Effect of Far-red:Blue LEDs on *Botrytis cinerea* Pers. morphogenesis and pathogenicity on strawberry (*Fragaria x ananassa* Duch.)

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DEDICATION

To the vibrant *Botrytis* community, who with their relentless pursuit of knowledge and unwavering commitment, have greatly advanced our understanding of this captivating fungal pathogen. I extend my deepest respect and admiration to the researchers, scientists, and enthusiasts who passionately devote their time and expertise to unraveling the intricacies of *Botrytis cinerea* Pers., further shaping the landscape of our knowledge.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
ANOVA	Analysis of variance
B	Blue
CE	Controlled environment
CEA	Controlled environment agriculture
DPI	Days post-inoculation
EC	Environnement contrôlé
FL	Fluorescent lamp
FR	Far-red
GDL	Gas discharge lamp
HPI	Hours post-infection
HPML	High-pressure mercury lamp
HPSL	High-pressure sodium lamp
IL	Incandescent lamp
IMP	Integrated pest management
J	Jules
JA	Jasmonic acid
LED	Light-emitting diode
MHL	Metal-halide lamp
PA	Protected agriculture
PAs	Proanthocyanidins
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PPFD	Photosynthetic photon flux density
R	Red
SA	Salicylic acid
SBA	Sabouraud agar
TCP	Totale en composés phénoliques
TPC	Total phenolic content
UV	Ultraviolet

VOCs	Volatile organic compounds
W	Watts

ABSTRACT

Controlled environment (CE) plant production systems have revolutionized the cultivation of strawberry fruits (*Fragaria* × *ananassa* Duch.), enabling year-round localized production to meet consumer demand irrespective of external environmental conditions. The use of light-emitting diode (LED) technology within CE strawberry production settings offers growers opportunities to optimize yields, enhance fruit quality, and influence plant development through precise control of lighting intensity and spectral composition. Additionally, manipulation of the spectral composition within a CE holds promise for managing economically important phytopathogens that affect strawberries. This study focuses on examining the potential of LED lighting as a method of cultural control for *Botrytis cinerea* Pers., a ubiquitous, hemi-biotrophic fungal pathogen that can cause significant economic losses on strawberry during both pre- and post-harvest stages. Specifically, this work explores the effects of mixed narrow-bandwidth lighting compositions consisting of Far-red and Blue light on the photomorphogenesis of a wild-type isolate of *B. cinerea* and evaluates their efficacy on immature, infected strawberry plants within a CE setting. In evaluating the photomorphogenic response of *B. cinerea*, the effects of three Far-red:Blue (1:5, 5:1, and 1:1) LED lamp diode ratios were assessed, comparing them to high-pressure sodium (HPS) lighting supplementing ambient light (White) and complete darkness (Dark) as controls. *In vitro* experiments demonstrated that all diode ratios effectively inhibited the hyphal growth, sporulation, and spore germination of the pathogen, indicating an interruption of asexual development and a plausible reduction in the proliferation of infectious propagules within a CE setting. Notably, an even diode ratio of 1:1 exhibited the highest efficacy in inhibiting lesion development on detached strawberry leaves compared to all other LED treatments and White or Dark controls. Furthermore, this study revealed that a Blue-dominant diode ratio of 1:5 significantly increased the total phenolic

content (TPC) in plant leaves sampled at 3 days post-infection, six-hours after the onset of a 16-hour photoperiod, which was absent in other treatment groups. Additionally, infected plants from other treatment groups exhibited substantial variation in TPC post-infection. This research provides additional context on the morphogenic response of *B. cinerea* under mixed Far-red and Blue narrow-bandwidth spectral compositions, offering valuable insights for future investigations. The effectiveness of LED lighting in suppressing the growth and development of *B. cinerea*, as demonstrated in this study, emphasizes the potential application of LED lighting as a tool for managing the strawberry-*Botrytis* pathosystem within a CE.

