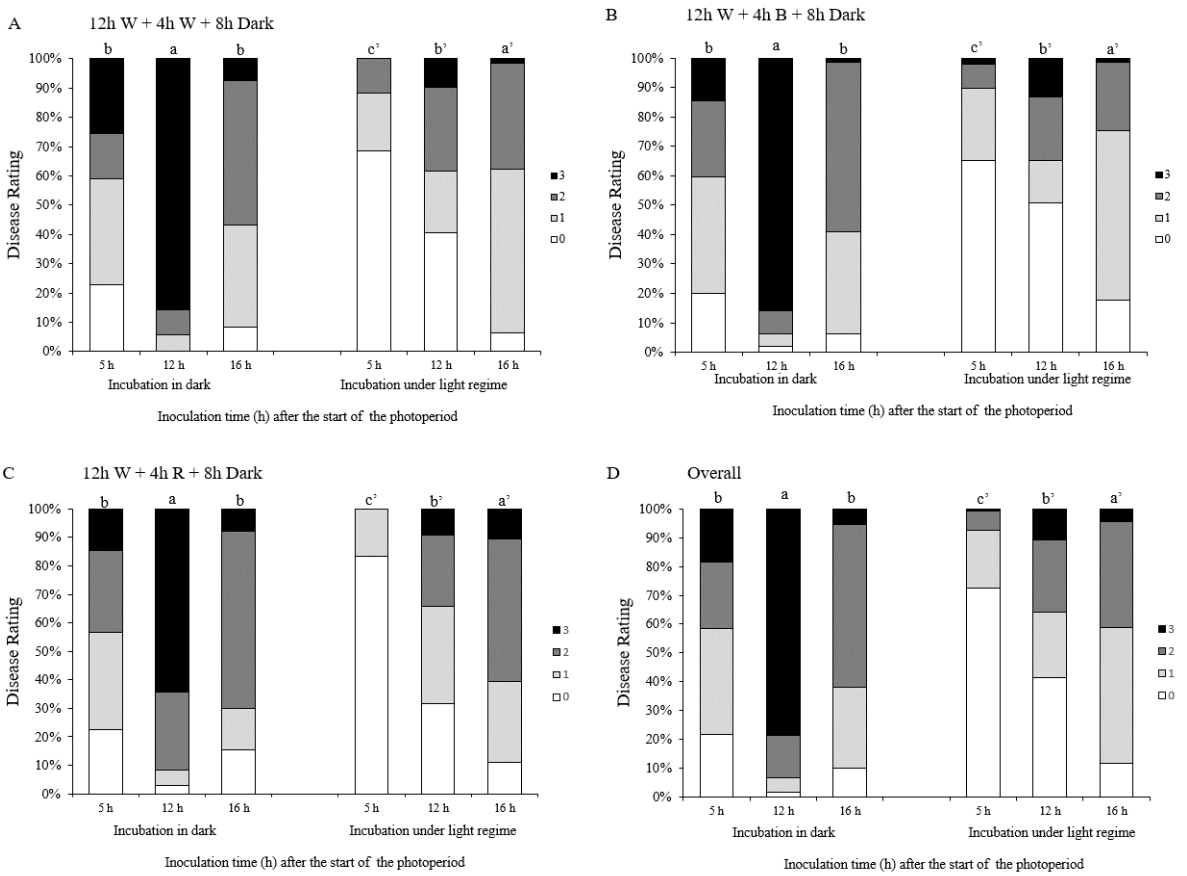


Figure 3.3 Effect of time of inoculation with *B. cinerea* (5 h, 12 h, or 16 h after the start of the photoperiod) on disease symptoms of strawberry leaves grown under different light conditions: A. 12 h W + 4 h W, B. 12 h W +4 h B, C. 12 h W + 4 h R, D. Overall disease symptoms independent of light conditions. Disease severity was assessed four days post inoculation using a 0-3 scoring system (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). Different letters (without prime for dark incubation and with prime for incubation under light regime) show significant difference among the treatments (n=12, Kruskall-Wallis followed by a post-hoc Dunn's test, $p \leq 0.05$). For all treatments, differences in disease severity between incubation in dark or under light regime were significant (Friedman Test, $p < 0.001$).

3.3.3 Influence of 12 h light quality treatment at the start of the photoperiod and time of inoculation on disease symptoms (Experiment 3)

The effect of prolonged light quality application (12 h) at the start of photoperiod on leaf susceptibility to *Botrytis* was investigated. Leaves were inoculated during the light quality treatment (10:00 h, 5 h after the start of the photoperiod), when light quality changed back to white light (17:00 h, 12 h after the start of the photoperiod) or after 4 h of white light (21:00 h, 16 h after the start of the photoperiod (Figure 3.1C). A noticeable decrease in leaf basal resistance under 12 h B + 4 h W, and an increase under 12 h R + 4 h W were noted in all treatments when infection developed in the dark (Figure 3.4A-C).

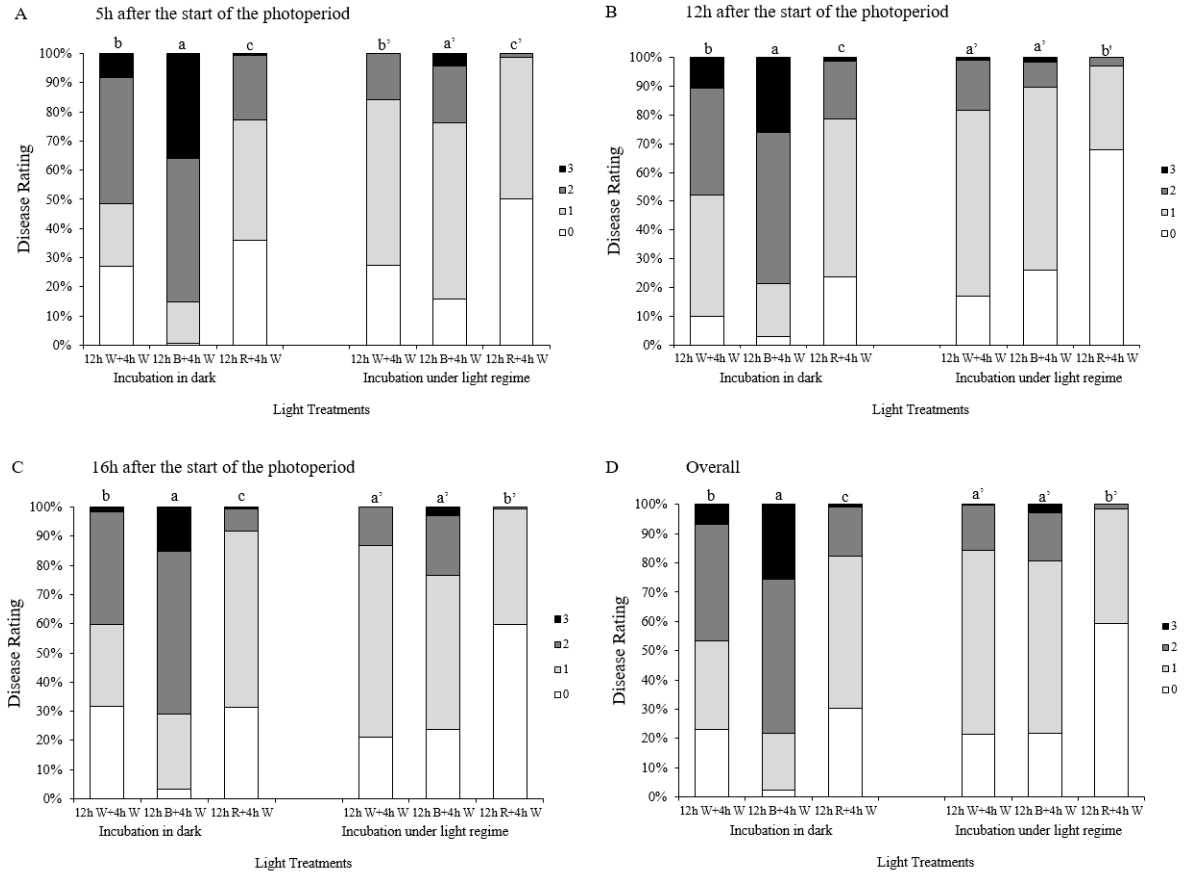


Figure 3.4 Effect of 12 h light quality treatments at the start of photoperiod and different times of inoculation on disease severity. A, inoculation at 5 h after the start of photoperiod; B, 12 h after the start of photoperiod; C, 16 h after the start of photoperiod; D, overall disease symptoms independent of inoculation time. Disease severity was assessed four days post inoculation using a 0-3 scoring system (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). Different letters (without prime for dark incubation and with prime for incubation under light regime) show significant difference among the light treatments (n=12, Kruskall-Wallis followed by a post-hoc Dunn's test, $p \leq 0.05$). For all treatments, differences in disease severity between incubation in dark or under light regime were significant (Friedman Test, $p < 0.001$).

However, blue and red light had different influential patterns when leaves remained under the light regime after inoculation. Blue light significantly increased disease severity in leaves inoculated 5 h after the start of the photoperiod, while red light reduced disease development in comparison with white light (Figure 3.4A). When leaves were inoculated 12 h and 16 h after the start of the photoperiod, only red light had a notable effect and decreased disease ratings (Figure 3.4B and C).

Again, disease symptoms were more severe in dark-incubated leaf discs as opposed to those incubated under the light regime. Overall, 12 h blue light significantly increased leaf susceptibility to *Botrytis*, while 12 h red light resulted in a decrease. When disease

developed under the light regime, only 12 h red light significantly reduced leaf susceptibility (Figure 3.4D).

Twelve hours of blue or red light at the start of photoperiod altered the effect of inoculation time on leaf susceptibility to *B. cinerea* in light-incubated leaves when compared to the white light treatment. White light-treated and incubated leaves were most resistant when inoculated 5 h after the start of the photoperiod, while red or blue light-treated and incubated leaves were most resistant when inoculation took place 12 h after the start of the photoperiod (Figure S3.3A-C). Overall, only a significant decrease in disease severity was observed in leaves inoculated 16 h after the start of photoperiod and incubated in the dark (Figure S3.3D).

3.3.4 Influence of 12 h light quality treatment at the start of photoperiod on diurnal variations in leaf metabolism

We explored the diurnal variations in hydrogen peroxide, flavonoids, phenolics, pigments and sugars in strawberry leaves subjected to the different light treatments in Exp. 3 (Figure 3.5 and S3.4).

Under white light, leaf hydrogen peroxide levels peaked at midnight (4 h after the start of night) and were lowest at both 8 and 16 h after the start of photoperiod. The diurnal fluctuation of hydrogen peroxide levels disappeared when given 12h blue light and altered when given12 h red light irradiance in the photoperiod. Irrespective of sampling times, the overall level of hydrogen peroxide was highest in the leaves from 12 h B + 4 h W, followed by 12 h W + 4 h W. The leaves grown under 12 h R + 4 h W lights had the lowest overall level of hydrogen peroxide (Figure 3.5A).

For each light treatment, the level of total phenolics peaked at 4 h after the start of photoperiod. Strawberry leaves treated with 12 h blue light had significant lower phenolic levels at 8 h and 12 h after the start of the photoperiod compared to 12 h white light. The overall levels of total phenolics in leaves exposed to 12 h blue light were considerably lower compared to white light-exposed leaves, independent of sampling time (Figure 3.5B).

The circadian fluctuation of hexoses was greatly influenced by 12 h blue/red light treatments. Strawberry leaves from 12 h blue or red light treatments showed different peaks from those from white light. Twelve hours of red light significantly increased the overall level of hexoses in strawberry leaves compared to white light, irrespective of sampling time (Figure 3.5C).

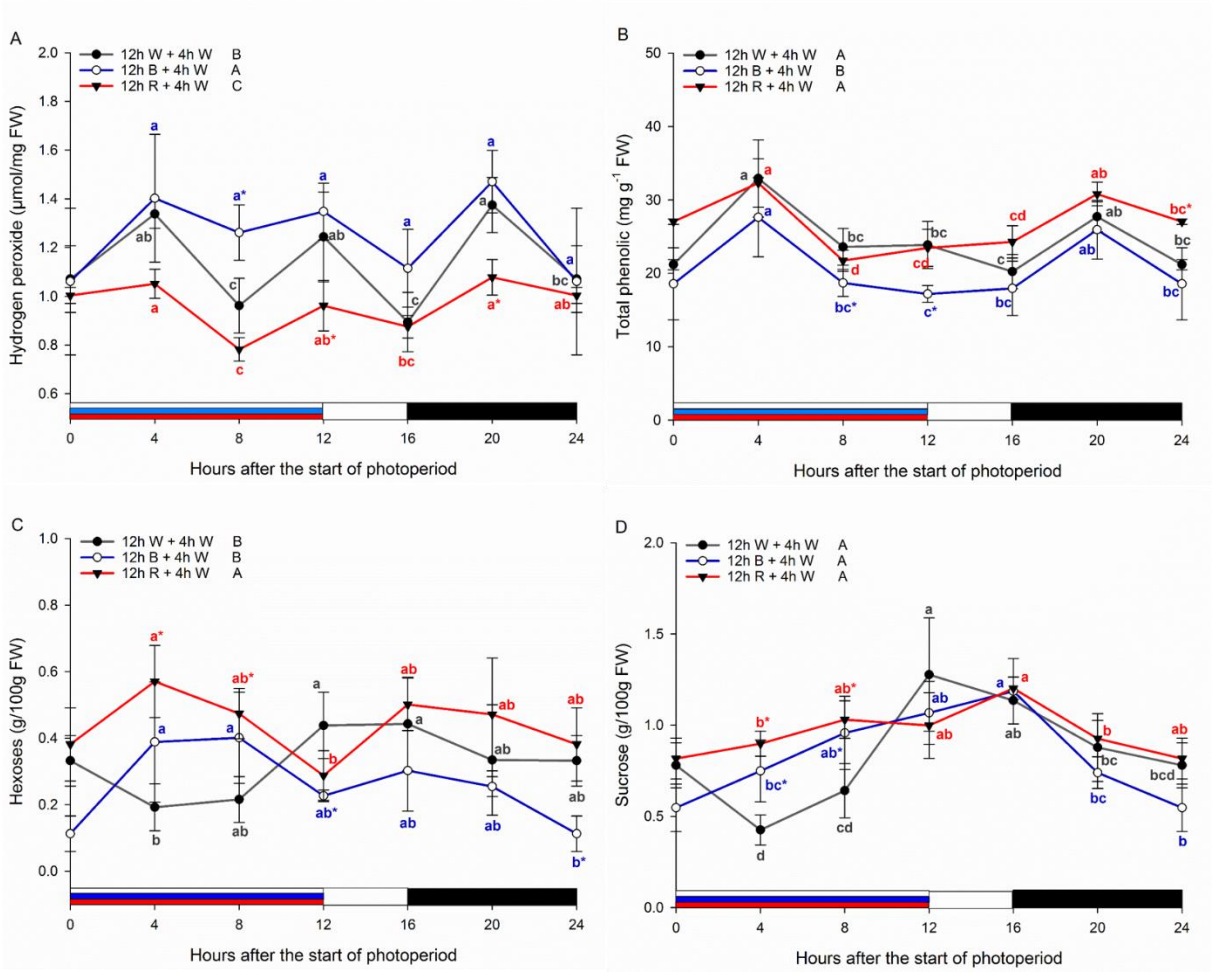


Figure 3.5 Diurnal variations of leaf metabolism in strawberry plants subjected to the different light qualities in Exp. 3. A, hydrogen peroxide; B, total phenolics; C, hexoses; D, sucrose. For each light treatment, leaves were sampled at 0h, 4h, 8h, 12h, 16h, and 20h after the start of the photoperiod. Data present the average levels from five replicates ± standard deviation. Different small letters with colors (black: 12 h W + 4 h W; blue: 12 h B + 4 h W; red: 12 h R + 4 h W) indicate statistically significant differences among sampling times for each light treatment based on Tukey's test ($p \leq 0.05$, n=5). * indicates significant effects from 12h blue or red light compared to 12h white light. The capital letters following legends show the significant difference between overall levels for each light treatment, irrespective of sampling times (Tukey's test, $p \leq 0.05$).

Twelve hours of blue or red light statistically increased the sucrose level at 4 h and 8 h after the start of photoperiod compared to white light. No overall significant differences in sucrose levels were found between the light treatments (Figure 3.5D). The levels of flavonoids and pigments showed significant diurnal variations, but were not influenced by the different light treatments (Figure S3.4).

3.3.5 Influence of light quality incubation on the infection processes of *Botrytis*

Light incubation irrespective of light quality greatly decreased the elongation of mycelium after spore germination compared to dark incubation. No large differences in spore germination were observed among the different incubations (Figure 3.6).

On dark-incubated leaf discs, spores started to germinate at 4 hpi. At 8 hpi, all observed spores had germinated, and the tubes developed into infectious hyphae (penetration). On light-incubated leaf discs, most spores had germinated at 8 hpi, but hyphae development was not yet observed. At 10 hpi, the mycelium from spore germination further developed in the dark incubation. The germinated tubes from light incubations started to extend, the length was around 100 µm. At 12 hpi, the germinated tubes from light incubations formed infectious hyphae and started to penetrate into the leaf.

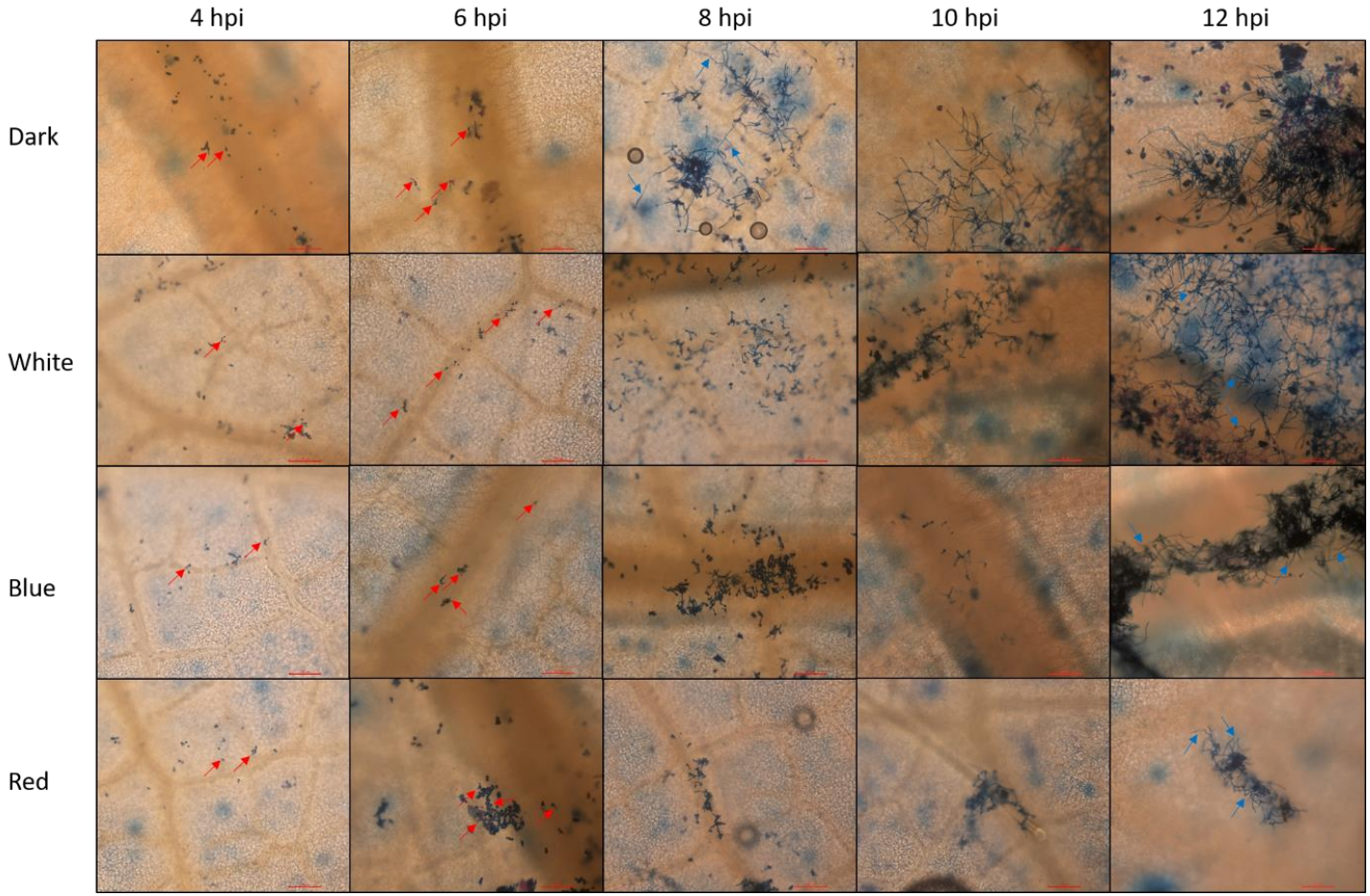


Figure 3.6 The infection process by *Botrytis* spores on leaf discs was observed at 4 hpi, 6 hpi, 8 hpi, 10 hpi, and 12 hpi using trypan blue staining. After spore inoculation, the leaf discs were parallelly incubated in the dark, and under white, blue, and red LED lights until sampling for trypan blue staining. Afterwards, the stained leaf discs were checked under microscope and images were captured with a ColorView Ⅲcamera and edited with the software package CELL-F. Scale bar is 100 µm. The red arrows indicate germinated spores, blue arrows indicate infectious hyphae. (Note: the blue background in the figure is the residue of trypan blue staining protocol).

3.4 Discussion

3.4.1 Influence of light on disease symptoms caused by *Botrytis cinerea*

This work revealed that strawberry leaves inoculated with *B. cinerea* showed less disease symptoms when incubated in the light compared to incubation in the dark (Figure 3.2, 3.3, and 3.5). Blue light treatment before inoculation increased disease severity in dark-incubated leaves, but this was not the case when leaves were incubated under the same light regime. Red light treatment enhanced leaf resistance to *B. cinerea* in both dark and red light-incubated inoculated leaves (Figure 3.2).

As an important environmental factor in almost all organisms, light influences the outcome of plant-pathogen interactions with direct effects on both the defense response in the host and the virulence of the attacking pathogen (Roden & Ingle, 2009). On one hand, light with different wavelengths has been reported to influence plant resistance to *B. cinerea* (Ahn et al., 2015; Islam et al., 1998; Kim et al., 2013; Xu et al., 2017). In strawberry, blue LED light irradiance increased leaf susceptibility, while red light decreased the susceptibility to *B. cinerea* (Meng et al., 2019). On the other hand, pathogen virulence is also directly mediated by light. The presence of light and deletion of the photoreceptor BcWCL1 (the orthologs of White Collar complex in *B. cinerea*) reduced disease symptoms caused by *B. cinerea* (Canessa et al., 2013). Therefore, the decreased disease severity in light incubation is the result from the effect of light on both strawberry and *B. cinerea*. This reduced disease severity by light presence is in agreement with the report in Arabidopsis that light/dark incubation caused 19% reduction in lesion area by *B. cinerea* in comparison with dark/dark conditions (Canessa et al., 2013).

In our study, strawberry leaves developed from blue light displayed a notable increased susceptibility to *B. cinerea* in dark incubation, while this increase in susceptibility disappeared when leaves were incubated under blue light/dark conditions, suggesting a direct effect of blue light on *Botrytis* infection. In *Botrytis*, at least eleven potential photoreceptors sense (near)-UV, blue, green, red, and far-red light signals, thus triggering various morphological changes (Schumacher, 2017). Moreover, continuous black light retarded mycelium growth of *B. cinerea* while sporulation was stimulated by near ultraviolet light (NUV), slightly inhibited by red and yellow light but inhibited by blue and green light (Tan & Epton, 1973). Likewise, Kim et al. (2013) described that blue LED light irradiance after inoculation with *B. cinerea* considerably inhibited symptom development in tomato compared to white LED light, resulting from the direct inhibition of blue light on *Botrytis* infection. Red light was much more efficient in decreasing disease development irrespective of light presence, because of its predominant enhancement of leaf basal resistance and

slight inhibition of *B. cinerea* (Meng et al., 2019; Tan & Epton, 1973). Red light is a promising light quality for plant production as it induced disease resistance in various crops, such as tomato resistance to both *B. cinerea* and *Pseudomonas syringae*, the resistance of bean and Arabidopsis to *B. cinerea*, and cucumber resistance to powdery mildew (Cerrudo et al., 2012; Khanam et al., 2005; Wang et al., 2010; Xu et al., 2017; Yang et al., 2015).

3.4.2 Effect of duration of light quality treatment on disease symptoms and plant resistance

Twelve hours of red light in a 16 h photoperiod increased leaf resistance in inoculated leaves under both dark and light/dark incubations, while 4 h red light was not sufficient (Figure 3.4 and S3.1). Twelve hours of blue light combined with 4 h white light increased leaf susceptibility to *Botrytis* (Figure 3.4).

Red light or a high percentage of red light in light combinations is reported to suppress disease development. The enhanced plant resistance to pathogens under red light is correlated with plant antioxidant systems such as total phenolics, and salicylic acid-mediated defense mechanisms (Meng et al., 2019; Shibuya et al., 2011). Red light or a high ratio of red light combined with far-red light increased resistance of cucumber plants to powdery mildew, and this was due to the induction of salicylic acid (SA) biosynthesis and expression of SA-regulating *PR-1* and *WRKY* genes (Shibuya et al., 2011; Wang et al., 2010). Moreover, red light restricted the spread of *Pseudomonas syringae* pv. *tomato* DC3000 (Pto DC3000) on tobacco plants by delaying the programmed cell death process (Moyano et al., 2020). Conversely, a reduced red:far-red ratio inhibited salicylic acid-dependent disease resistance to Pto DC3000 by the repression of SA-inducible kinases and defense-related genes in Arabidopsis (De Wit et al., 2013). Low red/far-red ratios reduced Arabidopsis resistance to *B. cinerea* via jasmonic acid (JA)-dependent responses (Cerrudo et al., 2012 and 2017; De Wit et al., 2013; Fernández-Milmanda et al., 2020). In these cases, phytochrome B inactivation by far-red light reduced plant immunity by upregulating *JAZ10*, one of the transcriptional regulators that repress JA responses through regulation of a sulfotransferase (ST2a) transcription (Cerrudo et al., 2017; Fernández-Milmanda et al., 2020; Moreno et al., 2009). Moreover, Courbier et al. (2020) recently reported that far-red light improved soluble sugar contents and *B. cinerea* disease severity via a JA-dependent pathway in tomato leaves. Therefore, it is likely that the development of *Botrytis* symptoms under red light was inhibited through a phytochrome-mediated JA-dependent signaling pathway. In contrast, blue light (12 h radiation in 16 h photoperiod) failed to activate PhyB-induced plant resistance as phyB perceives the changes in the red:far-red ratio (Ballaré, 2014), thereby leading to notably reduced leaf basal resistance.

Light quality has effects on leaf morphology, leaf physiology, photosynthesis, and leaf resistance. Among the different light qualities, red and blue light attract a lot of attention in artificial lighting research, as they play pivotal roles in plant development and in photosynthesis with the highest quantum yield under red light (McCree, 1971; Wang et al., 2016; Wang et al., 2008; Zhang et al., 2018). However, photosynthesis is impaired in plants grown for prolonged periods (weeks) under monochromatic red light, which can be inverted by a small fraction (7%) of blue light (Hogewoning et al., 2010; Trouwborst et al., 2016). Hence, effects of different ratios of red+blue light are largely documented. Nevertheless, a red/blue (1:1) light combination neutralized the resistance enhancement by red light and the resistance attenuation by blue light in strawberry (Meng et al., 2019). Furthermore, red and/or blue light combined with white LED improves the output and nutritional quality of crops (Dong et al., 2014; Lin et al., 2013; Zhang et al., 2019). Taken together, white light combined with red or blue light were used in this study. White LED exhibits obvious advantages on the optimization of plant light source and spectra, with a continuous spectrum and enhanced utilization rate. Previous studies demonstrated that white LED light guarantees a good quality and high yield of crops such as lettuce, tomato, and wheat in comparison with red, or red-blue LED light sources (Dong et al., 2014; Lin et al., 2013; Lu et al., 2012). However, studies about the effects of white LED combined with red or blue light on leaf resistance are limited. This study firstly documented that white LED combined with red LED increased leaf resistance in strawberry, which provides an important reference value for establishing lighting system in plant production for high yield, high quality as well as high resistance.

3.4.3 Diurnal effects on leaf resistance against *Botrytis cinerea* and correlation with circadian variations in leaf metabolism

Leaf resistance against *B. cinerea* showed diurnal variations in strawberry. There is increasing evidence that the circadian clock is important in regulating plant innate immunity. Ingle et al. (2015) documented that Arabidopsis resistance to *B. cinerea* peaked at dawn, thereafter resistance decreased during the light period, and bottomed when leaves were inoculated 15 h to 18 h after dawn, when light period turned to the dark. Accordingly, we investigated strawberry leaf resistance with inoculation at 5 h, 12 h, and 16 h after the start of photoperiod. Leaf susceptibility was highest when inoculated at 12 h after the start of photoperiod followed by dark incubation. When incubated under light/dark conditions, leaf displayed the highest resistance at 5 h after the start of photoperiod, intermediate resistance at 12 h after the start of photoperiod, and lowest resistance at 16 h after the start of photoperiod (Figure 3.3). This is partly in line with the demonstration by Ingle et al. (2015) in Arabidopsis. Light has previously been discussed to negatively affect the virulence of *B.*

cinerea, with reduced disease symptoms on strawberry leaves infected under diurnal conditions versus dark. However, the time-of-day variation in the success of *B. cinerea* infection is not simply due to the presence or absence of light (Ingle et al., 2015). The microbial clock in *B. cinerea* also played a role in the outcome of infection, and the pathogen was most virulent to Arabidopsis when inoculated at dusk (Hevia et al., 2015).

It should be noted that the time of inoculation does not correspond to the time of infection, as spore germination and penetration of plant tissue by *B. cinerea* hyphae must first occur. This process is influenced by several factors, including conidia concentration (Leroch et al., 2013), nutrient supplements (Audenaert et al., 2002), and plant surface (Holz et al., 2004). Therefore, we performed trypan blue staining in our study, and showed that this process takes place approximately 8 hpi in dark incubation and 12 hpi in light incubation irrespective of light quality (Figure 3.6). For the dark incubation, if inoculation was conducted 12 h after the start of photoperiod, then penetration occurred during midnight (20 h after the start of photoperiod), when strawberry leaves had the highest hydrogen peroxide content in the diurnal cycle. This benefited the successful infection, as H_2O_2 levels positively correlated with necrosis induced by *B. cinerea* (Govrin & Levine, 2000; Meng et al., 2019), thus leading to the greatest susceptibility at 12 h after the start of photoperiod in this study.

Circadian changes in leaf resistance may be partially explained by variations in leaf metabolites. Plants employ different mechanisms to defend themselves against pathogens at different times of the day (Zhang et al., 2013). This is partially due to the circadian regulation of reactive oxygen species, hormone signaling, sugars, and leaf metabolism in plants, as they all play a role in plant defense (Atamian & Harmer, 2016; Farré & Weise, 2012; Lu et al., 2017; Moghaddam & Ende, 2013). Here, we explored the diurnal variations of hydrogen peroxide, flavonoids and phenolics, sugars, and pigments, which all influence leaf basal resistance to *B. cinerea* in strawberry (Meng et al. 2019). Twelve hours of blue or red light in the photoperiod changed the circadian variations of hydrogen peroxide, total phenolics and hexoses (Figure 3.5). These can be the reasons for the increase in leaf basal susceptibility by 12 h blue light and decrease by 12 h red light in the photoperiod (Figure 3.4), as higher overall levels of hydrogen peroxide were found in leaves from 12 h B + 4 h W, and lower overall levels in leaves from 12 h R + 4 h W (Figure 3.5A). Moreover, the lower overall level of total phenolics in 12 h B + 4 h W light-leaves also may have contributed to the increase in leaf susceptibility, while higher hexose levels may have contributed to the decrease in leaf susceptibility from 12 h R + 4 h W (Figure 3.5B and C), as hexoses are needed to fulfill the energy and carbon requirements for the resistance response after infection (Truernit et al., 1996). The leaves from 12 h R + 4 h W with inoculations at 12 h and 16 h after the start of photoperiod displayed lower disease symptoms under light/dark

incubation compared to white light, this could be due to the low levels of hydrogen peroxide at 20 h after the start of photoperiod (8 hpi) and the highest total phenolic levels at the end of the dark period (8 hpi), respectively (Figure 3.5A and B).

3.5 Conclusion

Light is an important environmental factor that influences both the host and the pathogen during plant-host interactions. Red light increased leaf resistance to *Botrytis* irrespective of post-inoculation conditions. Four hours of red or blue light radiation in a 16 h photoperiod failed to alter leaf resistance, while a longer radiation of 12 h red or blue light succeeded in increasing or decreasing leaf resistance to *Botrytis*, respectively. Strawberry leaves showed significant diurnal variations in their susceptibility to *Botrytis*. The susceptibility was highest 12 h after the start of photoperiod followed by a dark post-inoculation phase, which coincides with the highest level of hydrogen peroxide at 8 hpi when the penetration happened. When disease development took place under the light regime, the shorter the light period after inoculation the stronger the disease symptoms. The alteration in circadian variations of leaf susceptibility by 12 h red or blue light radiation was linked to the changes in leaf metabolites. Higher hydrogen peroxide level and low total phenolics content in leaves derived from 12 h B + 4 h W light may have contributed to the increase in leaf susceptibility, while lower hydrogen peroxide, higher contents of total phenolics and hexoses may have contributed to the decreased leaf susceptibility of leaves developed under 12 h R + 4 h W light combination.

3.6 Supplementary Material

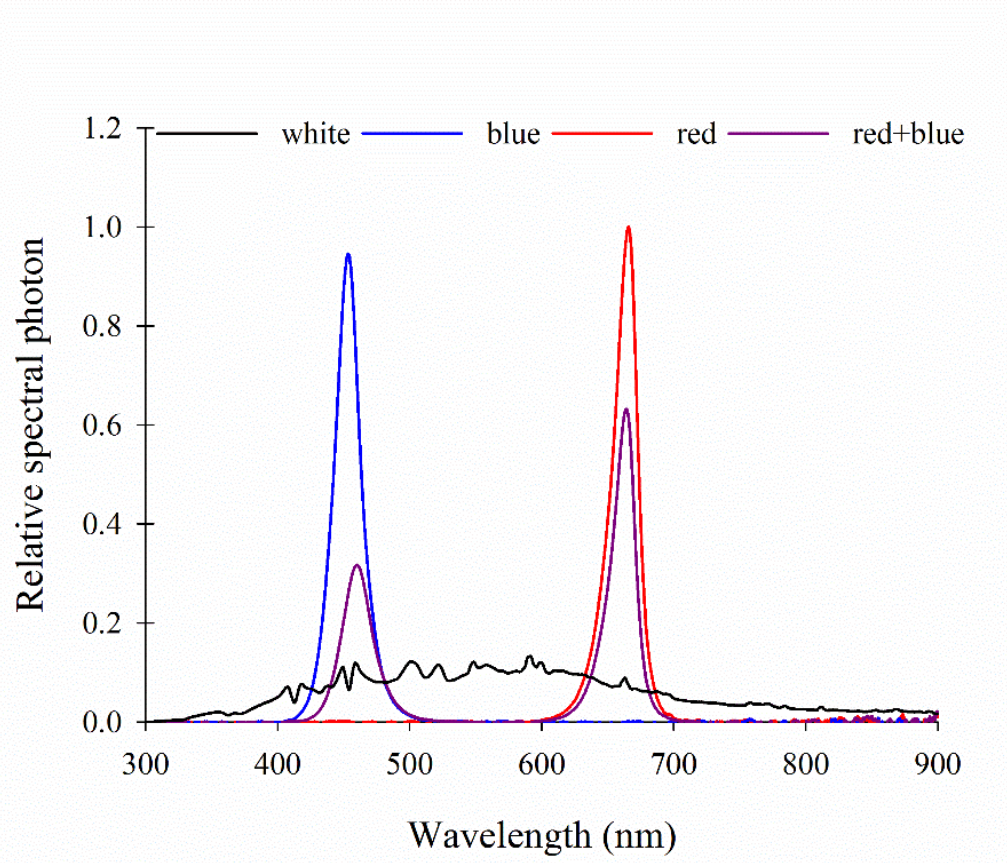


Figure S3.1 Relative spectral photon description of LEDs used for strawberry growth in all three experiments: White LED (white), Blue LED (blue), Red LEDs (red), Red plus blue (red+blue). Spectrum was measured with a JAZ spectroradiometer (Ocean optics, USA).

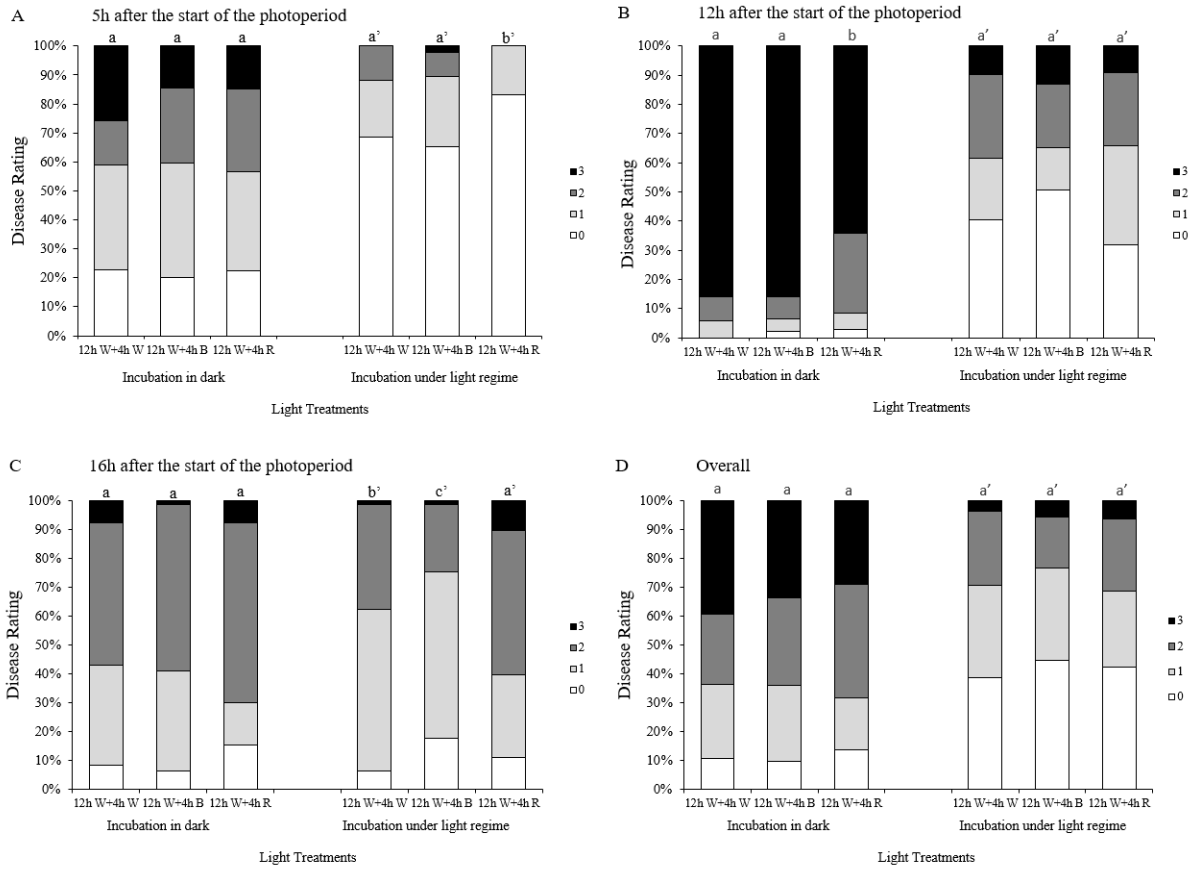


Figure S3.2 Effect of 4 h light quality treatments at the end of photoperiod on disease symptoms at different times of inoculation. A, inoculation at 5 h after the start of photoperiod; B, 12 h after the start of photoperiod; C, 16 h after the start of photoperiod; D, overall disease symptoms independent of inoculation time. Different letters (without prime for dark incubation and with prime for incubation under light regime) show significant difference among the treatments (n=12, Kruskall-Wallis followed by a post-hoc Dunn's test, $p \leq 0.05$). For all treatments, the difference between incubation in dark or under light regime was significant (Friedman Test, $p < 0.001$).

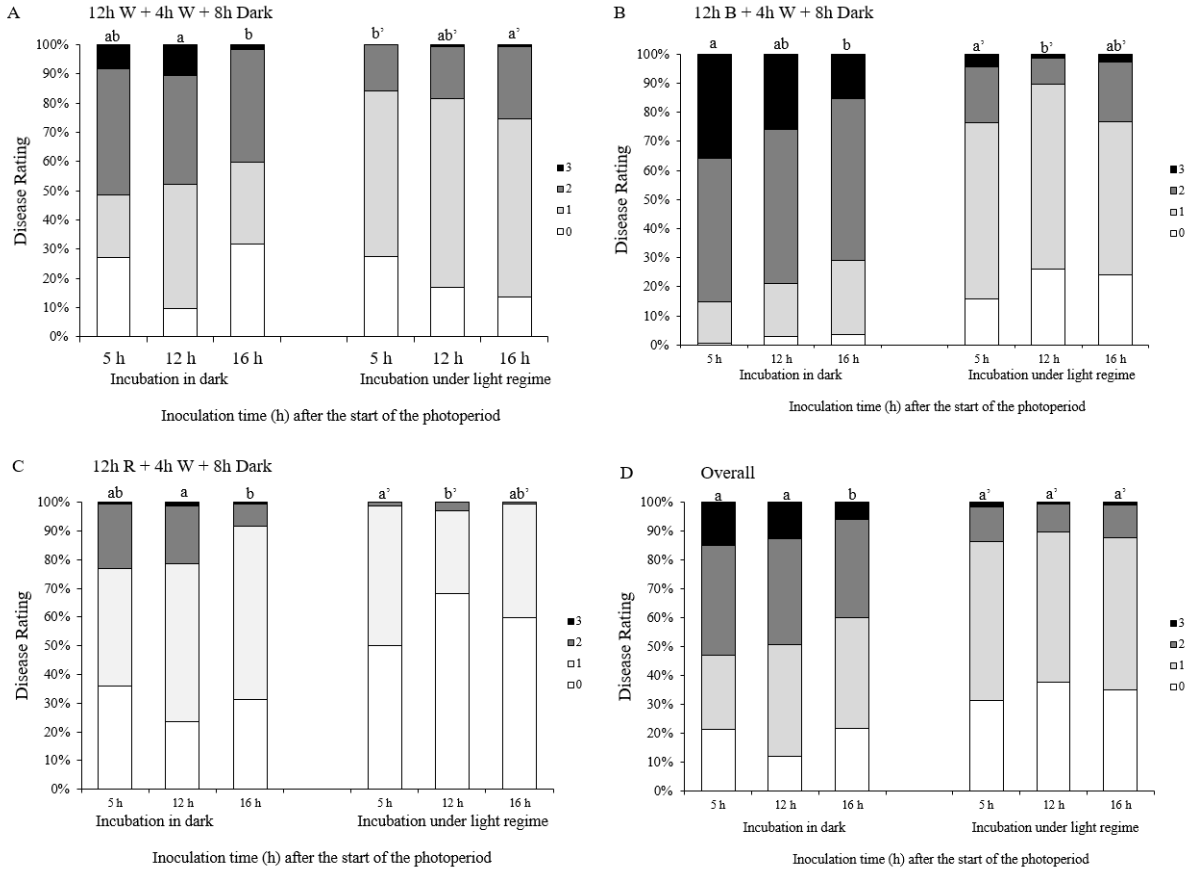


Figure S3.3 Effect of time of inoculation with *B. cinerea* (5 h, 12 h, or 16 h after the start of the photoperiod) on disease symptoms of strawberry leaves grown under different light conditions: A. 12 h W + 4 h W, B. 12 h B +4 h W, C. 12 h R + 4 h W, D. Overall disease symptoms independent of light conditions. Different letters (without prime for dark incubation and with prime for incubation under light regime) show significant difference among the treatments (n=12, Kruskall-Wallis followed by a post-hoc Dunn's test, $p \leq 0.05$). For all treatments, the difference between incubation in dark or under light regime was significant (Friedman Test, $p < 0.001$).

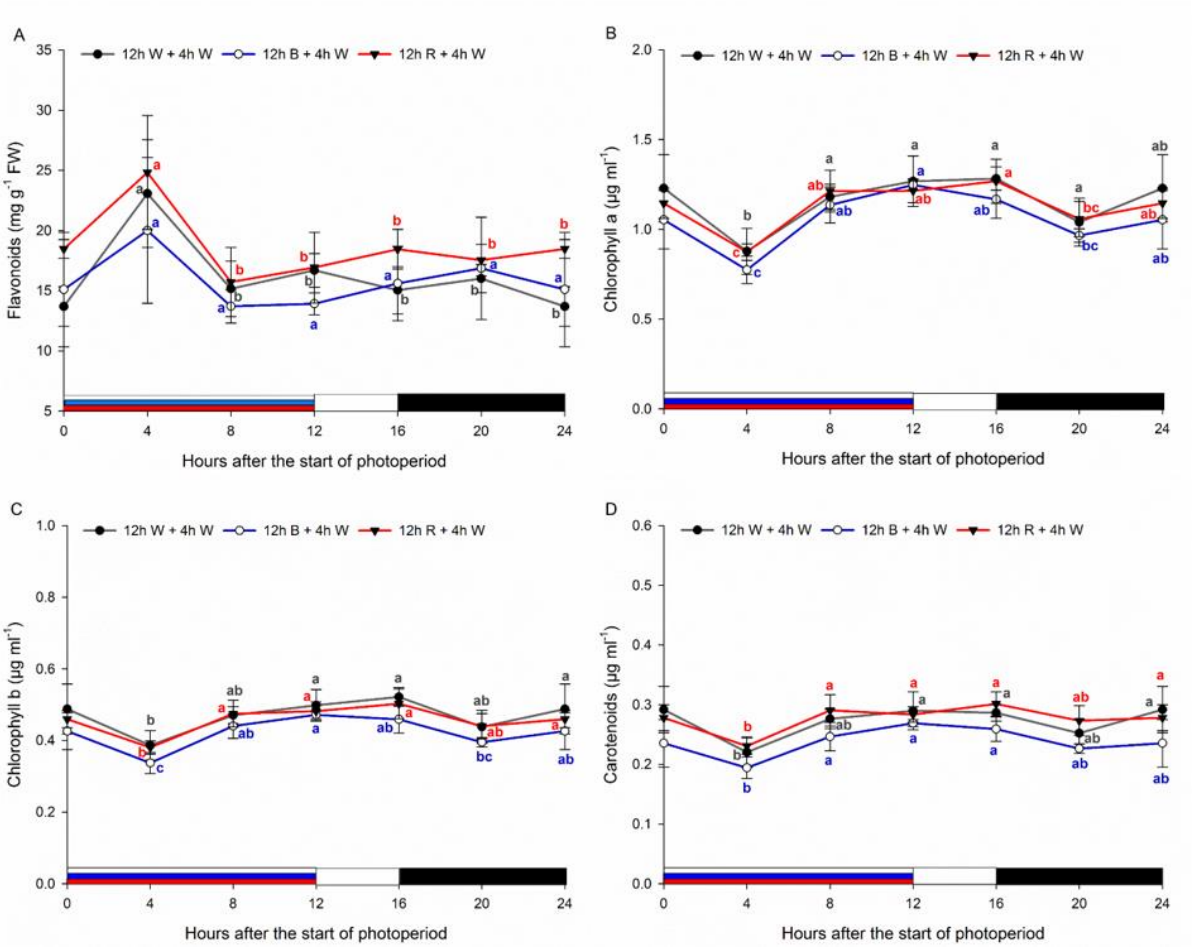


Figure S3.4 Strawberry leaves display diurnal variations in flavonoids (A), Chlorophyll a (B), Chlorophyll b (C), carotenoids (D), and 12h blue or red light have no effects on these diurnal variations. Leaves were sampled every four hours at 0h, 4h, 8h, 12h, 16h, and 20h after the start of photoperiod from each light treatment. Data present the average content from five replicates ± standard deviation. Different letters indicate statistically significant differences among treatments based on Tukey's test ($p \leq 0.05$, n=5).

Chapter 4

Phenotypic Variation of *Botrytis cinerea* Isolates is Influenced by Spectral Light Quality

This chapter is based on:

Lijuan Meng, Hanna Mestdagh, Maarten Ameye, Kris Audenaert, Monica Höfte, Marie-Christine Van Labeke (2020). Phenotypic variation of *Botrytis cinerea* isolates is influenced by spectral light quality. *Frontiers in Plant Science* 11: 1233.