RESUMÉ

Les systèmes de production végétale en environnement contrôlé (EC) ont révolutionné la culture des fraises (*Fragaria ananassa* Duch.), permettant une production localisée toute l'année pour répondre à la demande des consommateurs indépendamment des conditions environnementales externes. L'utilisation de la technologie des diodes électroluminescentes (DEL) dans les installations de production de fraises en EC offre aux producteurs la possibilité d'optimiser les rendements, d'améliorer la qualité des fruits et d'influencer le développement des plantes grâce à un contrôle précis de l'intensité de l'éclairage et de la composition spectrale. De plus, la manipulation de la composition spectrale au sein d'une EC est prometteuse pour la gestion des agents phytopathogènes d'importance économique qui affectent les fraises. Cette étude se concentre sur l'examen du potentiel de l'éclairage aux DEL comme méthode de lutte culturale contre *Botrytis cinerea* Pers., un agent pathogène fongique omniprésent et hémiobiotrophe qui peut causer des pertes économiques importantes avant et après la récolte des fraises. Plus précisément, ce travail explore l'effet des compositions d'éclairage à bande étroite mixtes constituées de lumière rouge lointain et bleue sur la photomorphogenèse d'un isolat naturel de *B. cinerea* et évalue leur efficacité sur les fraisiers immatures et infectés dans un EC. En évaluant la réponse photomorphogénique de *B. cinerea*, on a évalué les effets de trois ratios de diodes Rouge Lointain:Bleu (1:5, 5:1 et 1:1) en les comparant à l'éclairage à haute pression au sodium (HPS) et à l'obscurité complète comme témoins. Des expériences *in vitro* ont démontré que tous les ratios de diodes inhibaient efficacement la croissance hyphale, la sporulation et la germination de l'agent pathogène, indiquant une interruption du développement asexuel et une réduction plausible de la prolifération des propagules infectieuses dans un EC. En particulier, un ratio de diodes bleues dominantes (1:5) a montré la plus grande efficacité pour inhiber le développement de lésions sur

les feuilles de fraisier détachées par rapport à tous les autres traitements DEL et aux témoins éclairé ou d'obscurité. De plus, cette étude a révélé qu'un ratio de diodes bleues dominantes augmentait de façon significative la teneur totale en composés phénoliques (TCP) dans les feuilles de plantes échantillonnées 3 jours après l'infection, six heures après le début d'une photopériode de 16 heures, ce qui était absent avec les autres traitements. De plus, les plantes infectées provenant des autres groupes de traitement présentaient des variations substantielles au niveau de la TCP après l'infection. Cette recherche fournit un contexte supplémentaire sur la réponse morphogénique de *B. cinerea* sous des compositions spectrales mixtes de Rouge lointain et Bleu, offrant de précieuses perspectives pour des recherches futures. L'efficacité de l'éclairage aux DEL dans la suppression de la croissance et du développement de *B. cinerea*, tel que démontré par cette étude, met l'accent sur l'application potentielle de l'éclairage aux DEL comme outil de gestion du pathosystème fraisier-*Botrytis* au sein d'un EC.

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PREFACE

This work adheres to McGill University's "Guidelines Concerning Thesis Preparation" and adopts a manuscript-style format, which facilitates a comprehensive exploration of the efficacy of Far-red:Blue LED diode ratios as a cultural control method. The primary objective of this study is to advance our scientific understanding of lighting strategies for disease management in controlled environment plant protection settings. Through meticulous investigation, this research offers valuable insights into the effects of Far-red:Blue lighting ratios on the photomorphogenesis of *B. cinerea* and its impact on the strawberry-*Botrytis* pathosystem.

The thesis commences with an introduction (Chapter 1) and comprehensive literature review (Chapter 2) and is further comprised of two distinct research manuscripts presented as individual chapters (Chapters 3 and 4). Each chapter contributes interconnected information that enriches the overall narrative. Emphasis has been placed on establishing a cohesive presentation of the research findings by carefully bridging the works throughout this thesis with connected statements.

The following sections provide a general overview of the thesis and acknowledge the original authors who have made significant contributions to this work.

CONTRIBUTION TO ORIGNAL KNOWLEDGE

The following chapters of the present thesis unveil novel and original findings that contribute to our understanding of the photomorphogenic response of *B. cinerea* in relation to Far-red:Blue LED diode ratios, as well as the effectiveness of these treatments on infected strawberry plants. The contributions made in this research are as follows:

I. Investigation of Far-red:Blue Lighting Ratios: This study provided a thorough examination of the impact of Far-red:Blue lighting ratios (5:1, 1:1, and 1:5) on the photomorphogenesis of *B. cinerea* in a controlled environment. By exploring scaled ratios, it offered valuable insights into the effects of different lighting compositions on the pathogen's growth and development. While previous research has primarily focused on narrow-bandwidth lighting sources, this study stands out as the first report on the effectiveness of mixed narrow-bandwidth lighting consisting of Far-red and Blue wavelengths to disrupt the photomorphogenesis of *B. cinerea*.

II. Disruption of Pathogen Development: The findings revealed that all tested ratios effectively disrupted the asexual reproduction, growth, and development of *B. cinerea in vitro*. This contributes to our understanding of how lighting treatments can be utilized to impede the development of fungal pathogens.

III. *In planta* Efficacy: Building upon the *in vitro* results, this study evaluated the performance of lighting treatments on the pathogenicity of *B. cinerea* in detached leaves and immature strawberry plants. It demonstrated that a balanced ratio of 1:1 or a Blue dominant diode ratio of 1:5 exhibited the greatest efficacies in reducing the development of necrotic lesions on detached leaves, as well as inducing a potential plant defense response. This knowledge is

essential for developing practical strategies to mitigate disease progression in real-world agricultural settings.

CONTRIBUTION OF AUTHORS

Joshua Molligan served as the principal researcher for each manuscript, planning and designing the experimental designs under the supervision and guidance of Dr. Valérie Gravel. J. Molligan and Tessa Pulciano conducted greenhouse and laboratory experiments, as well as data collection. J. Molligan performed data analysis and authored the manuscripts. Dr. V. Gravel played a crucial role by providing valuable feedback on the manuscripts, offering technical assistance, and securing funding for all research endeavors. Their combined efforts and collaboration have greatly contributed to the success of this work.

Chapter 1: General Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a small fruit crop of great economical and commercial importance that has steadily gained popularity among consumers over the last few decades. This growth in consumer demand is largely attributed to its attractive flavor profile and reported health benefits. With a robust nutritional composition comprised of proximates, minerals, and vitamins, strawberries are one of the most well-studied fruits from a dietetic point of view (Afrin et al., 2016; Giampieri et al., 2012). Furthermore, numerous polyphenolic phytochemicals (flavonoids, tannins, and phenolic acids) isolated from strawberry have been shown to have inhibitive properties towards several types of disease, such as inflammation, neurodegeneration, diabetes, and cardiovascular diseases (Battino, 2020; Mazzoni et al., 2016).

An examination of global trends in the production of strawberries shows growth predominantly in temperate regions, to meet both domestic and exportation demands, while northern regions rely heavily on these importations due to seasonal constraints (Arias et al., 2020). In an effort to extend the restricted growing season, thus reducing dependence on importations by maximizing domestic yield, northern regions have steadily implemented various forms of controlled environment (CE) plant production to grow strawberries regardless of the ambient environment (Fernandez-Salvador et al., 2021; Samtani et al., 2019; Van Sterthem et al., 2017).

As CE plant production has become more prominent so has the implementation of various forms of electrical lighting, which are utilized as an indispensable tool for supplementing plant lighting. Over the past decades, new technological advances in light-emitting diode (LED) technology have emerged to replace the use of conventional or traditional lighting sources within CEs. Traditional lighting sources included incandescent lamps (ILs), fluorescent lamps (FLs), high-pressure mercury lamps (HPMLs), high-pressure sodium lamps (HPSLs), and metal-halide lamps (MHLs).

ILs generate light through thermal radiation, whereas the latter gas discharge lamps (GDLs) and FLs emit photons through electron excitation. Like sunlight, ILs, FLs, and GDLs are broad-spectrum lighting sources, meaning they have significant spectral emission in regions of the lighting spectrum that plants do not require nor utilize. The high expense of electrical energy required to power these lamps often makes these unutilized spectral emissions costly and impractical in CE plant production settings. Not only is the spectral quality generated by these lights non-specific, but their luminous efficacy, power requirement, and life span are considered to be largely inferior when compared to later emerging LED technology (Dutta Gupta, 2017).

Beginning with the development of high-brightness Blue LEDs (with a peak emission wavelength of 450 nm) by Shuji Nakamura in 1994, LED technology was realized to achieve specific wavelengths complimentary to the maximum absorption peak of plant photoreceptors (Nakamura and Fasol, 1997). Not only did this invention gain Nakamura the Nobel Prize in Physics later in 2014, but would lead to further advancements in diode fabrication, reducing cost and improving luminous (lm/W) and photon (μmol/J) efficiencies (Von Dollen et al., 2014). Given the ability of LEDs to provide narrow bandwidth emissions and a controllable spectral distribution, they can be used to alter varying aspects of plant photosynthetic reactions, morphological changes, and secondary metabolite synthesis (Bantis et al., 2016; Brown et al., 1995; Landi et al., 2020). Several studies have shown that these changes are often species-specific, suggesting a need to investigate further the interplay of wavelength and a plant's photobiological reaction to define a given species' optimal lighting spectra (Dougher and Bugbee, 2001; Paradiso and Proietti, 2022; Spaninks et al., 2020)