Abstract

Botrytis cinerea, a fungal pathogen that causes grey mold, displays a high degree of phenotypic diversity. Light-emitting diodes (LEDs) with specific light spectrum, which are increasingly used as lighting resource for plant greenhouse production, can also have an effect on the pathogens in this production system. In this study, we investigated the phenological diversity in fifteen *B. cinerea* isolates upon different light treatments. Daylight, darkness and LED lights with different wavelengths (white, blue, red, blue+red) were chosen as treatments. The fifteen *Botrytis* isolates differed in their mycelial growth rate, conidia production and sclerotia formation. Light quality had a limited effect on growth rate. All isolates sporulated under daylight treatment, red light resulted in lower sporulation, while white, blue, and blue+red light inhibited sclerotia formation in all isolates, and sporulation in most, but not all isolates. Pathogenicity of the *Botrytis* isolates was studied on two-week-old strawberry (*Fragaria* × *ananassa* 'Elsanta') leaves grown under white, blue and red LED lights. The isolates differed in virulence on strawberry leaves, and this was positively correlated to oxalic acid production by *B. cinerea in vitro*. Red LED light improved leaf basal resistance to all the tested *Botrytis* isolates. Blue light pretreatment resulted in decreased leaf resistance to some isolates. Furthermore, we used image analysis to quantify the virulence of the different *Botrytis* isolates based on changes in photosynthetic performance of the strawberry leaves: chlorophyll fluorescence (F_v/F_m), chlorophyll index (ChlIdx) and anthocyanin content (modified anthocyanin reflection index, mAriIdx). F_v/F_m showed a strong negative correlation with disease severity and can be an indicator for the early detection of grey mold on strawberry leaves.

4.1 Introduction

Botrytis cinerea Pers.:Fr the causal agent of grey mold disease is a filamentous, heterothallic fungus with a necrotrophic life style (Alfonso et al., 2000). This pathogen has a wide host range and infects more than 1000 plant species worldwide including vegetables, ornamentals, and fruits, leading to important yield and quality losses (Elad et al., 2016). *Botrytis cinerea* is a highly versatile pathogen. As a necrotroph, it can extract nutrients from dead or senescent plant material, but it can also infect living tissues via direct penetration or through natural openings or wounds (Williamson et al., 2007). Additionally, it can also grow saprophytically. *Botrytis* can be isolated from different plant species in nature and infection can be reproduced in the laboratory on a wide range of hosts. Yet, a certain degree of host specialization exists in this pathogen (Muñoz et al., 2002; Thompson & Latorre, 1999). For example, Cotoras and Silva (2005) reported that *B. cinerea* strains isolated from tomato were more virulent on tomato leaves than isolates from grapes. Furthermore, a study of 490 isolates from open-field crops by microsatellite loci suggested the occurrence of host-specific divergence of *B. cinerea* in perennial hosts (Asadollahi et al., 2013).

Botrytis cinerea isolates show phenotypic and genetic variability. Differences in colony morphology, mycelial growth, sporulation intensity, sclerotia formation, and pathogenicity have been described (Di Lenna et al., 1981; Khazaeli et al., 2010; Kumari et al., 2014; Kuzmanovska et al., 2012; Martinez et al., 2003; Pande et al., 2010). *B. cinerea* produces a battery of extracellular enzymes, including pectinases and pectin methylesterases (Reignault et al., 1994). *Botrytis* isolates with different pathogenic capabilities on various host produced different amounts of extracellular pectic enzymes (Di Lenna et al., 1981). High genetic diversity in *B. cinerea* populations has been revealed using a multiplicity of molecular techniques, such as PCR detection of transposable elements (Martinez et al., 2008), RFLP analysis of PCR-amplified loci (Baraldi et al., 2002; Muñoz et al., 2002), PCR amplification of microsatellite loci (Asadollahi et al., 2013; Isenegger et al., 2008), and randomly amplified polymorphic DNA (RAPD) analysis (Alfonso et al., 2000; Pande et al., 2010). Disease control is difficult because the pathogen has a broad host range and it can survive as mycelium and/or conidia or as sclerotia for extended periods. In addition, *B. cinerea* isolates differ in their sensitivity to fungicides and fungicide resistance is quickly obtained (Kretschmer & Hahn, 2008).

B. cinerea has eleven photoreceptors including two cryptochromes, four LOVs (light, oxygen, voltage), two opsins and three phytochromes to respond to different light conditions varying from near-UV, blue, green, red and far-red light (Schumacher, 2017). Blue light is sensed by the proteins that bind flavin via LOV or FAD (flavin adenine dinucleotide) domains such as

BcWCL1 and BcWCL2 (the orthologs of White Collar complex in *B. cinerea*), and BcVVD1 (the orthologs of vivid in *B. cinerea*) (Rodriguez-Romero et al., 2010). BcWCL1 interacts with BcWCL2 in the nuclei, forming the White Collar complex (WCC), which is required to respond to white light (Schumacher, 2012). Opsins are transmembrane proteins using retinal to sense green light, and phytochromes are histidine kinases using bilin to perceive red/far-red light (Rodriguez-Romero et al., 2010). Based on the light perception by these photoreceptors, *B. cinerea* senses surrounding light as a decision-tool for morphogenesis, as a guide for directed growth, as a stress factor for protection, and also as a time giver for the circadian clock (Schumacher, 2017). Studies have described the impact of light on mycelial growth, conidiation, sclerotial development, and tropic response, however, unclear results have been reported caused by *B. cinerea* isolate variability and different experimental conditions (Schumacher, 2017).

Strawberry (*Fragaria* × *ananassa*) is an important soft fruit crop that is popular all over the world. Grey mold is a serious disease in strawberry production and leads to important economic losses (Debode et al., 2015; Petrasch et al., 2019). *B. cinerea* can infect all plant parts of strawberry including leaves, fruits, flowers, petioles, and stems at every growth stage (Petrasch et al., 2019; Williamson et al., 2007). In year-round greenhouse strawberry production, light- emitting diodes (LED) are increasingly applied to increase the day length as well as the light intensity in winter. LED lighting offers the possibilities of spectral modulation thus influencing both morphology and metabolite content of the leaves. Commercial LED lamps typically combine blue and red wavelengths as these are highly absorbed by chlorophyll and thus promote photosynthesis and biomass production (Okamoto et al., 1996). Application of LED lighting also opens the possibilities to increase the strawberry's resistance to *B. cinerea.* Indeed, we previously showed that leaves that develop under monochromatic red light are more resistant to *Botrytis* infection compared to white, blue, and blue+red lights (Meng et al., 2019). Yet, in aforementioned study only one *Botrytis* strain was investigated. Hence, the investigation of the diversity in light-response of different *B. cinerea* isolates is of importance for the LED light application in greenhouse production.

Given the facts described above, we hypothesized that *B. cinerea* isolates will differentially respond to different light qualities. Therefore, we investigated the light-modulated phenotypic diversity of fifteen *B. cinerea* isolates. We characterized their pathogenicity on strawberry leaves under white, blue and red LED lights and hypothesized that a pretreatment of leaves with red light would reduce disease susceptibility irrespective of the isolate. To assess the virulence of the different *Botrytis* strains on strawberry leaves in an objective way, we investigated the potential of image-based early detection to quantify changes in the

photosynthetic performance of the leaves (quantum efficiency of photosystem II, chlorophyll index) and/or leaf defense compounds (modified anthocyanin reflection index).

4.2 Materials and Methods

4.2.1 *Botrytis cinerea* isolates

Fifteen *B. cinerea* isolates were used in this study (Table 4.1). Four *Botrytis cinerea* isolates (B1, B2, B6 and B7) are well-documented laboratory strains. Eleven gray mold isolates were collected from tomato, lettuce, apple and grape in Belgium. Purified isolates were cultivated on Potato Dextrose Agar (PDA, Becton, Dickinson, and Company) and subjected to long-term storage in 20% glycerol at −80°C.

Table 4.1 *B. cinerea* isolates used in this study.

Botrytis* isolates	**Host plant**	**Origin/geographic origin**	**References**
B1 (R16)	Grape	Result of the crossing SAS56×SAS405	(Faretra & Pollastro, 1991)
B2 (Bd90)	Grape	Bordeaux (France)	(Reignault et al., 1994)
B3	Lettuce	Belgium, 2018	this study
B4	Tomato	Belgium, 2018	this study
B5	Lettuce	Belgium, 2018	this study
B6 (B05.10)	Grape	Haploid strain resulted from a treatment with benomyl in Germany	(Quidde et al., 1998)
B7 (A336)	Grape	mutant of Bd90, from Bordeaux (France)	(Hamada et al., 1994)
B8	Lettuce	Belgium, 2018	this study
B9	Lettuce	Belgium, 2018	this study
B10	Apple	Belgium, 2018	this study
B11	Grape	Belgium, 2018	this study
B12	Lettuce	Belgium, 2018	this study
B13	Tomato	Belgium, 2018	this study
B14	Lettuce	Belgium, 2018	this study
B15	Lettuce	Belgium, 2018	this study

* Alternative names are indicated between brackets.

4.2.2 Light quality treatments to study *Botrytis* phenotypes

To investigate mycelial growth, sporulation and sclerotia production, the fifteen *B. cinerea* isolates were grown on PDA under different spectral light qualities at 20˚C. Six different light regimes were established using (1) daylight, (2) white LEDs (300-800 nm, 29% B, 39% G,

27% R and 5% FR, Philips, The Netherlands), (3) blue LEDs (400-500 nm, peak at 460 nm), (4) red LEDs (600-700 nm, peak at 660 nm), (5) red + blue LEDs (75%/25%, peak at 660 nm and 460 nm) (Figure S4.1) and (6) dark as control. A photoperiod of 16 h and a photon flux density of 40 µmol m^{-2} s^{-1} were provided by the LEDs. Natural daylight was provided at a lab bench (18 °C), with a natural day length of 15 h and average photon flux density of 10 µmol m^{-2} s^{-1}. The spectral light distribution and the light intensity were measured using a spectroradiometer (JAZ-ULM-200, Ocean Optics, US).

Mycelial plugs with 6 mm in diameter were inoculated in the center of Petri dishes (90 mm in diameter). The Petri dishes were assigned to one of the six light treatments. Six replicates per isolate and per light treatment were used. The radial growth per colony was measured daily on two perpendicular axes for 5 days or until it reached the edge of the plate. The growth rate (cm day^{-1}) was calculated as the average growth length increase per day.

To assess the sporulation and sclerotia production, PDA plates inoculated with 6 mm-mycelial plugs remained under the six light treatments for 15 days. Sporulation and sclerotia production were assessed visually and by microscopy. The class system was set as: Class 0 (no spores/sclerotia formation); Class Ⅰ (very few spores/sclerotia formation); Class Ⅱ (sparse sporulation/sclerotia formation); Class Ⅲ(average amount spores/sclerotia formation); Class Ⅳ(many spores/sclerotia formation); Class Ⅴ(abundant formation of spores/sclerotia). The classification system for sclerotia is illustrated in Figure S2. This was repeated three times with six replicates each time (n = 18).

4.2.3 Oxalic acid detection assay

Plugs of *B. cinerea* isolates were inoculated on Complete Medium (Canessa et al., 2013) and always maintained in the dark at 24 ˚C. This pH-indicating medium contains 0.1% bromothymol blue (SIGMA-ALDRICH) as indicator, and the medium color changes from green to yellow when an acid compound such as oxalic acid is produced. Medium acidification was evaluated after seven days by its effect on the medium pH. The pH was measured on the outside of the yellow circle using a flat pH electrode (SF113, VWR, Germany) which can test the pH directly via the surface of the medium. Four measurements were conducted for each plate and averaged, this was done in four replications per isolate.

4.2.4 Pathogenicity test on strawberry

Strawberry (*Fragaria* × *ananassa* ‘Elsanta’) leaves that developed under different light qualities were used for the pathogenicity tests with the fifteen *B. cinerea* isolates. The plants were potted in peat substrate (Van Israel nv, Belgium) and raised in a growth chamber with 70% relative humidity at 20 ˚C. The growth chamber was equipped with three light qualities:

white (W, 300-800 nm, Philips, The Netherlands), blue (B, peak at 460 nm, Philips, the Netherlands), and red (R, peak at 660 nm, Philips, The Netherlands) LED lights (Figure S4.1). A photoperiod of 16 h with the photosynthetic photon flux density at 100 µmol m^{-2} s^{-1} was provided. The plants were fertilized with Soluplant (N:P:K:Ca 19-8-16-4, Haifa, The Netherlands, EC=1.5 dS/m, pH=5.7) three times per week.

All fifteen isolates were tested on strawberry leaves grown under white LED light, and a subset of ten isolates was tested on leaves that developed under blue and red LED lights. *Botrytis* isolates were cultivated on PDA in Petri dishes in dark (24 ˚C). After 7-10 days, conidia were washed from the plates with ¼ Potato Dextrose Broth (PDB) solution containing 0.01% (v/v) Tween 20. After removing the mycelium fragments, spore titers were determined microscopically using a Thoma counting chamber. A final concentration of 5×10^5 spore mL^{-1} was used for inoculation. Leaf discs of one-cm in diameter from two-week-old strawberry leaves were cut the day before inoculation and placed in disposable 24-well plates with water. Each leaf disc was inoculated with 10 µL droplets of conidial suspension on the adaxial leaf surface. Incubation was at 22 ˚C under dark conditions. Disease symptoms were scored after 3 days. A 0-3 ordinal rating scale was employed for disease rating and disease index was calculated. Six leaf discs per leaf with four biological replicates were used in this study.

4.2.5 Image analysis

Non-invasive spectral phenotyping was applied to monitor the strawberry disease development by a platform that allows to visualize diverse physiological traits in real-time, based on specific absorption, reflection and fluorescence patterns in visible and near-infrared (NIR) wavelengths. The central part of the platform comprises a 3CCD 6Mp - 16 bit camera mounted on a Cartesian coordinate robot, equipped with 12 optical interference filters (CropReporter, PhenoVation B.V., Wageningen, Netherlands).

RGB (red green blue) images, reflectance spectra to calculate the anthocyanin index and chlorophyll index and the minimal fluorescence, F_0, and the maximum fluorescence, F_m, are captured by the camera. Images obtained from the phenotyping platform were processed via the “Data Analysis Software” program (PhenoVation B.V., Wageningen, the Netherlands).

The modified anthocyanin reflectance index (mAriIdx) was determined using following formula (Gitelson et al., 2009):

$$\mathrm{mAriIdx} = \left(\frac{1}{\rho_{550\mathrm{nm}}} - \frac{1}{\rho_{710\mathrm{nm}}}\right)\rho_{770nm}$$

The chlorophyll index (ChlIdx) was calculated using following formula (Gitelson et al., 2009):

$$\mathrm{ChlIdx} = \left(\frac{\rho_{770nm}}{\rho_{710\mathrm{nm}}} - 1\right)$$

where ρ_{550} is the reflectance in the first spectral band, which is maximally sensitive to anthocyanin content; ρ_{710} the reflectance in the second spectral band, which is maximally sensitive to chlorophyll content but not sensitive to anthocyanin content; and ρ_{770} the reflectance of the third spectral band, which compensates for leaf thickness and density.

The maximum quantum efficiency of photosystem II (F_v/F_m) was calculated using following formula (Baker, 2008):

$$F_v/F_m = (F_m - F_0)/F_m$$

Between measurements, the 24-well plates were placed in the dark allowing immediate quantification of the minimal (F_0), then saturating red light flashes of 3000 µmol m^{-2} s^{-1} were given and maximal fluorescence (F_m) was determined based on the OJIP induction curve according to the manufacturer's specifications (Björkman & Demmig, 1987; Kalaji et al., 2014).

4.2.6 Statistical analysis

Data were tested for normal distribution using the Kolmogorov–Smirnov test and for homoscedasticity of variances using Levene's test. The mycelial growth rate and disease rating among *B. cinerea* isolates were compared by the non-parametric Kruskal-Wallis test with Dunn test as the post hoc test. The effect of light quality on mycelial growth rate was analyzed by one-way-ANOVA. If significant differences were found, the Tukey test ($p \leq 0.05$) was carried out to establish significant differences between means, here a Bonferroni correction was applied when $n \geq 10$. The virulence of the *Botrytis* isolates assessed by the multispectral camera (F_v/F_m, ChlIdx, mAriIdx) were analyzed by one-way ANOVA for each light quality. As the light pretreatment affects both chlorophyll and polyphenol content (Meng et al, 2018), the effects of the light quality pretreatments were analyzed by ANCOVA using the images at the start (Day 0) as covariate, adjusted means were calculated using Bonferroni for confidence interval adjustment. All assumptions for performing ANCOVA including homogeneity of regression slopes were checked. All analyses were performed using SPSS version 26 (SPSS Inc., Chicago, USA).

4.3 Results

4.3.1 Phenotypic characterization of *B. cinerea* isolates

Significant differences in the mycelial growth rate were observed between the fifteen *B. cinerea* isolates, this for the dark control treatment (Figure 4.1, Table S4.1). B12 resulted in the lowest growth rate (0.45 cm/day) while B2 and B8 had a ± 3-fold higher growth rate (respectively 1.31 and 1.32 cm day^{-1}).

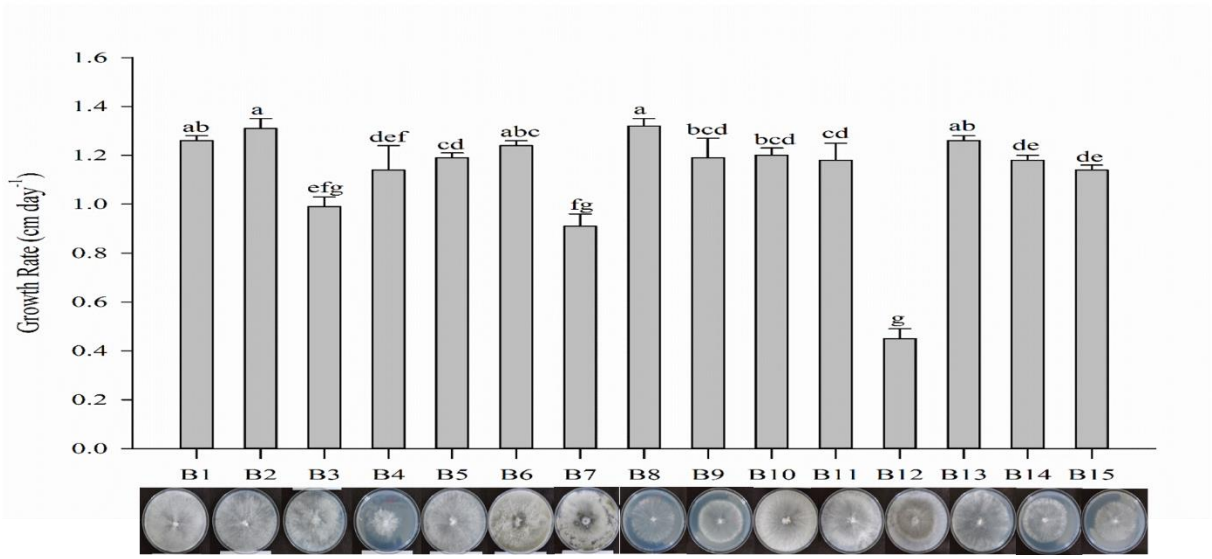


Figure 4.1 Mycelial growth rate of fifteen *B. cinerea* isolates in dark conditions. Data are presented as mean of six replicates with standard deviation. Different letters indicate statistical differences among *B. cinerea* isolates based on Kruskal-Wallis test with Bonferroni correction (Dunn test, $p \leq 0.0033$).

Light conditions greatly influenced the growth rate of *B. cinerea* (Figure 4.2, Table S4.1). For the isolates B2, B3, B4, B5, B7, B12, and B14, the mycelial growth was considerably higher under LED lights (white, blue, red, blue+red) compared to the dark and daylight treatment. The spectral quality of the LED treatment did not affect the growth rate of B2, B5, B7, B9, B10, B12, B13 and B15. However, compared to white LED light, a significant increase was observed in the growth rate of B8 under blue light, while red light enhanced the growth rate of B1, B11 and B14. The combination blue+red, decreased the mycelial growth of B3 and B11 considerably, while an increase was observed in B1. Additionally, B1 was the only isolate where the highest growth rate was observed in the dark while all other isolates had higher or equal growth rates than the dark treatment.

Sporulation and sclerotia formation varied among the fifteen *B. cinerea* isolates, and both were considerably affected by the light treatments used in this study (Figure 4.3). The fifteen isolates were grouped according to the presence/absence of sporulation and of sclerotia

formation under the light treatments (Figure 4.3). Four groups were defined based on their sporulation response. A first group clusters two lettuce and two tomato isolates (B3, B4, B13, B15) as they only produced spores under daylight and red LED light. A second group, including three lettuce and one grape isolate (B5, B8, B10, B14) sporulated in dark conditions as well as under daylight and red light. The third group (grape isolates B2 and B11) developed spores under daylight, red and blue light. The fourth group, including grape and lettuce isolates (B1, B6, B9, and B12) exhibited great variability in sporulation compared to the other isolates under the considered light conditions. Finally, the nonpathogenic mutant B7 (A366) forms a fifth group and produced spores in all six treatments.

No sclerotia formation was observed under white, blue, and blue+red LED lights, this for all isolates. Three groups could be distinguished based on the formation of sclerotia in dark, under daylight or under red light (Figure 4.3). No sclerotia were produced in B6 (B05.10) and B7 (A366) irrespectively the light or dark treatment, resulting in a first group. B1 (R16) formed a second group with only sclerotia formation in the dark. The third group is formed by the remaining twelve isolates including lettuce, tomato, apple and grape isolates, as they all produced sclerotia under dark, daylight, and red LEDs.

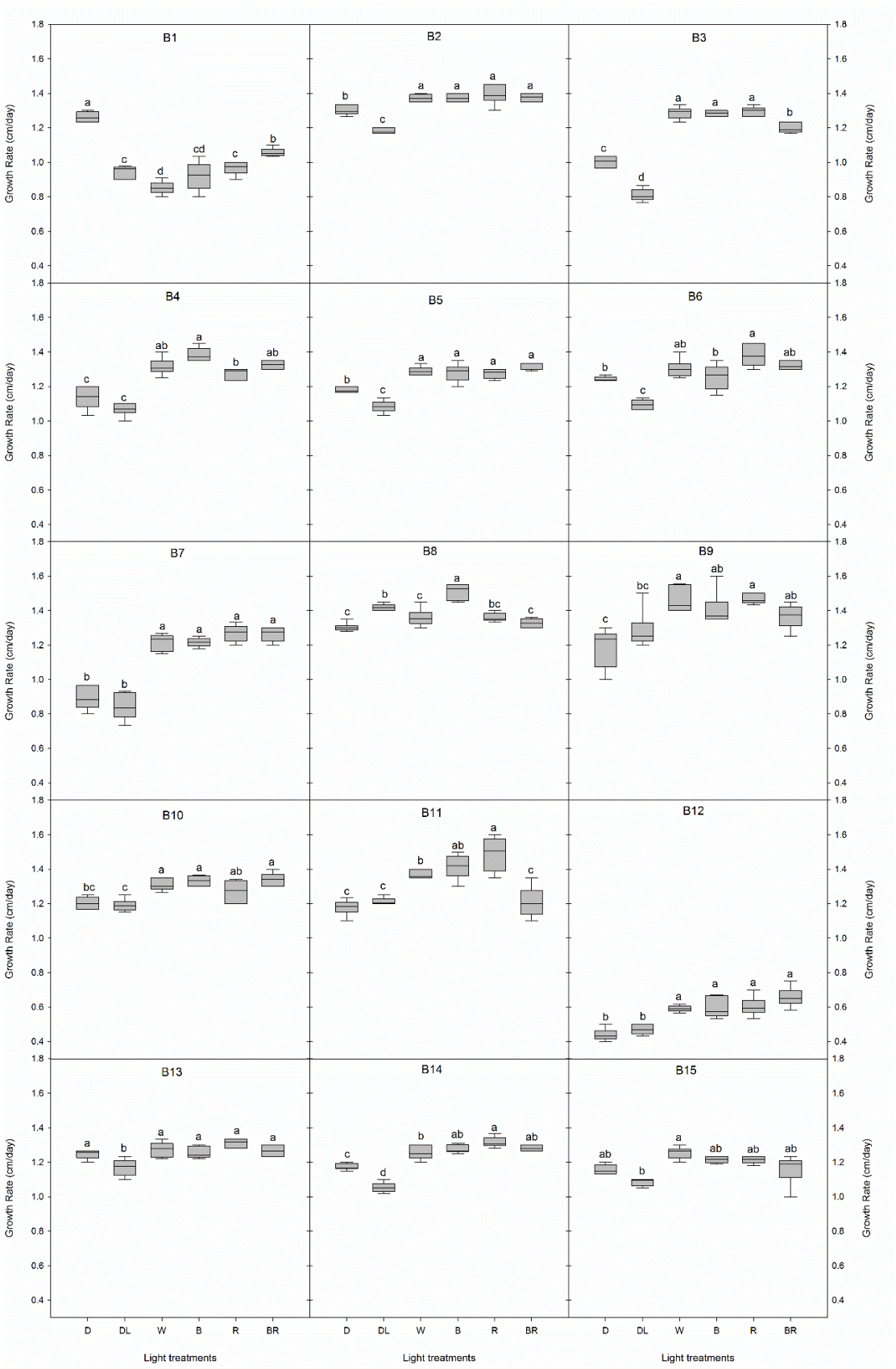


Figure 4.2 Effects of light quality on the growth rate of fifteen *B. cinerea* isolates. Six light treatments were analyzed namely dark (D), daylight (DL), white (W), blue (B), red (R), and blue+red (BR).