Among the challenges producers face when utilizing CE production systems is the proliferation of disease. Often, the environmental conditions needed for optimal plant growth are also conducive

to the growth and development of fungal pathogens. One of the most economically important fungal pathogens affecting strawberries in CE production is the ubiquitous Ascomycete *B. cinerea*, the causal agent of grey mold. Oftentimes, CE production systems provide the high humidity and moderate temperature requirements necessary for the germination of spores and subsequent hyphal growth of this pathogen, thus being conducive for disease incidence. Unlike other economically significant pathogens that impact strawberry, disease incidence of grey mold caused by *B. cinerea* affects not only the fruit but also the leaf and crown tissues. Additionally, it is worth noting that unripe fruit can harbor the pathogen in a quiescent state, remaining in a biotrophic latent phase until the ripening process begins. Delayed disease onset becomes particularly critical as the fruit ripens and is distributed, as it ultimately results in substantial fruit loss for both distributors and consumers alike.

Similar to plants, fungi have evolved light-dependent biological processes to respond and adapt to their environment. In particular, plant pathogenic fungi have evolved a complex response to lighting spectra that largely influences the infection process with their host. Light quality, including bandwidths within the Far-red and Blue spectra, plays a significant role in these processes. In crop production, growers commonly utilize Far-red and Blue light as these bandwidths are known to promote desirable qualities for plant growth. However, specific ratios of these bandwidths have not been extensively explored in relation to disease occurrence. It is important to investigate the effects of dominant mixed narrow-bandwidth diode ratios on pathogen development and disease incidence to determine their impacts. In the case of *B. cinerea*, although several studies have examined the effects of various narrow-bandwidth qualities, there is still a lack of comprehensive characterization studies focused on mixed narrow-bandwidth lighting sources. Therefore, it is crucial to evaluate the effects of different ratios of Far-red and Blue light

on the development of *B. cinerea* and its pathogenicity on strawberry to understand their potential implications.

1.1. Research objectives

Based on the previous introduction, this study aims to characterize initial key aspects of the strawberry-*Botrytis* pathosystem by incorporating various mixed narrow-bandwidth LED lighting treatments of Far-red and Blue light with respective diode ratios of 5:1, 1:1, and 1:5 within a CE. The objectives of this study include:

1. Assessing the photomorphogenic response of a wild-type isolate of *B. cinerea* by evaluating its hyphal growth, sporulation, and spore germination in an *in vitro* setting.
2. Investigating the efficacy of treatments and the defense response of strawberry during *B. cinerea* infection. This will involve measuring the progression of lesions on detached leaves and comparing changes in total phenolic content (TPC) between infected and non-infected young strawberry plants.

1.2. Research hypotheses

It is hypothesized that within a CE with Far-red:Blue LED diode ratios of 5:1, 1:1, and 1:5 there will be:

1. An inhibition in the morphogenesis of *B. cinerea*, with significant reductions in overall hyphal growth, sporulation, and spore germination exposed to all LED treatments compared to control treatments.
2. Reduction in the development of lesions on detached leaves of strawberry infected by *B. cinerea* exposed to all LED treatments compared to control treatments.

3. An increase in the TPC of strawberry plants under all LED treatments, regardless of infection, compared to control treatments.

Chapter 2: Review of Literature

2.1. State of strawberry production in Canada

The production and trade of strawberries (*Fragaria* × *ananassa* Duch.) in Canada have been steadily increasing for almost two decades, driven by the rising consumer demand. However, the industry faced a significant setback in 2013 due to a viral outbreak, resulting in a 50% loss in short-day crops (Haverstock, 2017). Since then, strawberry production has made a remarkable recovery and shown steady growth to date, with the province of Québec accounting for a significant portion, respectively contributing to 47% of cultivated area and 58% of marketed production (AAFC Pest Management Program, 2021). This recovery has led to a substantial rise in the total value of strawberry exports, increasing from $10.2 million in 2018 to $34.6 million in 2022. As of 2023, the farm gate value of strawberries in Canada stands at $144.5 million, making it the sixth highest among all Canadian fruits, however, it is important to note that despite this growth, imports in 2022 were valued at $667.2 million, being almost five times higher than domestic production (AAFC Horticulture Section, 2023).

Given the economic importance of strawberry production and trade in Canada, there is an increasing demand to enhance production methods through research and the development of new technologies. Approaches such as using high-yielding day-neutral cultivars, establishing collaborative breeding programs, and implementing tunnel and greenhouse production systems are some of the widely discussed strategies to boost Canadian production. However, it is crucial to address the issue of disease incidence, which often becomes a limiting factor of production increases. Therefore, exploring novel methods to combat pathogens that impose severe limitations on strawberry production in Canada is of high importance.

2.2. Progression towards controlled environment production settings

The global indoor farming market, valued at roughly $34 billion USD in 2022, is expected to grow at a compound annual growth rate of 12.9% between 2023 and 2030 (Grand View Research, 2023) Protected Agriculture (PA) refers to indoor farming practices that involve growing crops within protected structures, including open-air, hybrid, and enclosed structures like shade/hoop houses, high tunnels, greenhouses, and warehouses (McCartney and Lefsrud, 2018). Controlled Environment Agriculture (CEA) or Controlled Environments (CEs) are terms used to describe settings where growing conditions can be precisely controlled, making CEA/CE a subset of PA, where environmental factors such as temperature, humidity, and light can be closely regulated to ensure optimal plant growth.

In recent years, there has been a surge of interest in producing strawberries within PA settings, particularly using multi-bay greenhouses or warehouses. Various production methods have been tested, including vertical columns (Linsley-Noakes et al., 2006), suspended grow bags (Neocleous et al., 2010), and more recently, multilayer, horizontally-positioned hydroponic cultivation developed and employed by companies such as Growtec Solutions (ON, CA). These structures, conducive to large-scale commercial production, are leading the trend towards CE strawberry production.

Currently, several greenhouse industries in Canada have started producing strawberries. Anecdotal evidence suggests that approximately 40 acres of glass greenhouses in Canada use horizontally raised troughs with coconut-coir-based substrates for production (Samtani et al., 2019). In regions with more optimal climates, such as California, growers have introduced a similar substrate-based production system called tabletop production systems (Van Delm et al., 2016). These production systems can be implemented in simpler structures like high tunnels.

Structures such as multi-bay greenhouses and warehouses require finely adjusted CEs to meet production goals effectively. This often involves the use of supplemental plant lighting, with Light Emitting Diodes (LEDs) becoming increasingly prevalent. LEDs provide a narrow-bandwidth spectral output, allowing for precise control of the spectral distribution and offering energy-saving advantages compared to traditional gas-discharge lamps (Nakamura and Fasol, 1997). Adjusting the spectral composition within a CE using LED lamps enables producers to improve various aspects of production. These lamps primarily consist of Blue, Red, and Far-red diodes, which align with the peaks of the quantum yield curve for photosynthetically active radiation (McCree, 1971). Utilizing targeted bandwidth emissions has been shown to increase crop productivity, modify plant photosynthetic reactions, induce desired morphological changes, and enhance the synthesis of secondary metabolites (Bantis et al., 2016; Landi et al., 2020; Paradiso and Proietti, 2022). Additionally, the influence of different light qualities within various spectra directly affects plant defense responses (Ballaré, 2014).

As the global indoor farming market continues to expand, the shift towards CE strawberry systems signifies a promising future for this sector. By harnessing the advantages of precise environmental control and tailored lighting strategies, the potential for improved strawberry production, quality, and disease management becomes increasingly feasible.

2.3. Strawberry physiology: plant structure and growth habit

Strawberry plants can be classified as herbaceous perennials and consist of various components including adventitious roots, branched crowns, leaves, runners (stolons), flower clusters, and fruits (**Figure 1**) (Pritts et al., 1998; Sharma et al., 2019). The leaves of strawberry plants are trifoliate, composed of three leaflets, with stomata primarily located on the underside of the leaves within the lower epidermis (Pritts et al., 1998). Characteristically, plants tend to propagate asexually

through horizontally growing stolons, which develop adventitious roots. These adventitious roots can emerge along the nodes of stolons and aid in the formation of a new plantlet.

Auxiliary buds, present at the base of each petiole, are found on the primary crown or along the runner planes. This axillary meristematic tissue has the potential to differentiate into either a runner or a branched crown, but can also remain dormant depending on factors such as the developmental stage, environmental conditions, and genotype (Hytönen and Kurokura, 2020). Flower induction in strawberry plants is influenced not only by photoperiod and temperature but also by the concept of juvenility. Like other herbaceous perennials, young plants typically prioritize vegetative growth early in the season, with resources gradually allocated to reproductive structures as they mature.