Different letters indicate statistical differences between light treatments for each isolate based on Tukey's test ($p \leq 0.05$), except for B2 and B15 where a non-parametric Kruskal-Wallis followed by a post-hoc Dunn's test ($p \leq 0.05$) was performed.

Figure 4.3 Classification of sporulation and sclerotia formation in the fifteen *B. cinerea* isolates after 15 days under six light treatments: dark, daylight, white, blue, red, and blue+red LED, and classification based on F_v/F_m values at 4 dpi from infection in white-light-leaves. Classes of sporulation and sclerotia presented are from three replications with 6 plates for each replicate (n = 18). The classes of sporulation and sclerotia formation are: Class 0 (no spores/sclerotia formation); Class Ⅰ(very few spores/sclerotia formation); Class Ⅱ(sporulation/sclerotia formation sparse); Class Ⅲ(average amount of spores/sclerotia formation); Class Ⅳ(many spores/sclerotia formation); Class Ⅴ(abundant spore/sclerotia formation). The class of virulence is based on the significant letters of F_v/F_m at 4 dpi (Table 2) with exclusion of B6 which is indicated by a light grey color. The classes are: Class Ⅰ(significant letters starting with a); Class Ⅱ(significant letters starting with b); Class Ⅲ(significant letters starting with c); Class Ⅳ(significant letters starting with d); Class Ⅴ(significant letters starting with e). These classes are shown in colors (see the color bar).

Oxalic acid formation can be indicative as a virulence factor for *Botrytis* (Williamson et al., 2007). Media acidification due to organic acid formation was strongest for B4, B6, B12, B13 and B15 resulting in a decrease of more than 1.5 pH units compared to a non-inoculated control. The nonpathogenic mutant B7 (A366) did not acidify the medium in comparison to the control. All other isolates caused an intermediate acidification reducing the medium with 1 pH unit in comparison to the control (Figure 4.4). White, blue, and red LED irradiation had no significant effect on media acidification caused by different *Botrytis* isolates (Figure S4.8).

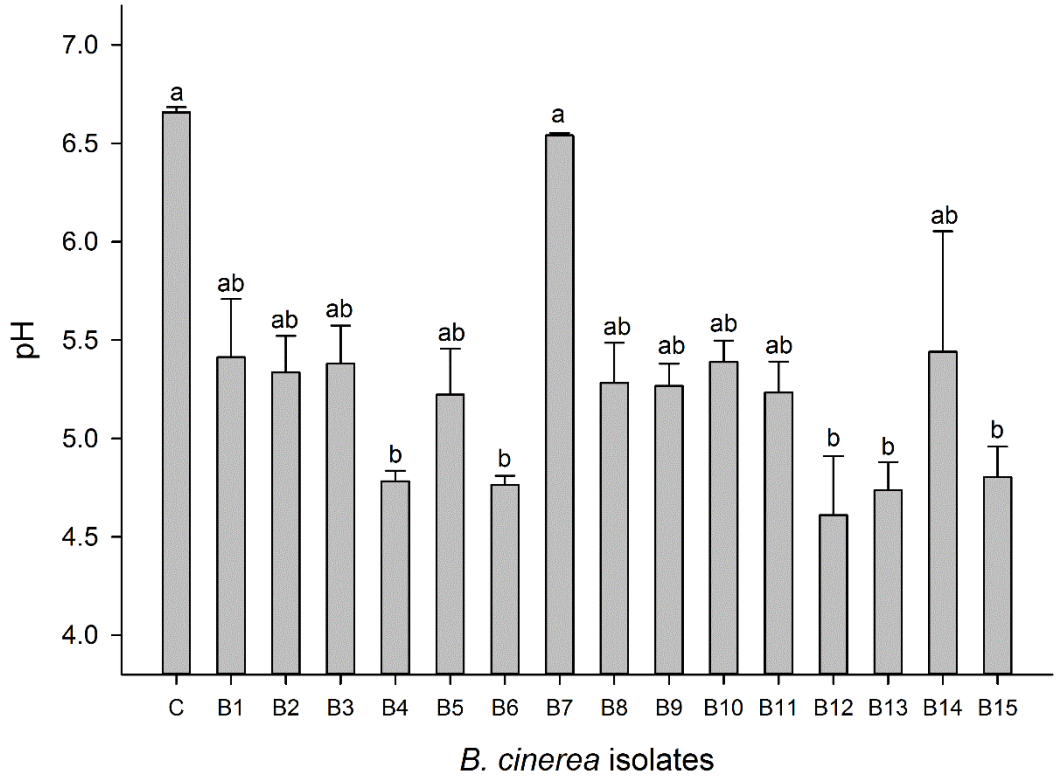


Figure 4.4 Oxalic acid production of *B. cinerea* isolates was assessed based on changes in the medium pH. Control treatment (C) is medium without *Botrytis* inoculation, all isolates were grown in dark. Data are shown as means ± SE (n=4). Different letters indicate statistical differences between *B. cinerea* isolates. Non-parametric Kruskal-Wallis followed by a post-hoc Dunn's test was performed with $p \leq 0.05$.

4.3.2 Inoculation assays on strawberry leaves

First, fourteen *Botrytis* isolates were tested by spore inoculation on white LED light-developed strawberry leaves. B6 could not be included because of its poor sporulation (Figure 4.5). Different degrees of virulence on strawberry leaves were observed between these 14 isolates. B4, B10, B13, and B15 were the most virulent, followed by B1, B2, B3, B5, B9, and B14. An intermediate virulence was observed for B8, B11 and B12. As expected, strawberry leaves were hardly infected by the non-pathogenic B7 (A366) strain.

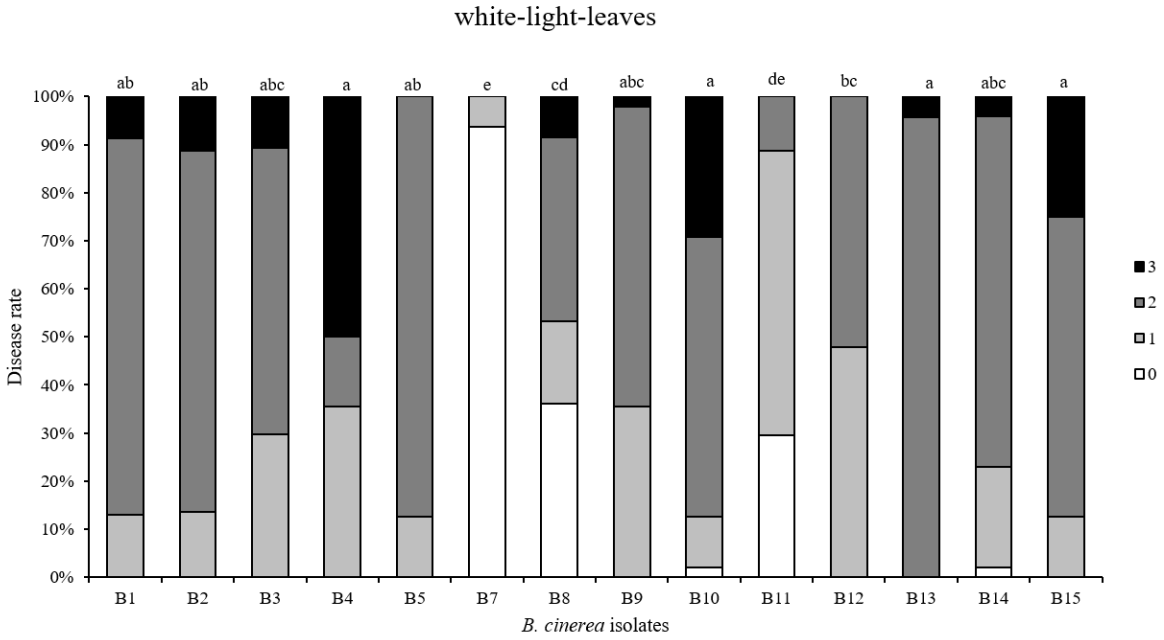


Figure 4.5 Disease severity caused by fourteen *B. cinerea* isolates spore-inoculated on strawberry leaves grown under white LED lights. Disease rating was scored 3 days post inoculation using four scoring categories (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). B6 was not tested because not enough spores were produced. Different letters indicate statistical differences among the isolates performed by Kruskall-Wallis test followed by a post-hoc Dunn's test with Bonferroni correction ($p \leq 0.0036$, $n = 4$).

Second, ten *Botrytis* isolates were tested on strawberry leaves that were developed under blue and red LED lights. The isolates showed significant variations in their virulence (Figure S4.3A and B). B7 (A366) was again the least aggressive, while B1, B2, and B13 were the most aggressive isolates and B15 resulted in moderate disease rating on the leaves that had developed either under blue or red light. Strawberry leaves that originated from red light were more resistant against the tested *Botrytis* isolates compared to leaves from white or blue light (Figure 4.6). Blue-light-developed leaves were more susceptibility to B1, B2, B9, B12, B13, while no remarkable difference was noted for isolates B3, B8, B10, B15, when compared to white-light-developed leaves. The isolate B7 (A366) also remained non-virulent on blue or red-light-developed leaves (Figure S4.3A and B).

Overall, B8 (lettuce isolate) was the least aggressive *Botrytis* isolate on strawberry leaves irrespective of light treatments, while B13 (tomato isolate) was the most aggressive one. Interestingly, B15 (lettuce isolate) resulted in the strongest disease symptoms on leaves from white light, however, blue- and red-light-developed leaves displayed moderate resistance to B15.

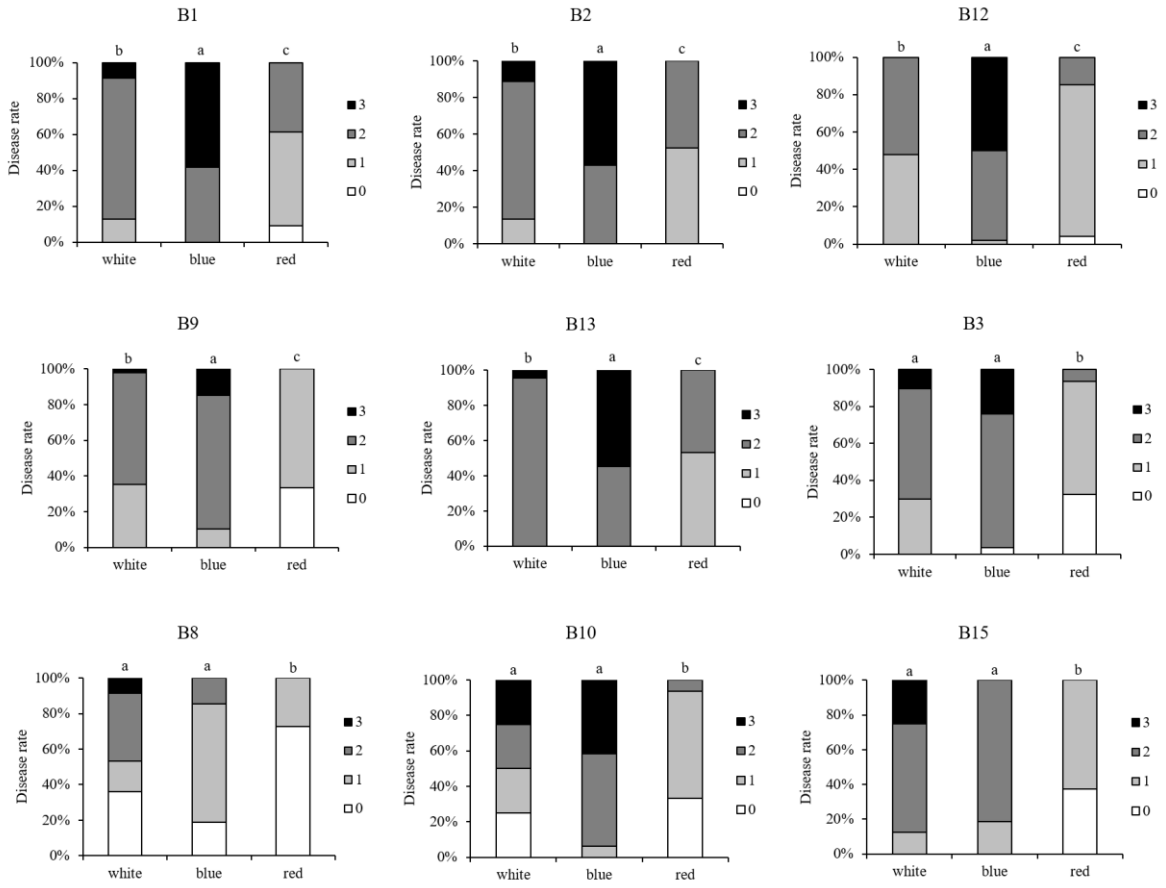


Figure 4.6. Comparison of virulence per isolate (including B1, B2, B3, B8, B9, B10, B12, B13, B15) with respect to the light pretreatments of the strawberry leaves. For each *Botrytis* isolate, virulence was tested on strawberry leaves grown under white, blue, and red LED lights, spore-inoculated leaf discs were dark incubated after inoculation and disease severity was compared between white-, blue-, and red-light-developed leaves. Disease rating was scored 3 days post inoculation using four scoring categories (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). Different letters indicate statistical differences among the isolates performed by Kruskall-Wallis followed by a post-hoc Dunn's test ($p \leq 0.05$).

4.3.3 Image-based assessment of *Botrytis* infection on strawberry leaves

For the image-based assessments of the progress rate of the disease after spore inoculation, changes in both chlorophyll fluorescence imaging (F_v/F_m) and stress indices (ChlIdx and mAriIdx) were assessed during 5 days (Figure 4.7, S4.4, S4.5 and S4.6). The phenotyping of the plant resistance is shown from 0 to 4 dpi for three representative *Botrytis* isolates: the non-pathogenic B7 strain, the intermediate aggressive B12, and the most aggressive B13 isolate as determined by visual scoring on white-light-developed leaves (Figure 4.5).

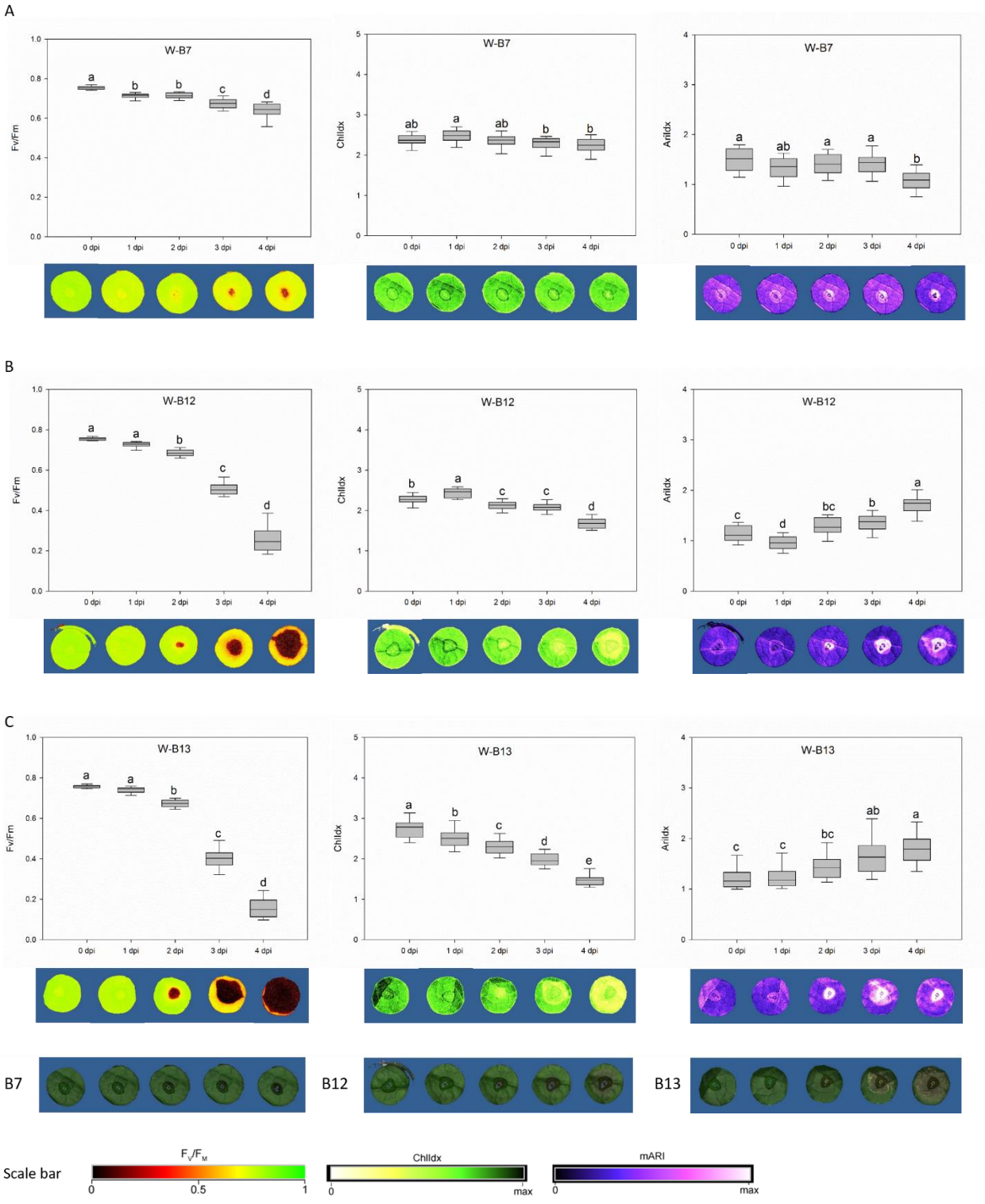


Figure 4.7. The variations of F_v/F_m, ChlIdx, and mAriIdx from 0 to 4 dpi caused by *B. cinerea* isolates B7(A), B12 (B), and B13 (C) were correlated with the development of disease lesion on white-light-leaves. The corresponding images are presented underneath the figures. Disease lesion led to darker F_v/F_m image with lower value, yellower ChlIdx image with lower value, and brighter mAriIdx image with higher level. RGB images of the inoculated leaf discs upon *Botrytis* isolates (B7, B12, B13) are shown before the scale bar. One-way ANOVA was applied for the statistical analysis (Tukey test, $p \leq 0.05$),

and data are shown by box plots with the median. Different letters indicate significant differences among the time points.

The non-pathogenic B7 displayed a minor but significant decrease in F_v/F_m from 0.755 at 0 dpi to 0.637 at 4 dpi and hardly any change in ChlIdx and mAriIdx (Figure 4.7A). For B12 a strong decline was observed in F_v/F_m from 3 dpi on, with values decreasing from 0.755 at 0 dpi to 0.263 at 4 dpi. Correspondingly, also ChlIdx displayed a significant decrease, while mAriIdx increased considerably from 0 dpi to 4 dpi (Figure 4.7B). The isolates B8, B9, B11, and B14 showed similar temporal changes as the intermediate aggressive isolate B12 for F_v/F_m, ChlIdx, and mAriIdx, except for the mAriIdx of B14 (Figure S4.4).

The most virulent isolate B13 caused the greatest decrease of F_v/F_m, this was already very strong at 3 dpi, while values further decreased to 0.172 at 4 dpi. Simultaneously a clear decrease in ChlIdx and increase in mAriIdx was found (Figure 4.7C). B1, B2, B3, B4, B10, and B15 grouped with B13 based on their virulence on strawberry leaves and they caused the same trends in F_v/F_m and ChlIdx. However, no significant increase was observed in mAriIdx for B1, B3, and B15.

The *Botrytis* isolates resulted in considerable variations in F_v/F_m, ChlIdx, and mAriIdx of the inoculated leaves at 4 dpi, this for all the pre-inoculation light quality regimes of the leaves (Table 4.2).

Leaves inoculated with the nonpathogenic strain B7 (A366) maintained the highest level of F_v/F_m and ChlIdx, while the lowest levels of mAriIdx were observed, this irrespective of the light quality treatments. Leaf inoculation with the virulent B13 resulted in the strongest decrease of F_v/F_m for white-light-leaves, but not for the other light quality pretreatments. Here, F_v/F_m was lowest after B12 inoculation of leaves grown under both blue and red light. Leaf yellowing and chlorophyll content, assessed by ChlIdx was lowest after B15 inoculation of white-light-leaves, B10 inoculation of blue-light-developed leaves, and B1 inoculation of red-light-developed leaves. Increase of anthocyanins (mAriIdx) was highest in both B9 and B13 in white-light-developed leaves, and in B12 in both blue- and red-light-developed leaves (Table 4.2).

Light pretreatment effects are thus clearly present and these effects are shown in Table 4.3. Overall red pretreated leaves have a significant higher F_v/F_m ($p < 0.001$) and ChlIdx ($p < 0.001$), while no difference between blue and white pretreated leaves is found. Only for strains B1 and B13 this positive effect of red light to maintain higher F_v/F_m levels was not observed, while this was the case of B2 with respect to ChlIdx. Overall, no significant effect of light pretreatment was found for mAriIdx ($p = 0.41$), indeed only for 3 out of 10 isolates an

effect of light pretreatment of the leaves was present, in B1 and B7 mAriIdx increased significantly while for B9 a significant decrease was found.

Additionally, overall comparison of anthocyanins with respect to the different light qualities (Table S4.2) or more specifically for three representative *B. cinerea* isolates (Table S4.3) were performed from 0 dpi to 4 dpi. Blue-light-developed leaves resulted in significant lower basal anthocyanin levels compared to leaves developed from white and red lights (Table S4.2). A higher anthocyanin content was observed in *Botrytis* isolates with higher virulence (Table S4.3).

As virulence of the isolates differed, correlations between the disease index and F_v/F_m, ChlIdx, and mAriIdx were explored (Figure 4.8). High correlations between disease index and F_v/F_m, were found, this irrespective of the light quality pretreatment (all $r \geq 0.978$, Figure 4.8A). Stress indices resulted in lower though still significant correlations between the disease index and ChlIdx although the correlation was lowest for red pretreated leaves ($r > 0.917$ for leaves from white light and blue light, while r is 0.824 for leaves from red light, Figure 4.8B). Correlations between disease index and mAriIdx were much lower ($r < 0.699$, Figure 4.8C).

We also checked the correlations between F_v/F_m and phenotypic characteristics of the different isolates such as growth rate (Figure 4.1) and medium acidification (Figure 4.4). Virulence and medium acidification were highly correlated (Figure S4.7A), while no correlation between virulence and mycelial growth rate was observed (Figure S4.7B).

Table 4.2 Effect of inoculation with different *Botrytis* isolates at 4 dpi on F_v/F_m, ChlIdx, and mAriIdx in strawberry leaves grown under white, blue, and red LED lights.