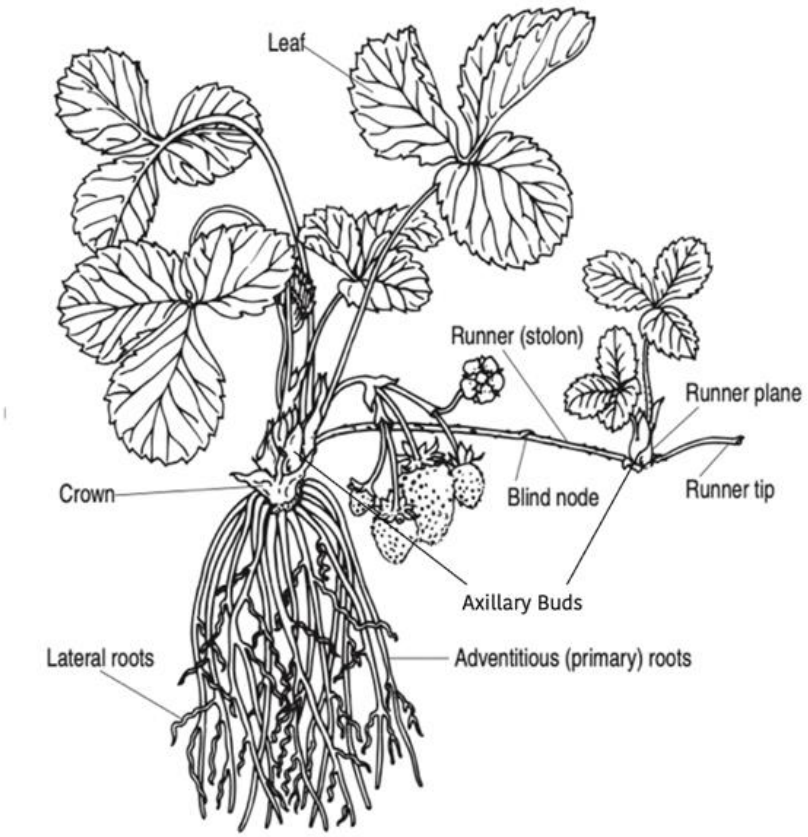

Figure 1. Anatomy of a strawberry plant. Adapted from Pritts et al. (1998).

Runner plants (plantlets) rely on the mother plant until the formation of lateral roots, at which point they can survive independently (Pritts et al., 1998). The timing, length, and concentration of root development depend on various factors including location, soil conditions, and soil type (Sharma et al., 2019). In commercial strawberry production, bare-root transplants or containerized plugs derived from asexually developed plantlets of runners are primarily used for planting. To produce containerized transplants, it is crucial to meet the cultural requirements necessary to induce root development from stolons, such as providing longer day length, adequate soil moisture, and high humidity. It is noteworthy that the entire commercial production of strawberries relies on asexually produced plant material.

2.4. Economically important diseases

Strawberry plants are susceptible to a wide range of disease-causing pathogens, including fungi, bacteria, and viruses. Each disease presents unique challenges in terms of diagnosis and control, as these pathogens can spread and affect plants in different ways. According to the AAFC Pest Management Program (2021) there are approximately 20 economically important diseases which affect strawberry in Canada. Among the fungal diseases studied, the most significant ones affecting strawberry crops are gray mold (*Botrytis cinerea* Pers.), anthracnose (*Colletotrichum* spp. Corda), powdery mildew (*Sphaerotheca macularis* (Wallr.) U. Braun & S. Takam.), and black root rot (*Pythium* spp. Pringsheim and *Rhizoctonia* spp. DC.). The incidence of these diseases varies greatly at the provincial level, primarily influenced by different environmental conditions. In the top three strawberry-producing provinces of Québec, Ontario, and Nova Scotia, gray mold is considered to be the only disease with a universally high rate of occurrence.

Among the various diseases affecting strawberry, gray mold caused by *B. cinerea* is universally recognized as the most economically destructive (Dean et al., 2012; Williamson et al., 2007). This

pathogen has a wide host range and causes estimated annual crop losses ranging from $10 to $100 billion globally (Hua et al., 2018). Strawberry fields are highly susceptible to severe epidemics, which can have devastating consequences for producers. A notable example occurred in Florida, USA, the largest strawberry production region in North America, where multiple multi-fungicidal-resistant isolates of *B. cinerea* led to significant crop loss and severe limitations in production capacities in 2012 (Amiri et al., 2013). Disease incidence is not limited to the production stages alone but also holds significant implications during post-harvest and processing stages. Therefore, further research is needed to develop novel strategies to mitigate disease incidence throughout various aspects of strawberry production.