Botrytis	**white**			**blue**			**red**		
isolates	**F_v/F_m**	**ChlIdx**	**mAriIdx**	**F_v/F_m**	**ChlIdx**	**mAriIdx**	**F_v/F_m**	**ChlIdx**	**mAriIdx**
B1	0.266±0.129 cde	1.635±0.357 bcde	1.590±0.234 ab	0.199±0.188 de	1.478±0.420 cd	1.456±0.323 b	0.342±0.130 d	2.072±0.389 c	1.860±0.439 ab
B2	0.318±0.169 bcd	1.780±0.375 bc	1.826±0.504 ab	0.148±0.097 de	1.383±0.275 cd	1.628±0.339 ab	0.376±0.157 cd	2.130±0.372 bc	1.761±0.376 abc
B3	0.218±0.066 de	1.517±0.183 cde	1.648±0.200 ab	0.350±0.248 bc	1.887±0.522 ab	1.519±0.458 b	0.498±0.153 bc	2.263±0.300 bc	1.435±0.379 bc
B4	0.223±0.116 de	1.441±0.264 de	1.567±0.348 ab	*	*	*	*	*	*
B5	0.275±0.067 cde	1.647±0.153 abc	1.820±0.423 ab	*	*	*	*	*	*
B7	0.637±0.042 a	2.243±0.194 a	1.095±0.280 c	0.599±0.038 a	2.166±0.209 a	0.841±0.214 c	0.712±0.022 a	2.765±0.275 a	1.320±0.314 c
B8	0.373±0.203 bc	1.719±0.368 bcd	1.457±0.269 abc	0.377±0.098 b	1.685±0.218 bc	1.289±0.220 bc	0.586±0.074 ab	2.434±0.254 ab	1.340±0.229 c
B9	0.301±0.095 cde	1.795±0.198 bc	1.845±0.245 a	0.206±0.082 cde	1.600±0.135 bcd	1.603±0.206 ab	0.502±0.102 bc	2.390±0.293 bc	1.365±0.259 c
B10	0.210±0.109 de	1.511±0.384 cde	1.722±0.507 ab	0.192±0.073 de	1.335±0.222 d	1.497±0.418 b	0.559±0.102 b	2.349±0.368 bc	1.478±0.394 abc
B11	0.444±0.090 b	1.881±0.187 b	1.523±0.213 abc	*	*	*	*	*	*
B12	0.263±0.071 cde	1.686±0.131 bcde	1.736±0.298 ab	0.095±0.038 e	1.573±0.161 bcd	2.017±0.394 a	0.354±0.071 d	2.149±0.207 bc	1.908±0.302 a
B13	0.173±0.108 e	1.489±0.178 cde	1.839±0.425 a	0.192±0.149 de	1.559±0.338 bcd	1.668±0.711 ab	0.387±0.176 cd	2.078±0.318 bc	1.736±0.635 abc
B14	0.375±0.080 bc	1.806±0.240 bc	1.440±0.320 abc	*	*	*	*	*	*
B15	0.222±0.109 de	1.378±0.282 e	1.400±0.490 bc	0.280±0.069 bcd	1.545±0.177 cd	1.605±0.445 ab	0.544±0.082 b	2.367±0.211 bc	1.491±0.353 abc

The statistical analysis was conducted by one-way ANOVA with Bonferroni correction (p=0.0036 for white-light-leaves, and p=0.005 for blue- and red-light-leaves) followed by Tukey's test. Data were collected from approximate 24 leaf discs of four replicates at 4 dpi and presented as mean ± S.D. Significant differences between *B. cinerea* isolates ware indicated with different letters. * means that the isolates were not tested on blue- or red-light-leaves.

Table 4.3 Effect of light quality on the virulence of the *B. cinerea* isolates assessed by chlorophyll fluorescence imaging (F_v/F_m) and image-based indices (ChlIdx, and mAriIdx) at 4 dpi. The assessment was performed for strawberry leaves that developed respectively under full spectrum white LED light (white), monochromatic blue LED (blue), and monochromatic red LED (red) light. As light quality influences metabolite levels in the leaves, ANCOVA was performed and adjusted means were calculated with Bonferroni as confidence interval adjustment and the covariates are the values of F_v/F_m, ChlIdx, and mAriIdx at 0 dpi.

***Botrytis* isolates**	**F_v/F_m**			**ChlIdx**			**mAriIdx**		
	white	**Blue**	**red**	**white**	**blue**	**red**	**white**	**blue**	**red**
B1	0.234 a	0.385 a	0.349 a	1.613 a	1.053 b	1.832 a	1.598 ab	1.432 b	1.818 a
B2	0.299 ab	0.108 b	0.413 a	1.786 a	1.441 a	1.842 a	1.816 a	1.626 a	1.749 a
B3	0.215 b	0.508 a	0.514 a	1.510 b	1.913 a	2.225 a	1.633 a	1.526 a	1.452 a
B7	0.639 b	0.592 c	0.714 a	2.327 ab	2.230 b	2.542 a	0.978 b	1.019 ab	1.261 a
B8	0.395 b	0.379 b	0.577 a	1.724 b	1.647 b	2.376 a	1.464 a	1.290 a	1.342 a
B9	0.306 b	0.208 c	0.498 a	1.882 b	1.596 c	2.264 a	1.779 a	1.702 a	1.374 b
B10	0.207 b	0.182 bc	0.558 a	1.541 b	1.342 b	2.336 a	1.686 a	1.604 a	1.486 a
B12	0.235 b	0.075 c	0.356 a	1.716 ab	1.581 b	1.974 a	1.735 a	2.045 a	1.826 a
B13	0.176 a	0.204 a	0.312 a	1.490 b	1.572 b	2.164 a	1.875 a	1.663 a	1.763 a
B15	0.189 c	0.306 b	0.545 a	1.449 b	1.545 b	2.597 a	1.379 a	1.598 a	1.486 a

Data are means of 24 replicates. Different letters indicate significant differences for each isolate between the light treatments per parameter.

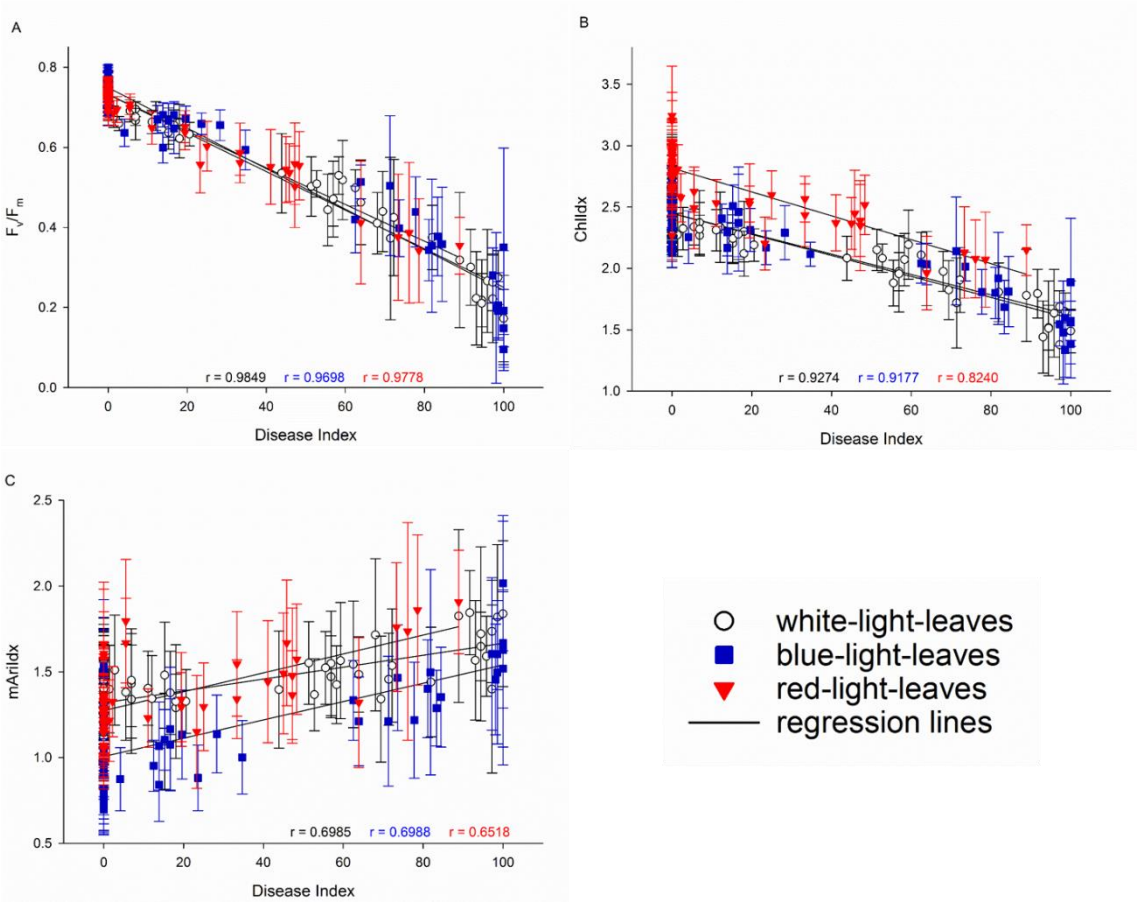


Figure 4.8 Correlations between disease index and F_v/F_m (A), ChlIdx (B), and mAriIdx (C) on strawberry leaves from white (in white), blue (in blue), and red (in red) LED lights. Values are presented as means with standard deviation shown by a vertical bar. The Pearson correlation r is shown in colors to indicate the corresponding light treatments.

4.4 Discussion

4.4.1 Phenotypic variability of *Botrytis cinerea* isolates is influenced by light quality

The fifteen *Botrytis* isolates in this study differed in their mycelial growth rate and in their ability to reproduce by conidia or form sclerotia (Figure 4.1, 4.2, and 4.3, Table S4.1). This intraspecies variation in *B. cinerea* is well documented (Martinez et al., 2003; Mirzaei et al., 2009) and can be influenced by environmental factors. As *Botrytis* is an important pathogen in greenhouse plant production, changes in the greenhouse environment might also lead to phenotypic variations. Here, we specifically focused on the increasing application of monochromatic red and blue light and its combination in lighting strategies with LED. Indeed, *B. cinerea*, possesses eleven photoreceptors to sense the surrounding light environment, which triggers various photoresponses influencing vegetative growth, sporulation, germination of spores and sclerotia formation (Schumacher, 2017). Generally, the short UV

A and B (300 - 420 nm) wavelengths retard the mycelial growth of *B. cinerea* (Tan & Epton, 1973). Here, the shortest wavelength we investigated was monochromatic blue light (460 nm) though this wavelength did not retard the growth rate of the studied isolates, in comparison to the full-spectrum white light. Light quality had a limited effect on the growth rate. Canessa et al. (2013) found that light reduced the daily growth rate of B05.10 (= B6) and attributed this to light-induced stress. It seems that our applied lower light intensities did not induce this light stress except for the strain B1 which showed the highest mycelial growth rate under dark conditions. The reduced mycelial growth rate under daylight might be explained by the lower temperature (18°C while 20°C in the climate rooms) as indicated by Tan and Epton (1973).

Early studies showed that sporulation happens exclusively in light, it is strongly stimulated by near-UV light and slightly stimulated by red light, while blue light is ineffective (Tan & Epton, 1973). This light dependent effect on sporulation was later confirmed for strain B05.10 (= B6) by Canessa et al. (2013) where broad spectrum light (400-720 nm) induced sporulation but blue light inhibited the formation of conidiophores. Also in our research all isolates sporulated under full spectrum daylight (from class I to class V). Monochromatic red light resulted in similar or lower sporulation in most isolates (12 out of the fifteen) when compared to daylight, this supported the role of red light in stimulating sporulation reported by Tan and Epton (1973). Sporulation under white LEDs was low, only B1 and B7 sporulated under this light source (both class II) although the light spectrum is very similar to daylight. However, daylight is rich in far-red light (700-800 nm, Figure S4.1), which promotes sporulation (Tan, 1975).

Here, the capacity of blue light to inhibit sporulation was only observed in nine of the fifteen isolates. Blue light stimulated sporulation in B6 (B05.10) and two other grape isolates (B2 and B11), in the nonpathogenic strain B7 (A366), and in two lettuce isolates (B9 and B12). The fact that B05.10/B6 produced very few spores (Class I) under blue light but also hardly sporulated under daylight, might indicate that despite its genetic stability (Canessa et al., 2013) the strain has mutated. This is also supported by the fact that this strain did not form sclerotia in the dark. Adding red light (600 - 700 nm) to the blue spectrum did not enhance sporulation as only three isolates sporulated under these conditions. Stewart and Long (1987) reported that *Botrytis* strains could also show varying degrees of sporulation in the dark. This is also confirmed in our study where 8 out of 15 strains sporulated in the dark. Although blind strains that exhibit the same phenotype under light and darkness are found in nature, this was not the case for our field collections. Only B7, the nonpathogenic mutant A366 produced conidia under each light quality as well as under dark conditions and, as described by Kunz et al. (2006), was not able to form sclerotia.

Early studies indicated that sclerotia formation for survival exclusively occurs in cultures in constant darkness. Yet, this can be affected by small broad light dosages. Fewer sclerotia were formed when irradiated for 30 min compared to 15 min, and none were observed when irradiated for more than 60 min. Furthermore, sclerotia formation was found to be promoted by red and infrared light (Tan & Epton, 1973). In this study, blue, blue+red, and white lights inhibited sclerotia formation in the fifteen *B. cinerea* isolates. This inhibition is due to the blue light fraction as under red light sclerotia were formed in twelve out of the fifteen isolates. In this study also daylight at a very low light fluence (10 µmol m^{-2} s^{-1}) induced sclerotia promotion which can be caused by the enrichment of the longer wavelengths (red light or the far-red light) in the indoor daylight spectrum (Tan & Epton, 1973).

It is clear that phenotypic responses of *B. cinerea* isolates to light quality are very diverse and sometimes conflicting with earlier publications. These conflicting results might be due to the fact that limited strains were studied though probably also light intensity might be an interacting factor with light quality. Also day length effects cannot be excluded, most studies apply a photoperiod of 12 h while in this research a photoperiod of 16 h, based on greenhouse lighting duration was applied.

4.4.2 Virulence variation in *B. cinerea*

B. cinerea isolates in this study displayed significant variations in their virulence. Virulence diversity in *B. cinerea* isolates has been studied in various locations and on various plants around the world (Kerssies et al., 1997; Kumari et al., 2014; Martinez et al., 2005; Mirzaei et al., 2009). Variation in virulence of *B. cinerea* is often due to differences in cell wall degrading enzymatic activities and in the secretion of other virulence factors such as oxalic acid (Derckel et al., 1999). Higher oxalic acid accumulation leads to higher aggressiveness of the pathogen and reversely, lower secretion of oxalic acid is associated with lower virulence (Kunz et al., 2006; Sun et al., 2019). Various degrees of medium acidification caused by oxalic acid production of *B. cinerea* isolates were also observed in this study (Figure 4.4). B4, B13, and B15 with highest virulence on strawberry leaves secreted more oxalic acid. In contrast, B7 (A336), the nonpathogenic mutant, showed no oxalic acid production and did not cause disease. Medium acidification and virulence were highly correlated (Figure S4.7A). Yet also other virulence factors such as cell wall degrading enzymes, toxins, and secondary metabolites (Sharma & Kapoor, 2017) can explain differences in virulence. However, it seems that *B. cinerea* isolates with strong virulence favored less sporulation and produced more sclerotia (Figure 4.3).

4.4.3 Strawberry leaves developed under red light displayed enhanced resistance to *B. cinerea* isolates

Higher resistance to all the tested *B. cinerea* isolates (except for the non-pathogenic B7) was found in strawberry leaves grown under red LEDs (Figure 4.6). Meng et al. (2019) showed that red LED light increased strawberry leaf basal resistance against R16 (B1 in this study). Resistance improvement by red light was associated with lower hydrogen peroxide levels in the red-light-leaves of strawberry (Meng et al., 2019). It might also be linked to phytochrome modulated defense signaling where low red:far-red ratios repress the JA response to *Botrytis* (Ballaré, 2014; Cerrudo et al., 2012). In contrast, blue-light-leaves were more susceptible to some of the isolates, including B1, B2, B9, B12, and B13 (Figure 4.6) and had a lower level of anthocyanins compared to leaves grown under white and red lights (Table S4.2). Here, both the increased hydrogen peroxide level as well as the reduced anthocyanin level might explain the increased susceptibility (Bassolino et al., 2013; Meng et al., 2019).

4.4.4 F_v/F_m is the best indicator for early detection of *B. cinerea* on strawberry leaves

Virulence of the *Botrytis* isolates on strawberry leaves derived from different light quality pretreatments was also assessed by imaging. The decrease in F_v/F_m synchronized with the development of lesion size, which was visualized on fluorescence images in dark red (Figure 4.7 and S4.4, S4.5). Strong correlations between F_v/F_m and disease index were observed in strawberry leaves derived from white ($r = 0.985$), blue ($r = 0.97$) and red ($r = 0.978$) LEDs (Figure 4.8).

F_v/F_m responded well to the development of *Botrytis* disease in strawberry leaves. Both biotic and abiotic stress factors decrease the efficiency of photosynthesis and suppress the variable fluorescence of dark-adapted chlorophyll-containing leaves correspondingly. F_v/F_m decreases along with the increasing effect of stresses (Gorbe and Calatayud, 2012; Rolfe & Scholes, 2010). This reduction in F_v/F_m suggests destructive changes in chloroplasts and photosystem II caused by *B. cinerea* infection. Here ChlIdx was clearly not as sensitive as F_v/F_m, only at higher disease indices (> 50%) this parameter decreased. Furthermore, F_v/F_m could discriminate virulence of the *B. cinerea* isolates. Higher virulence resulted in a stronger decrease in F_v/F_m, and lower virulence in smaller F_v/F_m reduction. In this study, the infectious symptom by *B. cinerea* were predominantly observed at 2 dpi based on chlorophyll fluorescence image. Chaerle et al. (2007) also showed that chlorophyll fluorescence imaging can be used for early detection of *Botrytis* infection. Autofluorescence signals (F_{440}/F_{740}) after *Botrytis* inoculation on grape berries could only be recorded 4 days after infection (Bélanger

et al., 2011), which is a delay of 2 days in comparison with F_v/F_m and 1 day in comparison with ChlIdx response.

Anthocyanins are water-soluble pigments responsible for the red colors in leaves. Different abiotic stresses enhance the biosynthesis of anthocyanins in leaves, and anthocyanins act as antioxidant and reactive oxygen scavengers (Landi et al., 2015). Heim et al. (1983) described that zones of anthocyanin accumulation often surround restricted lesions where a plant disease has been successfully contained, whereas low anthocyanin levels often occur in susceptible combinations of maize. Additionally, anthocyanins levels are negatively correlated with the susceptibility to *B. cinerea* in tomato fruit (Bassolino et al., 2013). *B. cinerea* infection causes hydrogen peroxide accumulation which leads to the accumulation of anthocyanin at the infectious site (Chalker-Scott, 1999; Govrin & Levine, 2000). In this study, foliar anthocyanins are indicated by mAriIdx. Along with the pathogen invasion, anthocyanins accumulated at the infectious site as visualized in Figure 4.7, S4.4 and S4.5. Despite the antioxidative role of anthocyanins responding to stresses, few studies focus on its development along with pathogen infection by imaging technology. Here the blue-light-leaves which had an increased susceptibility to *Botrytis*, showed significant lower basal anthocyanin levels compared to leaves derived from white and red lights (Table S4.2). On the other hand, *B. cinerea* isolates with higher virulence induced a higher anthocyanin content (Table S4.3), this was due to the higher accumulation of hydrogen peroxide caused by *Botrytis* infection. Furthermore, weaker correlations between *Botrytis* disease index and anthocyanin level are observed, compared to F_v/F_m and ChlIdx. Therefore, it seems that AriIdx is more a supporting observation, but less useful for the early detection.

4.4.5 Horticultural implications

B. cinerea causes significant losses in plant greenhouse production, storage, shipping and marketing, which makes control of grey mold very important (Michailides & Elmer, 2000; Karchani-Balma et al., 2008; González et al., 2009). To control this pathogen cultural, chemical and biological methods, as well as plant resistance breeding are used (Pande et al., 2006). However, the complexity and variability of this pathogen are reasons that make control difficult (Mirzaei et al., 2009). Awareness of the existence of pathogen variability together with the new insights in photobiological responses of both pathogen and plants may contribute to more efficient non-chemical methods of control. Here it is confirmed that red LED irradiance improved leaf resistance not only to one *B. cinerea* strain, but to all the isolates in this study. Therefore, red LEDs have potential to be used in plant production systems to control grey mold but this should be further investigated in greenhouse

conditions. Moreover, effects of red LED irradiance on *Botrytis* infections on strawberry flowers and fruits need to be assessed.

Many biotic stress symptoms start as spots or patches within a crop. Such symptoms could be discovered with imaging techniques at an early stage, when no visible symptoms are yet apparent. The possibility of early stress detection allows timely treatment to prevent pathogen spread within the crop and greenhouse, which would result in limited yield loss and reduced chemical usage. Today robots equipped with sensors are under investigation in greenhouse production. A UV-Robot with UV-C radiation is developed to control powdery mildew in horticulture (Mazar et al., 2018). This could also be equipped with imaging sensors, as early detection of disease is beneficial from both economic and environmental perspectives. In this study we evaluated imaging sensors in a highly controlled environment, without interference from other environmental factors such as wind, fluctuating light intensities, and temperature. Moreover, in horticultural production systems, not only environmental factors change dynamically but also crop-dependent factors such as leaf morphology and orientation, leaf waxes and hairs, and leaf density might influence the response. Therefore, the potential of early detection of grey mold by chlorophyll fluorescence imaging in horticulture needs further validation in a greenhouse environment (Gorbe & Calatayud, 2012; Mahlein, 2016).

4.5. Conclusion

Here it is clearly shown that *B. cinerea* isolates differently respond to different light qualities in phenotypes such as mycelial growth, sporulation, and sclerotia formation. Despite differences in virulence, red light considerably improved leaf basal resistance against all the tested *B. cinerea* isolates, while blue light pretreatment increased leaf susceptibility to some of them. Disease development caused by different *B. cinerea* isolates is highly correlated with F_v/F_m (maximal PSII quantum efficiency), meaning that this indicator can be used to objectively quantify *B. cinerea* disease severity and may also be useful for early detection of plant stress related to grey mold infection. Overall, red LED light has potential to control grey mold in greenhouse production, and image sensors could be developed into a new technology for early disease detection.

4.6 Supplementary material

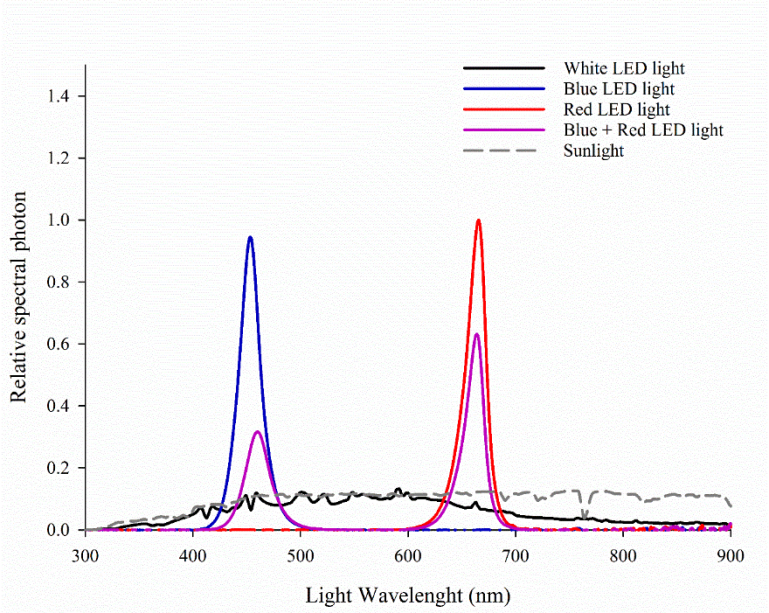


Figure S4.1 Relative spectral photon description of LEDs used for morphology test of *B. cinerea* isolates and strawberry growth: White LED (white), Blue LED (blue), Red LEDs (red), Red plus blue (red+blue). Spectrum was measured with a JAZ spectroradiometer (Ocean optics, USA).

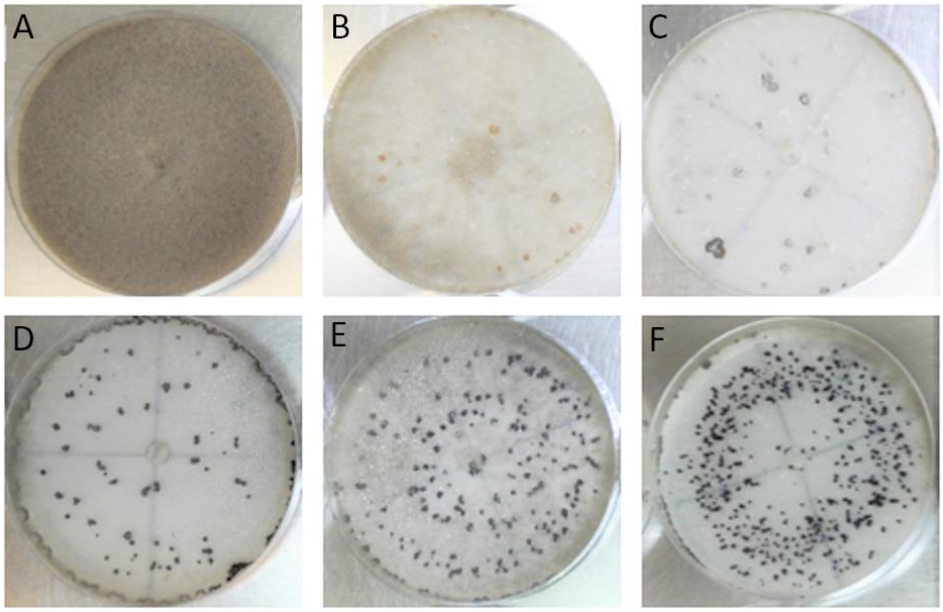


Figure S4.2 Classification of sclerotia production by *B. cinerea* isolates. A: Class 0 (no sclerotia formation); B: Class Ⅰ(very few sclerotia formation); C: Class Ⅱ(sclerotia formation sparse); D: Class Ⅲ(average amount sclerotia formation); E: Class Ⅳ(many sclerotia formation); F: Class Ⅴ(abundant sclerotia formation).

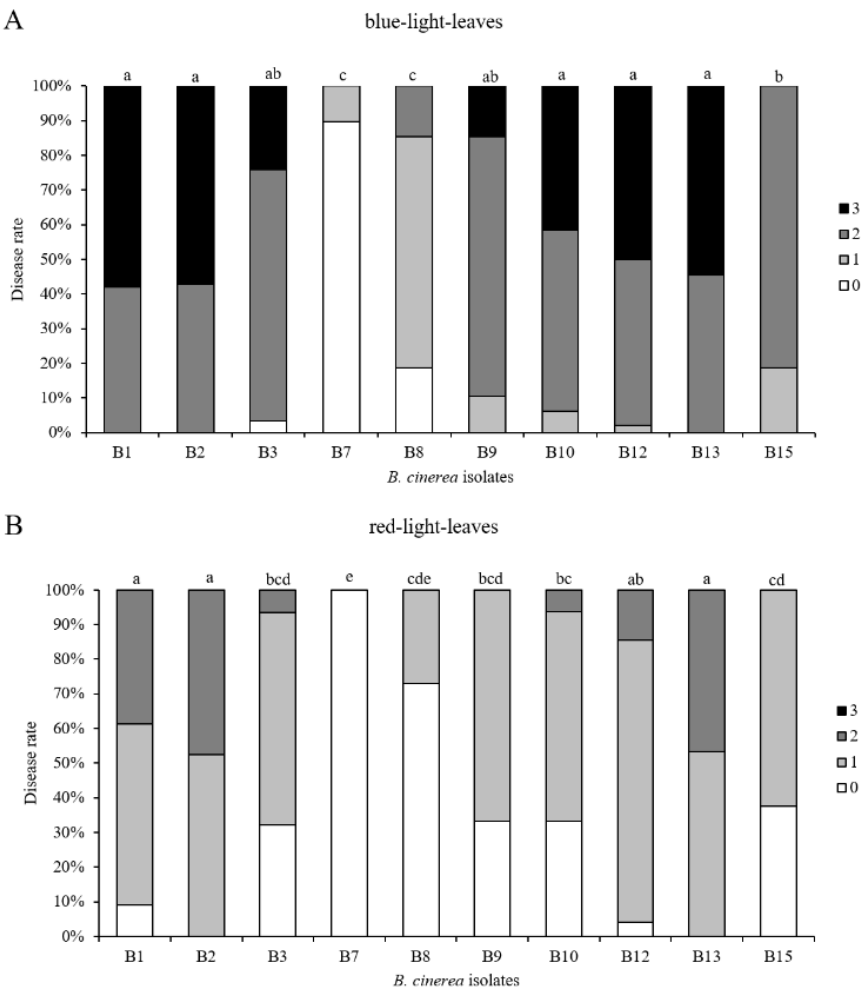
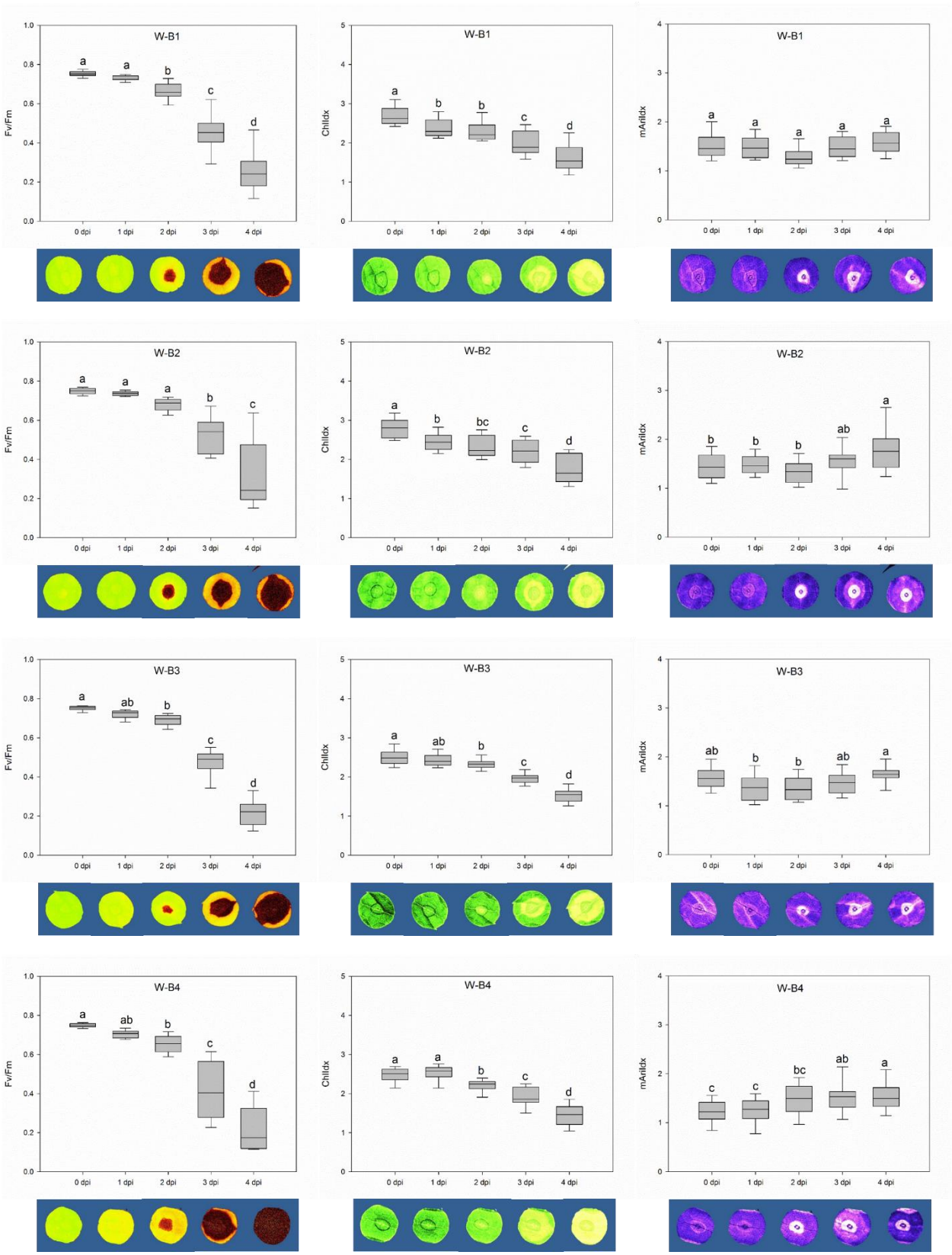
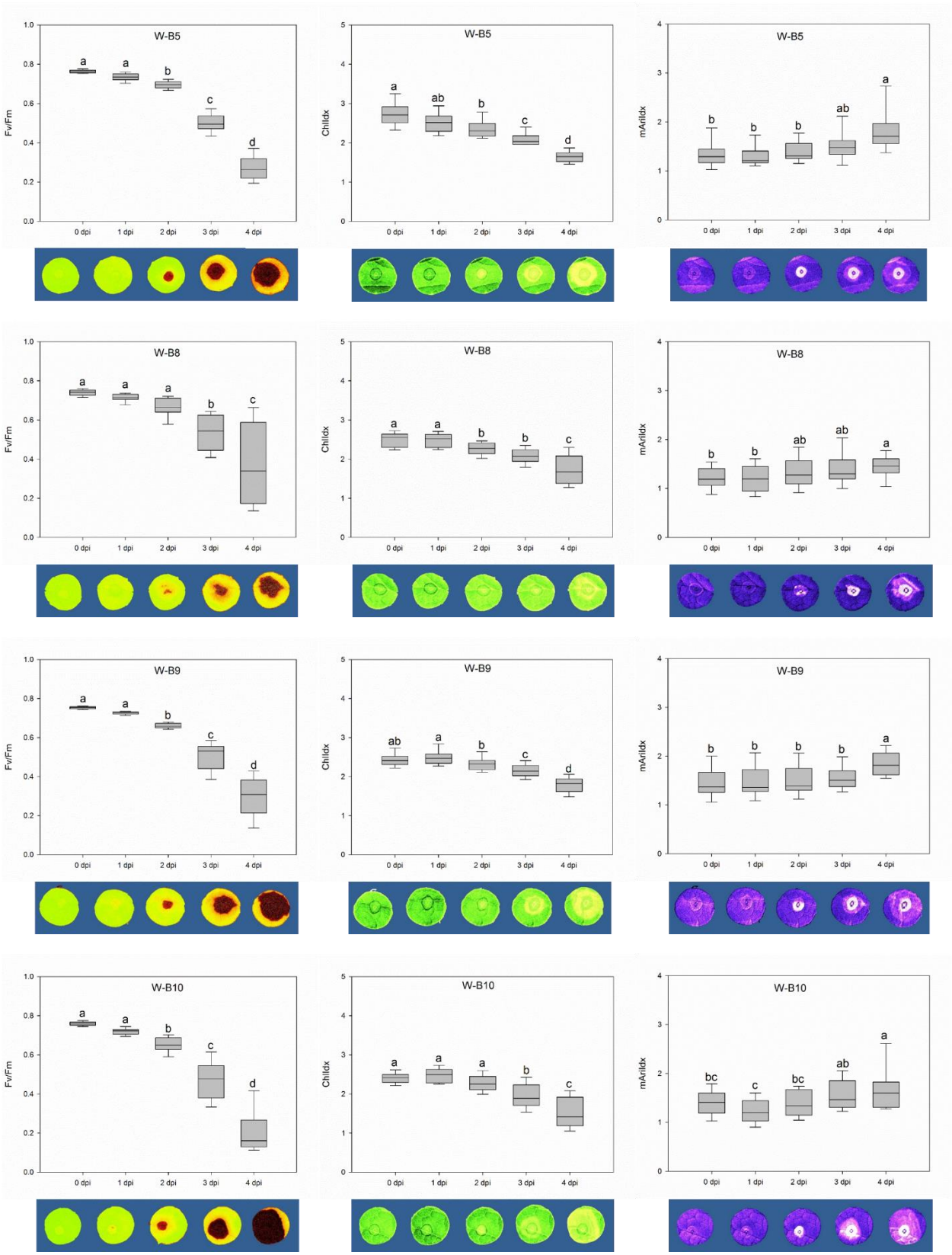


Figure S4.3 Pathogenicity of ten *B. cinerea* isolates on strawberry leaves developed under blue and red LED lights by spore inoculation. Disease rating was scored 3 days post inoculation using four scoring categories (0, resistant; 1, slightly spreading lesion; 2, moderately spreading lesion; 3, severely spreading lesion). Different letters indicate statistical significance among the isolates performed by Kruskall-Wallis followed by a post-hoc Dunn's test with Bonferroni correction ($p \leq 0.005$, $n = 4$).


W-B1
Fv/Fm
Chlidx
mAnthidx
W-B2
W-B3
W-B4
0 dpi
1 dpi
2 dpi
3 dpi
4 dpi


W-B5
Fv/Fm
a a b c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B5
ChlIdx
a ab b c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B5
mAriIdx
b b b ab a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B8
Fv/Fm
a a a b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B8
ChlIdx
a a b b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B8
mAriIdx
b b ab ab a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B9
Fv/Fm
a a b c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B9
ChlIdx
ab a b c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B9
mAriIdx
b b b b a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B10
Fv/Fm
a a b c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B10
ChlIdx
a a a b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
W-B10
mAriIdx
bc c bc ab a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi

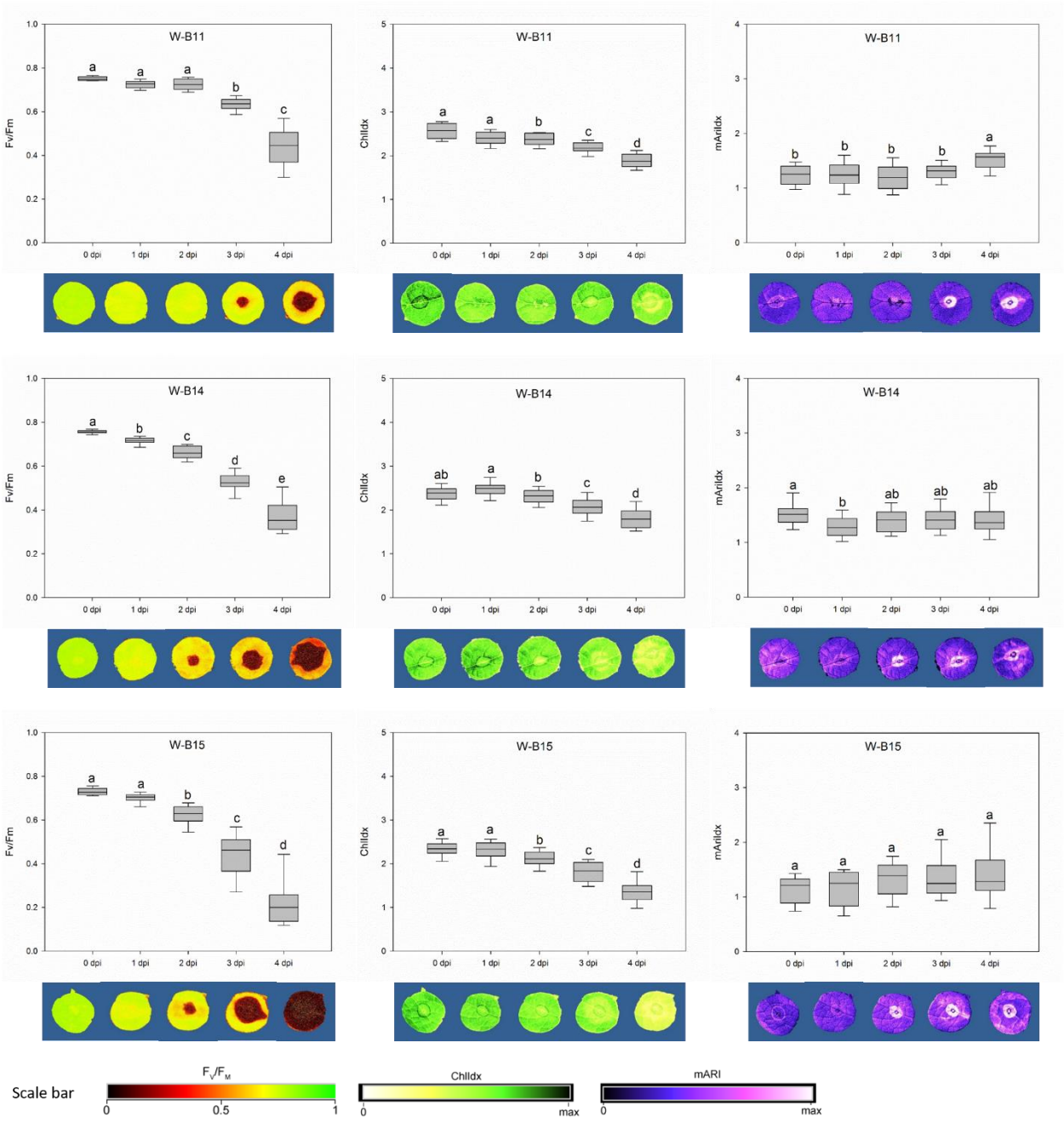
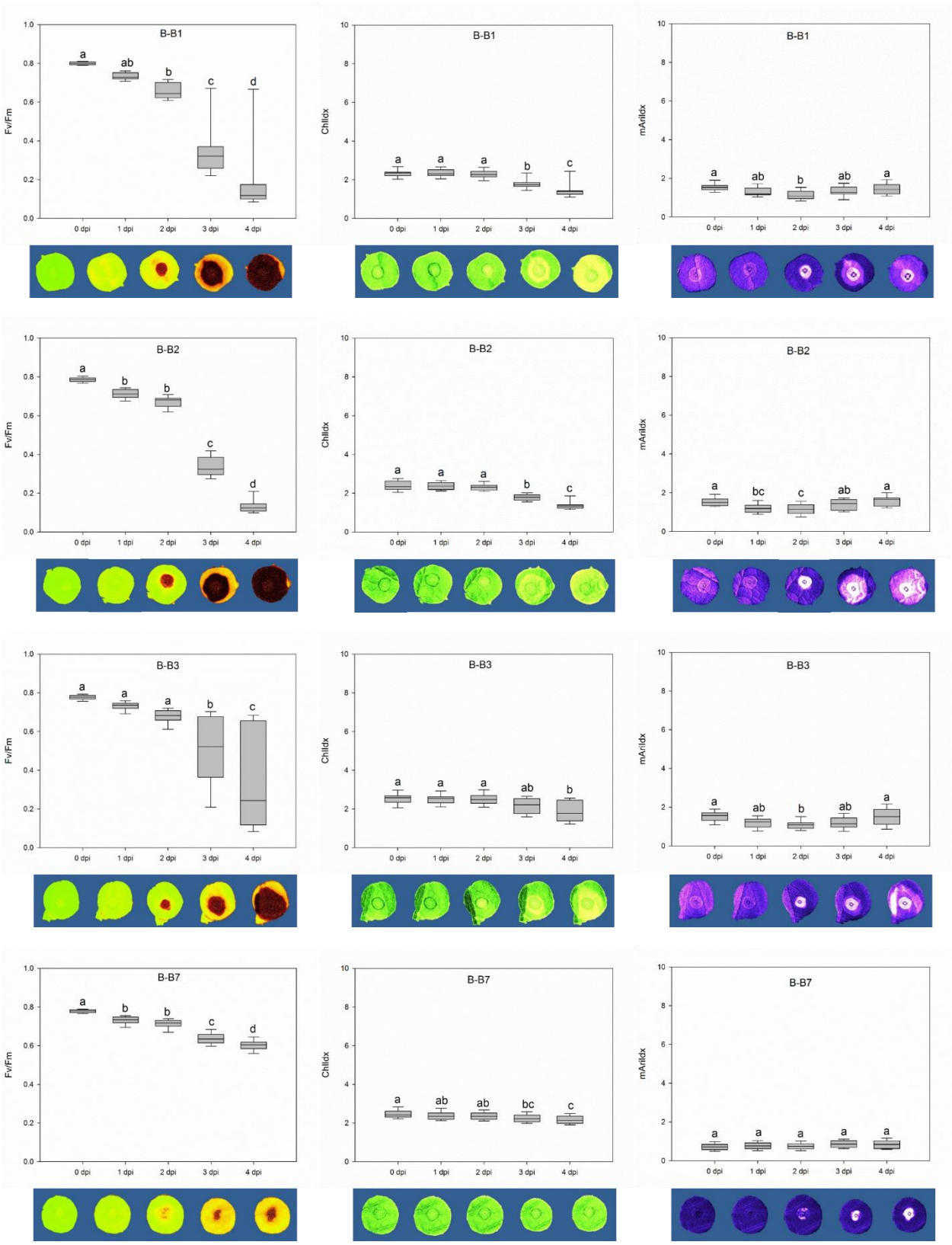
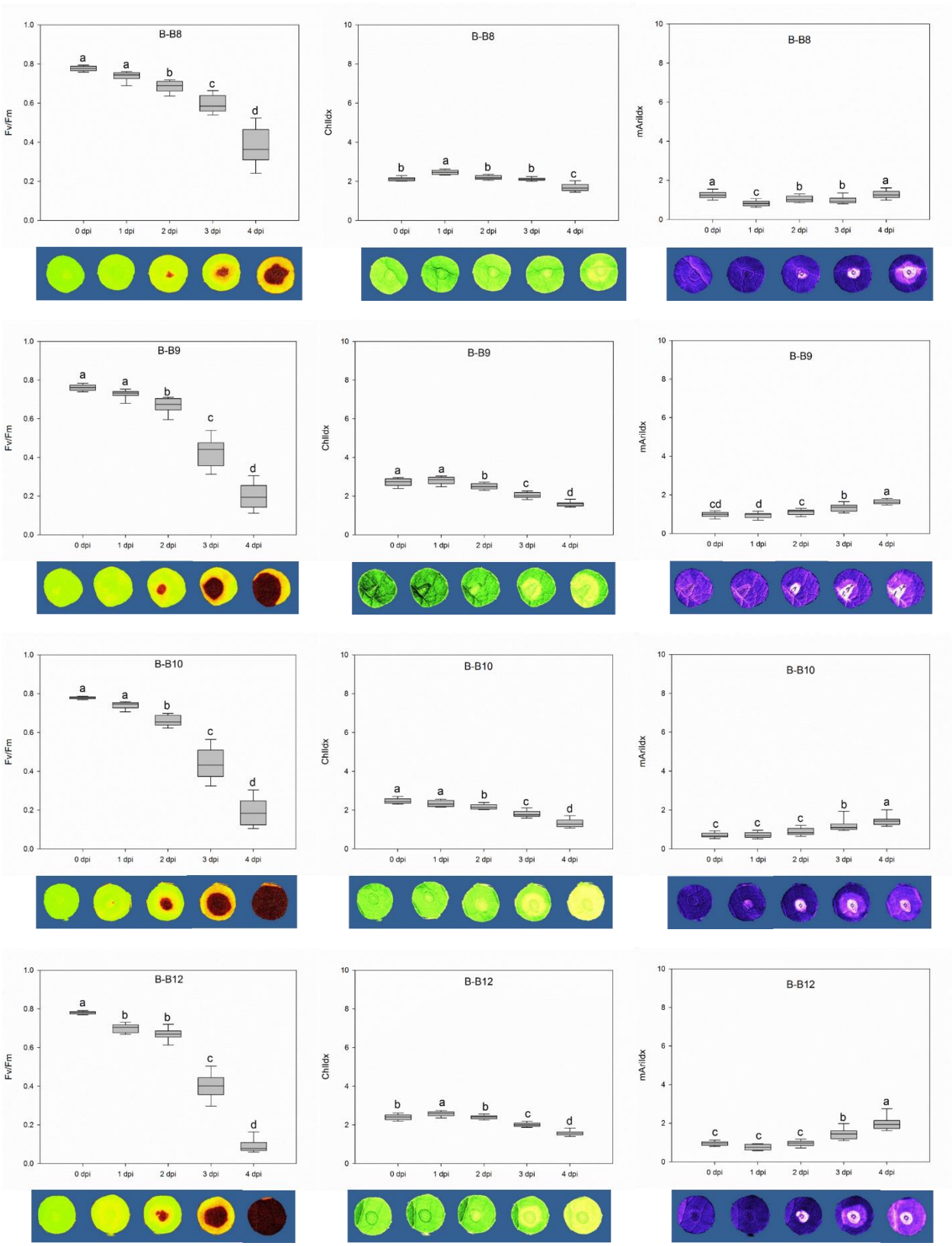


Figure S4.4 The variations of F_v/F_m, ChlIdx, and mAriIdx from 0 to 4 dpi caused by B1, B2, B3, B4, B5, B8, B9, B10, B11, B14, and B15 were correlated with the development of disease lesion on white-light-leaves. The corresponding images are present underneath the figures. Disease lesion led to darker F_v/F_m images with lower value, yellower ChlIdx images with lower value, and brighter mAriIdx images with higher values. One-way ANOVA was applied for the statistical analysis (Tukey test, $p \leq 0.05$), and data were shown by box plots with the median. Different letters indicate significant differences among the time points.