2.5. The pathogen: *Botrytis* spp. & *Botrytis cinerea*

The Ascomycete fungal genus *Botrytis* belongs to the family of *Sclerotiniaceae* and currently comprises roughly 35 identified species, a hybrid, and a species complex (Walker, 2016). To date, it could be precluded that there may be numerous unidentified species given that most identified species come solely from industrialized and developed regions. In the last five years alone, five novel host-specific species have been identified, aiding an exponential trend of novel-species identification (Ferrada et al., 2016; Garfinkel et al., 2019; Saito et al., 2016; Zhong et al., 2019).

Species within the *Botrytis* genus are commonly characterized as generalist necrotrophic pathogens, as they induce host cell death through the secretion of enzymes that catalyze cell lysis. This process allows the pathogens to absorb intracellular nutrients (Bi et al., 2022). However, it has also been reported that the pathogen can exist in a quiescent state within host tissues, exhibiting characteristics of a hemi-biotrophic lifestyle. In some cases, prolonged endophytic infections have been documented (Barnes and Shaw, 2003; Sowley et al., 2010).

Botrytis species are known to infect a wide variety of plant species across more than 170 families in the plant kingdom. Some species, like *B. fabae*, have a narrow host range, infecting specific plants such as broad beans. On the other hand, species such as *B. cinerea* have a broader host range, capable of affecting approximately 200 plant species, including both monocotyledonous and dicotyledonous plants (De Miccolis Angelini et al., 2016). The devastating effects of *B. cinerea* are particularly concerning for small fruit crops and leafy greens such as strawberries, grapes, and lettuce. This is partly due to the pathogen's ability to establish endophytic infections within the host, leading to a quiescent infection stage that causes significant losses post-harvest. In addition to the crop losses experienced during production, post-harvest losses are common in these crops as well.

Botrytis spp., as with other Ascomycetes, reproduces both sexually and asexually, although sexual reproduction has yet to be sighted in nature. This occurs via the fertilization of sclerotia by microconidia, resulting in the production of an apothecium that bears ascospores. The sexually produced propagules (ascospores) of various *Botrytis spp.* have been reported to induce primary infections, although these findings are rare and dated (Godfrey, 1923; Kublitskaya and Ryabtseva, 1972). This is not indicative that sexual reproduction is entirely uncommon, but it is still generally accepted that the predominant form of inoculum dispersal is through asexually produced propagules, or conidiophores. The survival of conidia is heavily influenced by temperature extremes, moisture, and sunlight exposure (Williamson et al., 2007). The dispersal of inoculum is thought to be primarily ariel, driven by air movement, or through water droplets that move within the crop canopy. As such, the pathogen's population density is generally determined through the quantification of airborne conidia or assessing disease incidence within a production setting (Carisse, 2016). Dormant fungal structures can persist as overwintering mycelium on plant debris

or as sclerotia (highly melanized and compact mycelium). Either of these dormancy structures is suitable to produce conidiophores post-dormancy period and they play an integral role in the disease cycle. This subsequent production of conidia post-dormancy will provide the inoculum necessary for initial colonization.