B-B1
Fv/Fm
a
ab
b
c
d
0 dpi
1 dpi
2 dpi
3 dpi
4 dpi
B-B1
ChlIdx
a
a
a
b
c
B-B1
mAriIdx
a
ab
b
ab
a
B-B2
Fv/Fm
a
b
b
c
d
B-B2
ChlIdx
a
a
a
b
c
B-B2
mAriIdx
a
bc
c
ab
a
B-B3
Fv/Fm
a
a
a
b
c
B-B3
ChlIdx
a
a
a
ab
b
B-B3
mAriIdx
a
ab
b
ab
a
B-B7
Fv/Fm
a
b
b
c
d
B-B7
ChlIdx
a
ab
ab
bc
c
B-B7
mAriIdx
a
a
a
a
a


B-B8
Fv/Fm
Chlidx
mAnldx
B-B9
B-B10
B-B12
0 dpi
1 dpi
2 dpi
3 dpi
4 dpi

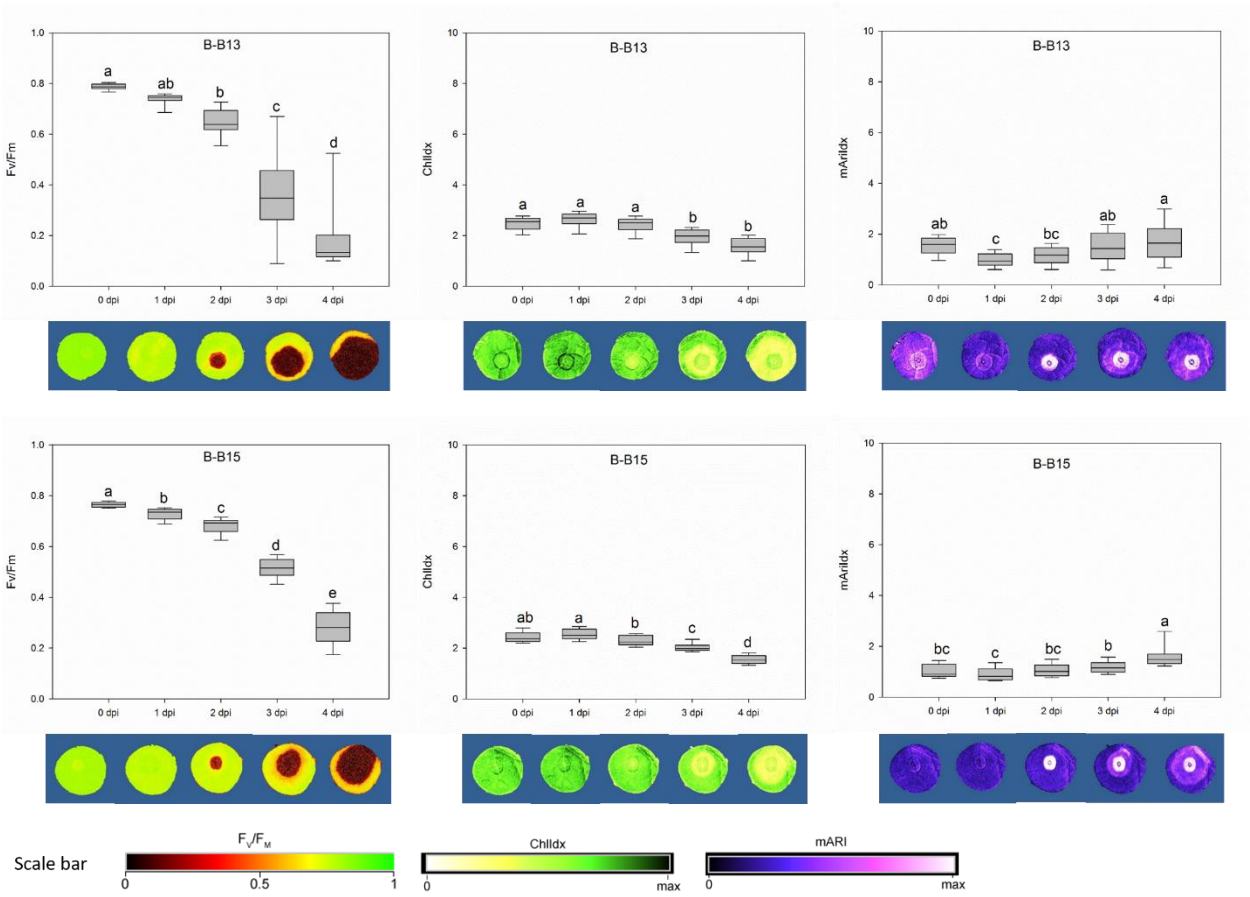
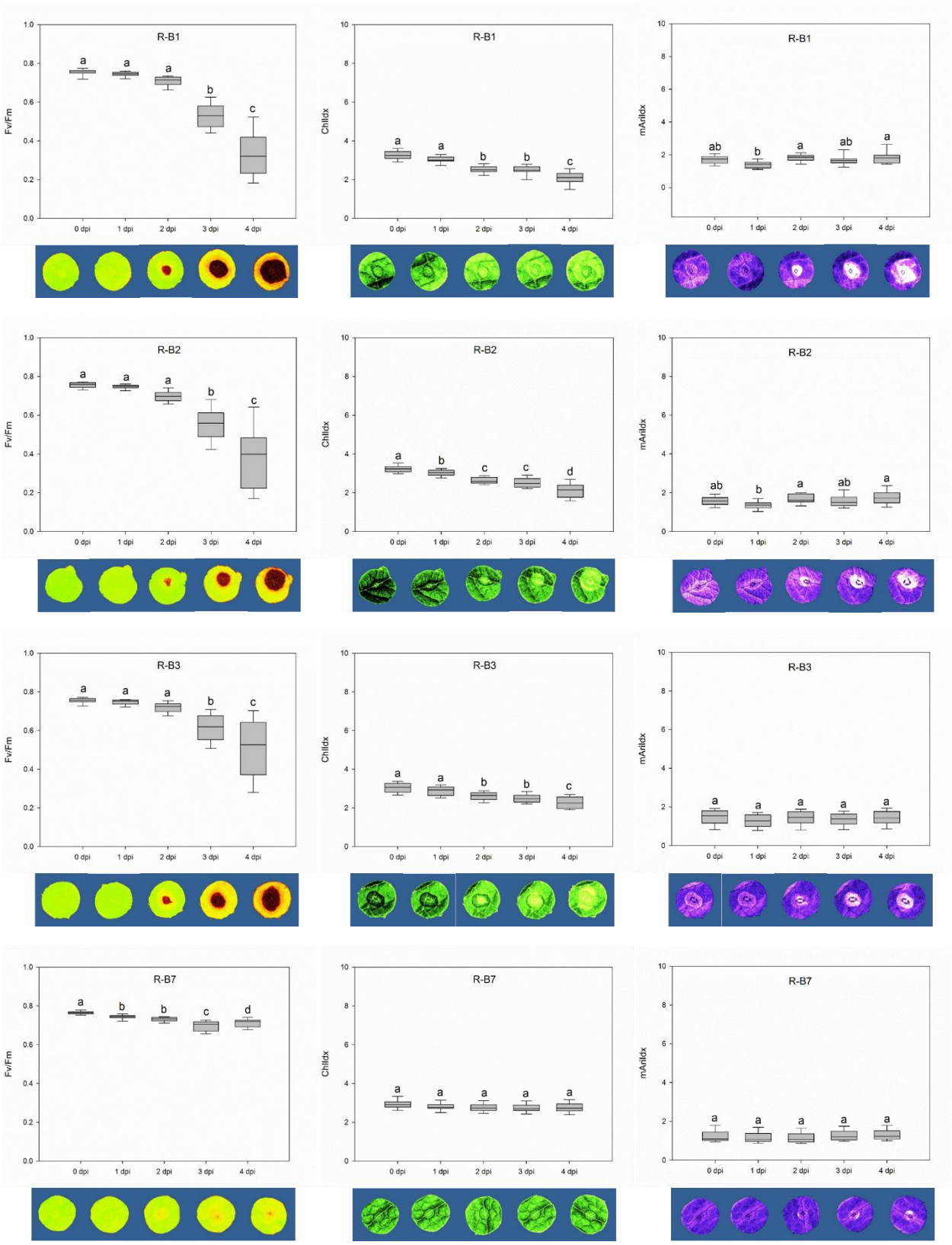
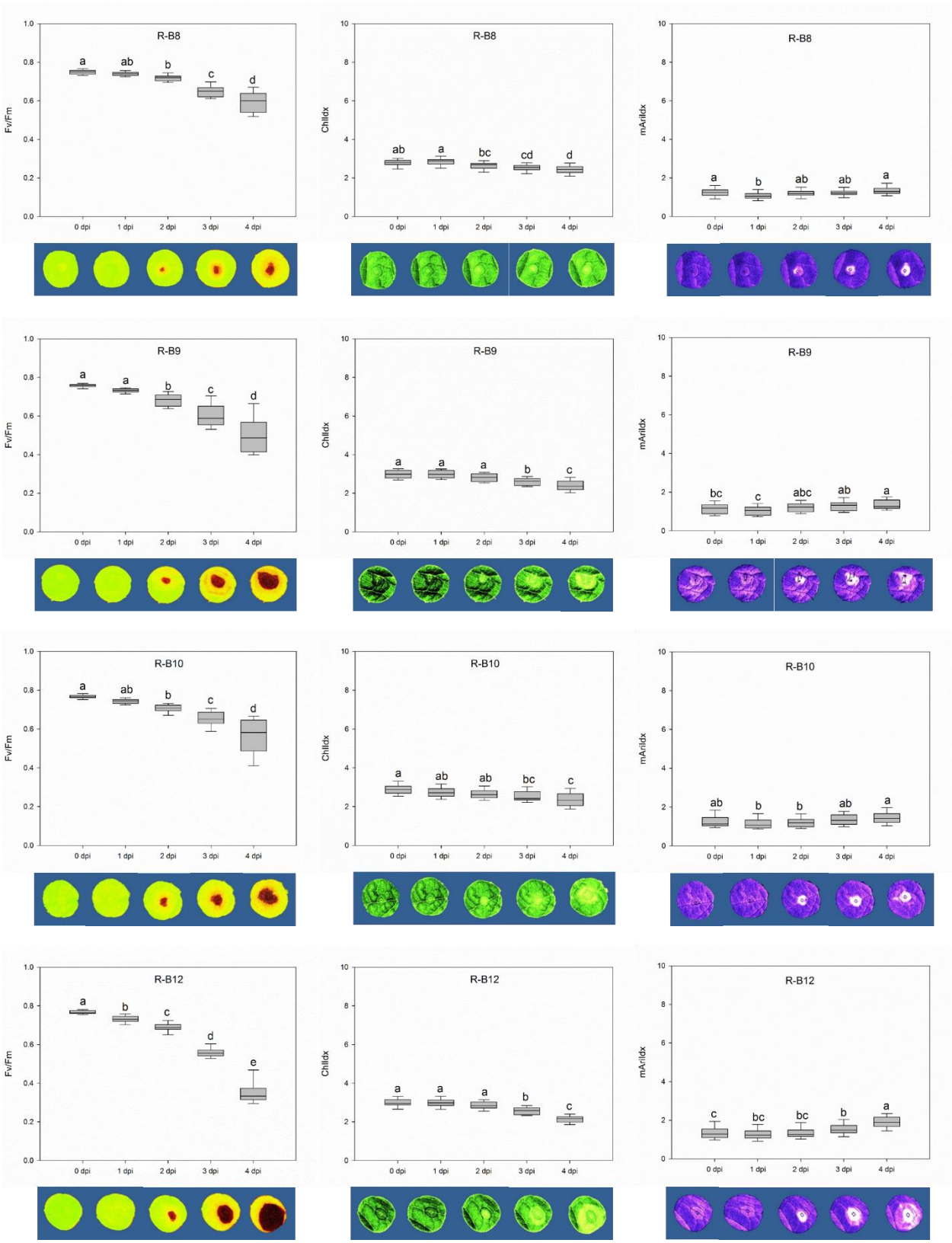


Figure S4.5 The variations of F_v/F_m, ChlIdx, and mAriIdx from 0 to 4 dpi caused by B1, B2, B3, B7, B8, B9, B10, B12, B13 and B15 were correlated with the development of disease lesion on blue-light-leaves. The corresponding images are present underneath the figures. Disease lesion led to darker F_v/F_m images with lower value, yellower ChlIdx images with lower value, and brighter mAriIdx image with higher values. One-way ANOVA was applied for the statistical analysis (Tukey test, $p \leq 0.05$), and data were shown by box plots with the median. Different letters indicate significant differences among the time points.


R-B1
Fv/Fm
a a a b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B1
ChlIdx
a a b b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B1
mAriIdx
ab b a ab a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B2
Fv/Fm
a a a b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B2
ChlIdx
a b c c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B2
mAriIdx
ab b a ab a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B3
Fv/Fm
a a a b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B3
ChlIdx
a a b b c
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B3
mAriIdx
a a a a a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B7
Fv/Fm
a b b c d
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B7
ChlIdx
a a a a a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi
R-B7
mAriIdx
a a a a a
0 dpi 1 dpi 2 dpi 3 dpi 4 dpi


R-B8
Fv/Fm
a
ab
b
c
d
0 dpi
1 dpi
2 dpi
3 dpi
4 dpi
R-B8
ChlIdx
ab
a
bc
cd
d
R-B8
mAriIdx
a
b
ab
ab
a
R-B9
Fv/Fm
a
a
b
c
d
R-B9
ChlIdx
a
a
a
b
c
R-B9
mAriIdx
bc
c
abc
ab
a
R-B10
Fv/Fm
a
ab
b
c
d
R-B10
ChlIdx
a
ab
ab
bc
c
R-B10
mAriIdx
ab
b
b
ab
a
R-B12
Fv/Fm
a
b
c
d
e
R-B12
ChlIdx
a
a
a
b
c
R-B12
mAriIdx
c
bc
bc
b
a

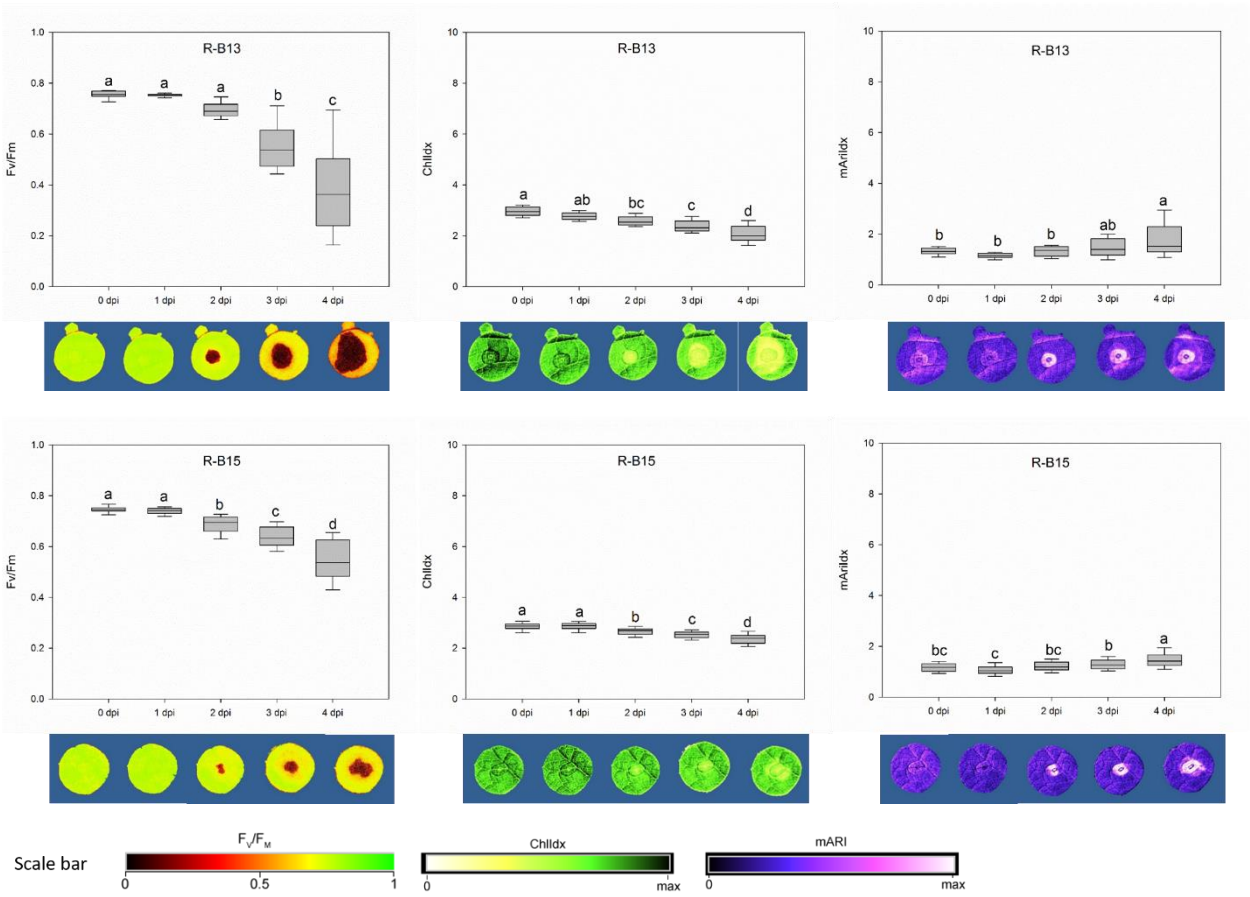


Figure S4.6 The variations of F_v/F_m, ChlIdx, and mAriIdx from 0 to 4 dpi caused by B1, B2, B3, B7, B8, B9, B10, B12, B13 and B15 were correlated with the development of disease lesion on red-light-leaves. The corresponding images are present underneath the figures. Disease lesion led to darker F_v/F_m images with lower value, yellower ChlIdx images with lower value, and brighter mAriIdx images with higher values. One-way ANOVA was applied for the statistical analysis (Tukey test, $p \leq 0.05$), and data were shown by box plots with the median. Different letters indicate significant differences among the time points.

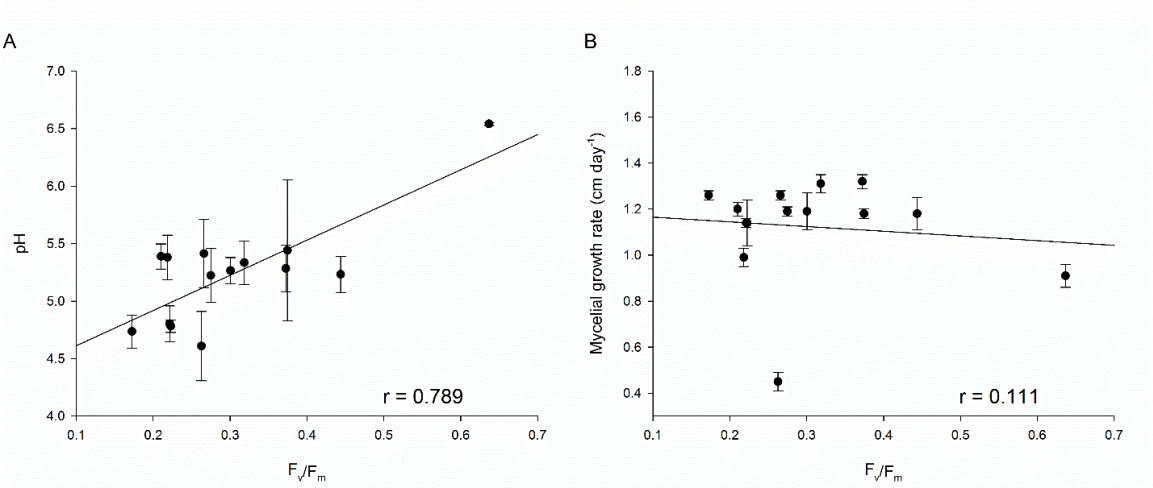


Figure S4.7 The relationship between F_v/F_m and pH (**A**) and mycelial growth rate (**B**). F_v/F_m values observed at 4 dpi from infection in white-light leaves were used for the Pearson correlation (r). Values are presented as means with standard deviation shown by a vertical bar.

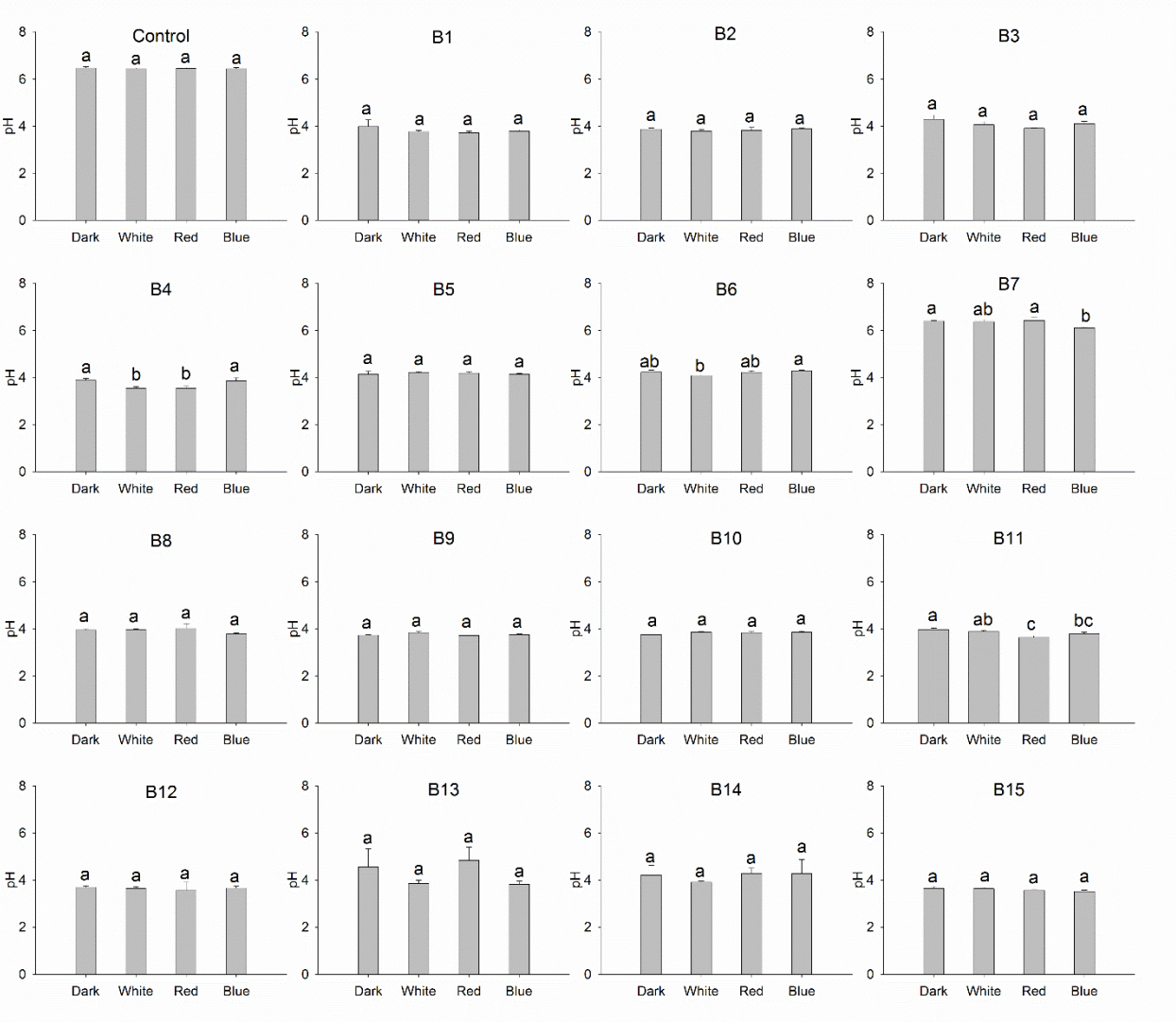


Figure S4.8 Effect of light quality on medium acidification caused by fifteen *Botrytis* isolates. Control and inoculated plates were incubated under different light treatments (dark, white, red, and blue) in 16 h/8 h light/dark conditions. Medium pH was shown to indicate the degree of acidification. Data are shown as means ± SE (n = 6). Different letters indicate significant differences between light treatments. One-way ANOVA for B2, B4, B5, B6, B7, B10, B11, and non-parametric Kruskal-Wallis test followed by a post-hoc Dunn's test for the other isolates was performed with $p \leq 0.001$.

Table S4.1 Mycelial growth rate of *B. cinerea* isolates under different light conditions.

B. cinerea Isolates	Growth Rate (cm day^{-1})											
	Dark		Daylight		White		Blue		Red		Blue+Red	
B1	1.26±0.02	ab	0.94±0.08	ef	0.86±0.10	ef	0.90±0.12	f	0.94±0.10	e	1.07±0.03	gh
B2	1.31±0.04	a	1.18±0.02	c	1.37±0.03	ab	1.37±0.03	a	1.40±0.09	ab	1.38±0.03	a
B3	0.99±0.04	efg	0.82±0.05	fg	1.29±0.05	cd	1.29±0.02	cd	1.29±0.04	cd	1.21±0.04	fgh
B4	1.14±0.10	def	1.06±0.05	d	1.32±0.08	bc	1.38±0.06	a	1.28±0.04	cd	1.32±0.03	abcd
B5	1.19±0.02	cd	1.08±0.02	d	1.30±0.03	cd	1.27±0.08	cde	1.28±0.04	cd	1.31±0.02	cde
B6	1.24±0.02	abc	1.10±0.03	d	1.32±0.08	bc	1.23±0.10	de	1.40±0.09	ab	1.32±0.03	abcd
B7	0.91±0.05	fg	0.84±0.10	fg	1.22±0.05	def	1.21±0.02	def	1.29±0.05	cd	1.28±0.04	cdef
B8	1.32±0.03	a	1.42±0.03	a	1.37±0.08	ab	1.57±0.06	a	1.37±0.03	ab	1.32±0.03	abc
B9	1.19±0.08	bcd	1.24±0.02	ab	1.47±0.08	a	1.43±0.14	a	1.47±0.03	a	1.35±0.10	a
B10	1.20±0.03	bcd	1.19±0.02	bc	1.32±0.03	bc	1.32±0.03	c	1.23±0.06	cd	1.33±0.06	a
B11	1.18±0.07	cd	1.20±0.00	abc	1.37±0.03	ab	1.42±0.10	a	1.45±0.13	a	1.20±0.13	efg
B12	0.45±0.04	g	0.49±0.02	g	0.59±0.03	f	0.59±0.07	f	0.62±0.08	e	0.66±0.09	h
B13	1.26±0.02	ab	1.19±0.05	c	1.28±0.05	cd	1.26±0.04	de	1.32±0.02	bc	1.26±0.04	def
B14	1.18±0.02	de	1.07±0.03	de	1.27±0.03	cde	1.28±0.02	cde	1.31±0.02	bc	1.28±0.02	cdef
B15	1.14±0.02	de	1.09±0.02	d	1.26±0.02	cde	1.22±002	ef	1.22±0.02	de	1.14±1.13	fgh

Values are means of six replicates ± standard deviation; different letters indicate significant differences based on Kruskal-Wallis test with Bonferroni correction ($P \leq 0.0033$).

Table S4.2 Overall anthocyanin content at 0, 1, 2, 3, 4 dpi of leaves pretreated with different light qualities: white, blue, and red.

Light treatments	0 dpi	1 dpi	2 dpi	3 dpi	4 dpi
white	1.356 a	1.266 a	1.327 a	1.459 a	1.580 a
blue	1.129 b	0.960 c	1.072 b	1.271 b	1.560 a
red	1.343 a	1.172 b	1.335 a	1.389 a	1.552 a

As light quality influences metabolite levels in the leaves, ANCOVA was performed and the covariates are the values of AriIdx at 0 dpi. Different letters indicate significant differences for overall isolates between the light treatments (One-way ANOVA, Tukey's test, $p \leq 0.05$).

Table S4.3 Comparison of anthocyanin content between the representative *Botrytis* isolates: B7 (non-pathogenic strain), B12 (intermediate virulence), and B13 (highest virulence) for white-light-leaves, B7 (non-pathogenic strain), B15 (intermediate virulence), and B2 (highest virulence) for blue- and red-light leaves.

strains	white				strains	blue				red			
	1 dpi	2 dpi	3 dpi	4 dpi		1 dpi	2 dpi	3 dpi	4 dpi	1 dpi	2 dpi	3 dpi	4 dpi
B7	1.217 a	1.310 a	1.305 b	1.049 b	**B7**	0.787 b	0.738 b	0.844 b	0.871 b	1.217 a	1.179 b	1.296b	1.358b
B12	1.026 a	1.340 a	1.434 b	1.769 a	**B15**	0.908 b	1.067 a	1.209 a	1.607 a	1.109 a	1.258 b	1.326a b	1.558a b
B13	1.275 a	1.473 a	1.743 a	1.852 a	**B2**	1.180 a	1.151 a	1.445 a	1.585 a	1.287 a	1.605 a	1.523a	1.657a

Data are means of 24 replicates. To limit the influence of anthocyanin content in different individual leaves, ANCOVA was performed and the covariates are the values of mAriIdx at 0 dpi. Different letters indicate significant differences for each day (One-way ANOVA, Tukey's test, $p \leq 0.05$).

Chapter 5

General Discussion and Future Perspectives

5.1 General discussion

Greenhouse cropping systems have developed rapidly worldwide to meet the increasing demand for soft fruit, vegetables or ornamentals. Light is the most important environmental factor in these production systems. Supplementary light is applied to extend the day length as well as the growing season with the aim to maximize yield. LEDs as the newest type of artificial lighting are increasingly used as lighting source in greenhouses owing to their high efficiency in promoting plant growth and development as well as the energy advantages they have over conventional light sources. Moreover, LEDs with specific wavelengths can also influence plant resistance to various attackers, which is a promising aspect for environmental-friendly disease control in greenhouse production. On the other hand, also the attacking pathogens perceive light signals by means of their photoreceptors to fine-tune their adaptation. Therefore, supplementary lighting sources in greenhouse production play an inescapable role in the outcome of plant-pathogen interactions.

This doctoral research studied the influences of specific light qualities on strawberry, *Botrytis cinerea*, and their interaction. In this final chapter, we will discuss the main conclusions of the three experimental chapters and give some mechanistic insights. At last, some perspectives on future research are given.

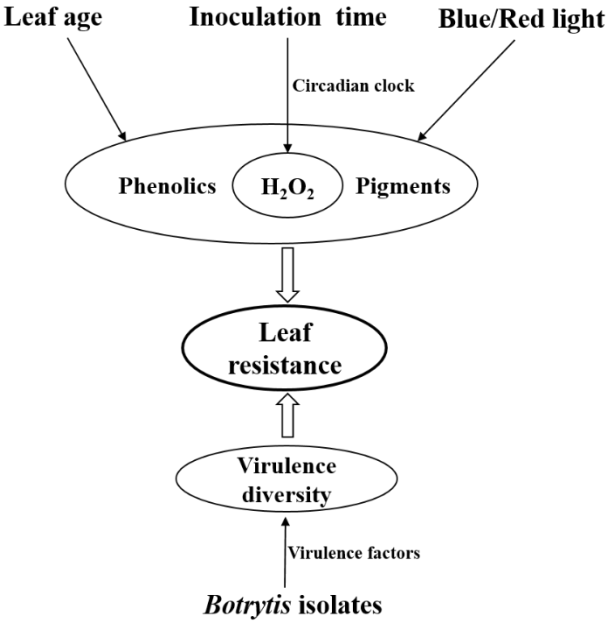


Figure 5.1 Overview of factors (leaf age, inoculation time, blue/red light, *Botrytis* isolates) studied in this thesis that influence leaf resistance to *Botrytis cinerea* in strawberry.

Based on the findings in this doctoral research, leaf age, inoculation time, and light quality (blue/red light) have significant effects on leaf resistance in strawberry. On the other hand,

Botrytis isolates with different virulence result in different disease severity, this indirectly affects leaf resistance (Figure 5.1).

Leaf age is an important determining factor of stress responses due to the link between stress susceptibility and a range of metabolic changes depending on leaf development (Coleman, 1986). In strawberry, leaf age significantly influenced leaf resistance to *B. cinerea*. Leaf resistance gradually and significantly increased from 1-week-old to 4-week-old leaves, and decreased in 5-week-old leaves (Table 1.1 in **Chapter 2**). Leaf resistance upon leaf age negatively correlated with H_2O_2 and flavonoid levels, and positively correlated with the content of pigments such as chlorophyll and carotenoids (Table 1.1 in **Chapter 2**). This coincides with the knowledge that high H_2O_2 levels benefit the successful infection by *B. cinerea* (Govrin & Levine, 2000). The correlation between leaf resistance and flavonoids is mainly explained by the high flavonoid content in the susceptible 1-week-old leaves. As for the pigments, lower chlorophyll content contributes to the enhanced sensitivity of plants to pathogens (Demotes-Mainard et al., 2016). Therefore, leaf age mediates leaf resistance by affecting the biosynthesis pathways of H_2O_2, flavonoids, and pigments.

The effect of inoculation time on leaf resistance is caused by effects of the circadian clock on leaf resistance. Plant responsiveness to both abiotic and biotic stresses is under circadian control. Timing of inoculation showed diurnal variation in leaf susceptibility to *B. cinerea*. Susceptibility was highest in leaves inoculated in the evening (12 h after the start of photoperiod) followed by incubation in dark (Figure 3.3 in **Chapter 3**). This higher susceptibility correlated with the highest H_2O_2 levels, regulated by the circadian clock, at the moment of *B. cinerea* penetration (8 hours post inoculation) (Govrin & Levine, 2000; Lai et al., 2012). When disease developed under light/dark conditions, the shorter the light period after inoculation, the stronger the disease symptoms (Figure 3.3 in **Chapter 3**). This is due to the disease inhibition by the presence of light, which will be discussed later. On the other hand, the effects of the circadian clock on leaf resistance can be altered by changes in the environment, such as light signals (Gómez & Simón, 1995). In this study, 12 h of blue/red light in the photoperiod altered the circadian variation of leaf resistance. This was correlated with alterations in leaf metabolites, such as H_2O_2, total phenolics and hexoses.

Light quality significantly influences leaf resistance. In this doctoral research, blue and red light were studied. As leaf resistance and the strawberry leaf-*B. cinerea* interaction are the central focus of this doctoral research, we will primarily discuss the effect of light quality on the interaction between strawberry leaves and *B. cinerea*, but also refer to the phenotypic effects of light quality on strawberry and *B. cinerea*, separately.

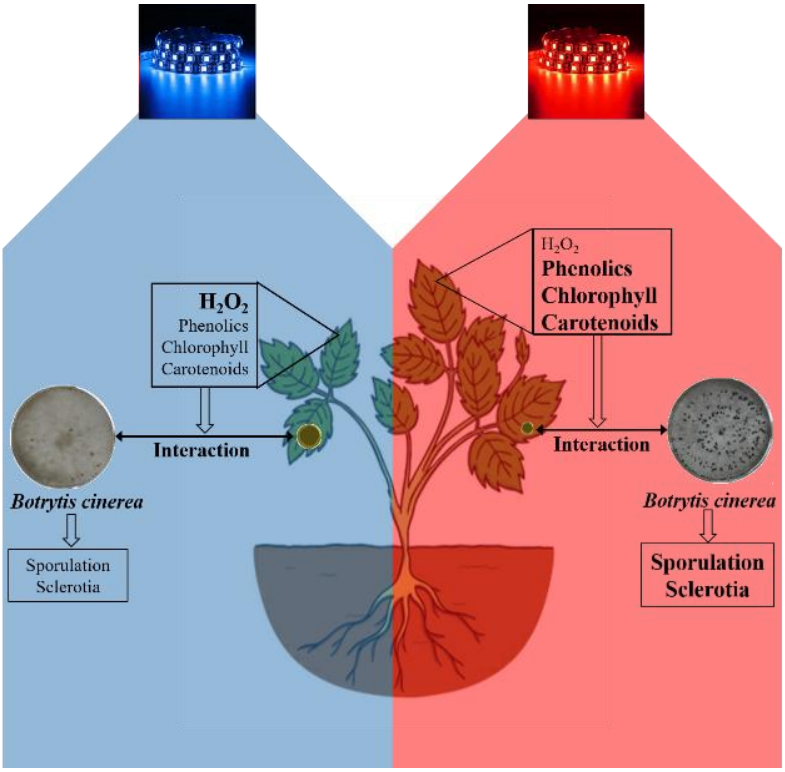


Figure 5.2 The effects of blue/red light on strawberry, *Botrytis cinerea*, and the outcome of their interaction. Red light increased leaf number, the contents of phenolics, chlorophyll and carotenoids, decreased H_2O_2 levels in strawberry leaves, it stimulated sporulation and sclerotia formation in the fungus, and it enhanced leaf resistance to *B. cinerea*. Blue light decreased leaf number, the contents of phenolics, chlorophyll and carotenoids, increased H_2O_2 levels in strawberry leaves, it inhibited sporulation and sclerotia formation in the fungus, and it decreased leaf resistance to *B. cinerea*.

The effects of light quality on plant resistance after inoculation with *B. cinerea* have been studied, while the influence of light quality on leaf basal resistance has hardly been investigated. First of all, the effects of pre-treatment with different light qualities on leaf basal resistance were explored in strawberry. Red light pre-treatment increased, while blue light decreased leaf basal resistance to *B. cinerea* (Table 2.1 and Figure 2.3 in **Chapter 2**). Secondary metabolites that play important roles in plant response to abiotic and biotic stresses are greatly modulated by the light spectrum (Bennett & Wallsgrove, 1994; Darko et al., 2014). Here, light quality considerably influenced the levels of H_2O_2, phenolics and flavonoids, proline, pigments, as well as activities of antioxidative enzymes. Leaf basal resistance was highly correlated with the contents of H_2O_2, total phenolics, chlorophyll and carotenoids (Figure 2.7 in **Chapter 2**). Blue-light-grown leaves with high susceptibility to *B. cinerea* resulted in high levels of H_2O_2, low contents of total phenolics, chlorophyll and carotenoids. On the contrary, red-light grown leaves with high resistance to *B. cinerea* resulted in low levels of H_2O_2, high contents of total phenolics, chlorophyll and carotenoids. As discussed above, H_2O_2, phenolics, pigments are important determining factors of the

outcome of leaf-*B. cinerea* interaction. Light quality affects leaf basal resistance by regulating the basal contents of H_2O_2, total phenolics, and pigments (Figure 5.2).

Plants are grown under light/dark cycles. Light presence definitely affects the outcome of plant-pathogen interactions. Moreover, the effects of light/dark incubation on infection of *B. cinerea* were investigated. Light presence during incubation inhibited *Botrytis* infection, irrespective of light quality, compared to dark incubation. The influence of light on the outcome of plant-pathogen interactions is correlated with the direct effects on both plant defense response and the virulence of the pathogen (Roden & Ingle, 2009), as both plants and *Botrytis* process photoreceptors to perceive the environmental light signals (Galvão & Fankhauser, 2015; Schumacher, 2017). Under light regime incubation, blue light had no significant effect on disease development, red light again decreased disease severity when compared to white light (Figure 3.2 in **Chapter 3**). The different outcome by blue light from dark incubation is mainly due to the direct effect of light on *B. cinerea*. Blue light irradiation was reported to directly inhibit *Botrytis* disease (K. Kim et al., 2013). The further inhibition by red light irradiation in disease development benefits from positive phototropism in *B. cinerea* caused by red light, leading to unsuccessful penetration (Islam et al., 1998). In conclusion, disease inhibition under red light pre- and post-treatment is due to both the improvement of leaf basal resistance (direct effects of red light directly on the plant) and unsuccessful infection of *B. cinerea* (direct effect of red light on the pathogen) (Figure 5.2).

Despite the positive effect of red light on leaf resistance, monochromatic red light irradiation may lead to a dysfunctional photosynthesis, while the combination with white LED light can improve the output and nutritional quality (Dong et al., 2014; Hogewoning et al., 2010). The effects of blue or red LED light combined with white LED on the outcome of strawberry leaf-*B. cinerea* interaction were investigated. Twelve hours of blue and red light in a 16 h photoperiod significantly increased and decreased, respectively, leaf susceptibility in inoculated leaves under both dark and light/dark conditions, while 4 h blue/red light was not sufficient to cause the alterations (Figure 3.4 in **Chapter 3**). The phytochrome B-regulated jasmonate signaling pathway is the key regulator in plant defense response and plays a very important role in the competition between the shade avoidance syndrome and the disease syndrome (Ballaré & Austin, 2019; Moreno et al., 2009). The increase in leaf resistance by 12 h red light is probably due to the activation of PhyB by the high proportion of red light (12 h out of 16 h), while the decrease of leaf resistance by blue light may be due to the inactivated phyB under longer irradiation of blue light, as phyB perceives the changes in red:far-red ratio (Ballaré, 2014). On the other hand, the alteration in leaf resistance by 12 h blue/red light is caused by the alteration in secondary metabolites (Figure 3.5 in **Chapter 3**), such as H_2O_2 and total phenolics, as discussed above, they are highly correlated with leaf

resistance. In conclusion, a high proportion of red light in combination with white light that can stimulate the phyB-dependent jasmonate pathway is able to improve leaf resistance to *B. cinerea* as well as guarantee a normal plant growth.

B. cinerea is a fungal pathogen with a high degree of virulence diversity and this will be discussed later. Due to the variability in virulence, fungicide efficiency differs upon *Botrytis* isolates (Leroch et al., 2011). Therefore, it is interesting to study light quality effects on leaf resistance to various *Botrytis* isolates. In Chapter 4, the effects of light quality pre-treatment on leaf resistance was tested using different *B. cinerea* isolates. Red light pre-treatment increased leaf basal resistance to all the tested *B. cinerea* isolates (Figure 4.6 in **Chapter 4**). This again confirmed the effective role of red light in improving leaf resistance. Thus, *Botrytis* disease control by red LED light can be developed in greenhouse production.

In summary, pre- and/or post-treatment of light quality, light combinations, and various *Botrytis* isolates were studied with respect to blue/red light. Overall results showed the enhancement of leaf resistance by red light, thus confirming the promising role of red LED light in the control of grey mold.

In addition to the effect of light quality on plant-pathogen interactions, light quality also has direct phenotypic effects on plant, and pathogen, separately. In strawberry, red light increased leaf initiation rate, blue light had no significant effect, while a combination of blue and red light inhibited leaf initiation (Figure 2.2 in **Chapter 2**). However, this is not the case in *Alternanthera* and cucumber seedlings (Hernández & Kubota, 2016; Macedo et al., 2011). Thus, the effect of light quality on leaf initiation is plant-dependent.

As to *B. cinerea*, phenotypic variability of different isolates is influenced by light quality. The fifteen *Botrytis* isolates in this study differed in their mycelial growth rate and in their ability to reproduce by conidia or to form sclerotia. This intraspecies variation in *B. cinerea* is well documented and can be influenced by environmental light (Mirzaei et al., 2009; Tan & Epton, 1973). Although *B. cinerea* responds to light signals in vegetative growth by processing eleven photoreceptors, the effect of light quality on mycelial growth in this study is limited. Both sporulation and sclerotia formation were mediated by the light treatments used in this study (Figure 4.3 in **Chapter 4**). Generally, sporulation happens exclusively in light, it was strongly triggered by near-UV light, and slightly stimulated by red light, but inhibited by blue light (K. K. Tan & Epton, 1973). Here, the inhibition of blue light on sporulation was only observed in nine of the fifteen isolates. Adding red light to blue light did not enhance sporulation. Monochromatic red light resulted in similar or lower sporulation which is due to the slight stimulation by red light. Daylight with full light spectrum induced sporulation in all isolates, while white LED light with a similar spectrum hardly induced sporulation, this might

be explained by day length. The daylight treatment was conducted in winter in Belgium, characterized by short day lengths, while the white LED light was applied as a 16 h photoperiod. Additionally, blue, blue+red, and white lights inhibited sclerotia formation in all isolates. This inhibition is due to the blue light fraction as under red light sclerotia were formed in most isolates. Daylight in winter at a very low light fluence induced sclerotia formation which can be caused by the enrichment of the longer wavelengths (700-800 nm, Figure S4.1 in **Chapter 4**) in the indoor daylight spectrum (Tan & Epton, 1973). These phenotypic responses of *B. cinerea* isolates to light quality are very diverse and sometimes conflicting with earlier articles, hence, more *Botrytis* strains should be studied for light quality responses, and light intensity as well as day length should be taken into account.

In addition to the phenotypic variability, *Botrytis* isolates in this study also displayed significant variations in virulence (Figure 4.5 in **Chapter 4**). Virulence diversity of *B. cinerea* is often due to differences in activities of cell wall invasive enzymes, and secretion of virulence factors, such as oxalic acid, toxins, ROS (Nakajima & Akutsu, 2014). We investigated oxalic acid production by *B. cinerea* isolates *in vitro*. Various degrees of medium acidification caused by oxalic acid production of *B. cinerea* isolates were observed (Figure 4.4 in **Chapter 4**), this can partly explain the variability in virulence. Moreover, *Botrytis* isolates with higher virulence on strawberry secreted more oxalic acid, while the non-pathogenic isolate hardly produced any oxalic acid. This coincides with the positive correlation between oxalic acid production and virulence (Kunz et al., 2006; Sun et al., 2019). This variability of virulence in *Botrytis* isolates led to differences in disease severity on strawberry leaves. In other words, *Botrytis* isolates affect leaf resistance via virulence diversity, which is an indirect regulation (Figure 5.1).

Due to the serious economic losses caused by grey mold, it is necessary to detect *Botrytis* infection at an early stage. In this study, we quantified the virulence of different *Botrytis* isolates using a phenotyping platform. The changes in photosynthetic performance of strawberry leaves: chlorophyll fluorescence (F_v/F_m), chlorophyll index and anthocyanin index were recorded upon disease development. F_v/F_m showed a strong negative correlation with disease severity (Figure 4.8 in **Chapter 4**). This is owing to the photosynthesis disruption caused by *B. cinerea* infection, leading to F_v/F_m decrease along with the increasing effect of stress (Makarova et al., 1998). Therefore, F_v/F_m can be an indicator for the early detection of grey mold on strawberry leaves.

In conclusion, red light showed high potential in disease control of grey mold in strawberry leaves, irrespective of various *Botrytis* isolates. Red light combined with short irradiation of white light in the photoperiod is beneficial not only for leaf resistance but also for normal

plant growth. This study also provides more information about the phenotypic response of *Botrytis* isolates to different light conditions. Moreover, F_v/F_m is a useful indicator for early detection of grey mold based on imaging techniques.

On the other hand, based on our findings, recommendations can be given to growers and researchers. Red LED light is recommended for growers as the main lighting source in combination with sunlight to control *B. cinerea* disease in strawberry in greenhouse production. Leaf age and time of inoculation should be taken into account by researchers when doing assessments of plant disease severity. The use of young leaves is recommended owing to their high susceptibility. Studies on *Botrytis* should consider differences in sporulation capacity and virulence among isolates. For the development of grey mold early detection techniques, the F_v/F_m indicator is highly recommended.

5.2 Future perspectives

This study mainly focused on the effects of light quality on leaf resistance against *B. cinerea* in strawberry. *Botrytis* can infect every part of the plant at every stage and disease results mainly from latent infections of flower parts, which develop into rot once fruit begins to ripen. The white flower bud, the open flower, and senescent flower stages are most susceptible to infection, whereas flowers at the green bud stage are relatively resistant. It would be interesting to study the effect of light quality on the resistance of flowers at different stages to *B. cinerea*. Perspectives for applied greenhouse production could focus on the whole production phase beyond the vegetative growth phase. If artificial lighting is applied through the whole strawberry production period, it would also be interesting to investigate the light quality effect on flowering number and time, fruit forming and quality, as well as post-harvest fruit resistance to *Botrytis*. On the other hand, plants are attacked by various intruders even in greenhouse production. It is also necessary to study light quality effects on other diseases in strawberry, such as powdery mildew. Furthermore, several studies also reported the positive role of UV-B light in controlling herbivores. This opens the possibilities to use light quality to control herbivores, which also should be included in future research.

Our research is at the beginning stage of exploration of light quality effects on strawberry resistance to *B. cinerea*. Based on the increasing application of blue and red LED lights in greenhouse production, we have primarily studied effects of monochromatic blue, red, and dichromatic blue+red LED lights on leaf resistance. Furthermore, blue or red light combined with white LED light was investigated and 12 h red light followed by 4 h white light was found outstanding in increasing leaf resistance. If we translate this to greenhouse production, then the fraction of natural light (=white light) is limited. Therefore, it would be reasonable to further study the effect of this combination on the other aspects of strawberry production,

including resistance of flowers, flowering number and time, fruit yield and quality as well as post-harvest fruit resistance. On the other hand, due to the advantages of red light in promoting resistance and of white light in plant growth and development, it is interesting to explore other combination strategies of red and white light for strawberry production with high resistance, high yield and high quality. In addition, as blue or far-red lights promote flowering in strawberry compared to red light and the amount of light supplied is an important factor in flowering, light recipes for a given developmental stage combining light quality (eq. red+white light with blue or far-red light) with light intensity, should be investigated for better performance of both resistance and flowering in strawberry. This is especially of interest with the recent developments in vertical farming and multilayer production of strawberry.

From our results, monochromatic red light increased, while monochromatic blue light decreased leaf resistance to *B. cinerea*, and dichromatic blue+red (1:1) light neutralized the resistance enhancement by red light and the resistance attenuation by blue light in strawberry. It is a very interesting phenotype that deserves a further investigation of the mechanisms involved, such as the phytochrome B-jasmonate pathway (Cerrudo et al., 2012; Moreno et al., 2009), biosynthesis of hydrogen peroxide and secondary metabolites. The underlying mechanisms involved may help us to optimize the lighting strategies for a better outcome in greenhouse production systems.

Early detection of disease could reduce the application of fungicide and yield loss, which is beneficial from both the economic and environmental perspectives. Chlorophyll fluorescence imaging (F_v/F_m) was observed to be highly correlated with *Botrytis* infection. In addition, robots with UV-C radiation have been developed to control powdery mildew in horticulture (Mazar et al., 2018). Hence, the development of robots with imaging sensors for early detection of disease could be interesting for the greenhouse growers.

Overall, LED research is still at the beginning of its potential, especially in disease control. This thesis mainly focused on blue, red lights and their combination, however, other efficient LED lights within the visible light (such as green light) might become available resulting in better regulation of plant growth, development and disease resistance. Our existing knowledge is far from the real world of light in the regulation of plants, hence more research should be conducted to better understand the world of light.

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Publications

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