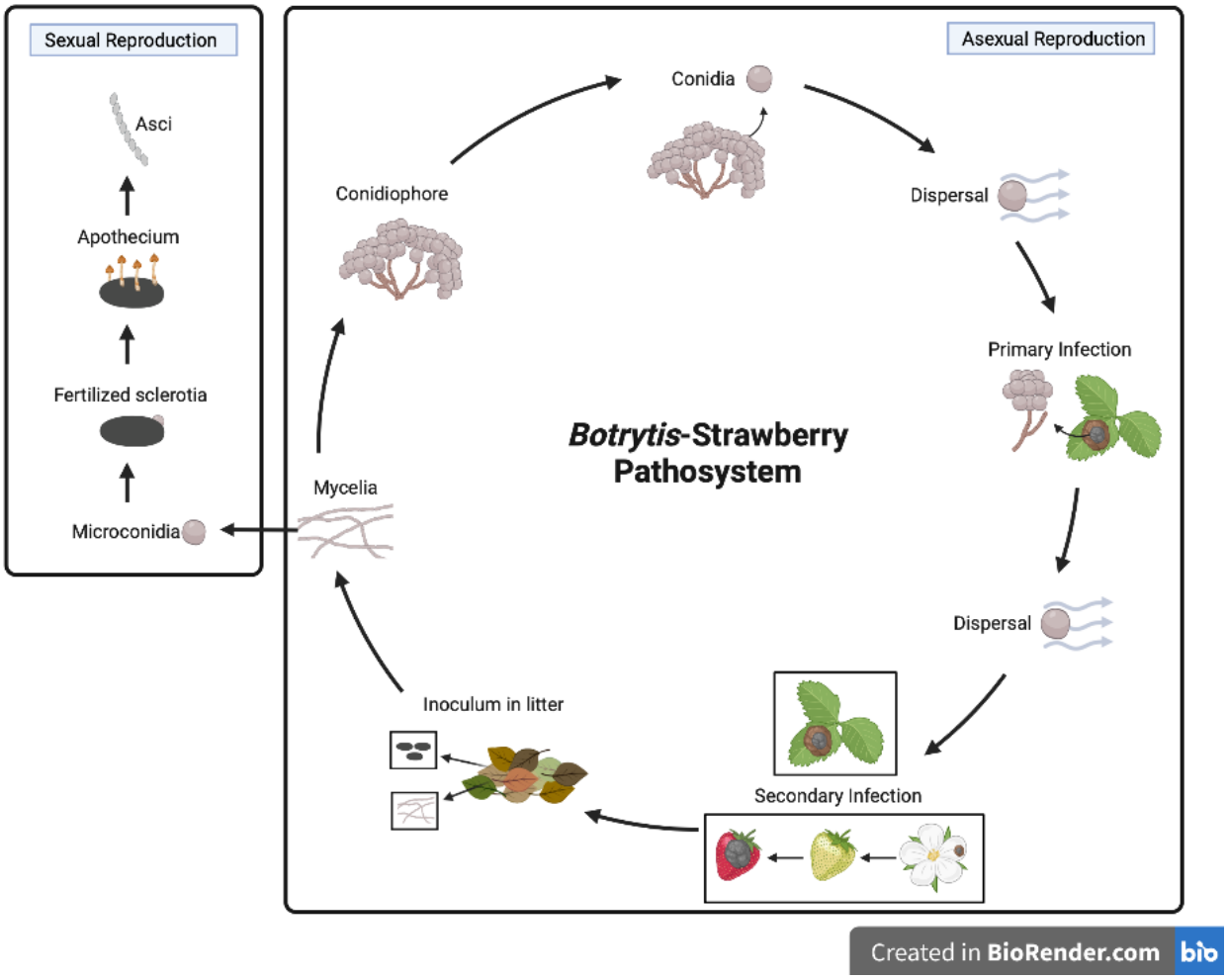

Figure 2. Strawberry-*Botrytis* pathosystem. Image created with BioRender (https://www.biorender.com).

Upon contact with the host cuticle, conidia become attached to the substratum due to an adhesive mucilaginous sheath which also envelopes the germination tube (Jarvis, 1977). As the germination tube elongates, the formation of an appressorium (or penetration peg) occurs from the distal end. Enzymatic secretion-assisted penetration was first suggested when McKeen (1974) observed the dissolution of the host cuticle resulting in a sharp clean pore, absent of indentation of the cuticle or epidermal wall. During host colonization, the fungus is not capable of hyphal branching within the host alone and will secrete toxins and enzymes to assist hyphal penetration by further degrading plant tissue (Cantoral and Collado, 2011). This initial spread of the pathogen can be limited in damage, although the subsequent and rapid production of conidia enables a heightened rate of spreading.

The production of conidia and exponential dissemination of the pathogen can result in the exponential formation of new colonies. This asexual method of replication in *B. cinerea* is often attributed as the basis of genetic diversity within populations. During the formation of conidiophores, each spore undergoes genomic replication, in which spontaneous mutations can occur, leading to changes in the genetic makeup of the pathogen. These genetic variations further result in phenotypic changes, such as altered virulence or fungicidal resistance, among others, including the disposition of *blind* isolates. These are isolates that exhibit the same phenotype regardless of lighting conditions (Canessa et al., 2013; Schumacher, 2017). Genetic diversification of the fungus resulting from asexual reproduction allows for adaptations to changing environmental conditions, host defenses, and fungicidal treatments. From a control perspective, the pathogens' affluence for adaptability can make it challenging to manage disease occurrence, as

the effectiveness of fungicides or environmental controls may vary depending on the genetic makeup of the pathogens' population.

Apart from fungicidal applications, various cultural methods have been used for mitigating disease incidence. Disease mitigation of *B. cinerea* is usually achieved through an integrated pest management (IPM) approach with some combination of the following: (1) reducing planting density, (2) managing the crop canopy to allow for aeration, (3) fertigation with increased levels of potassium and calcium and reduced amounts of nitrogen, (4) the use of soil mulch and passive solar heating of unheated greenhouses, (5) avoiding harvesting on rainy days, and (6) appropriate timing of fungicidal applications (Elad et al., 2016). Primarily, ventilation systems and plant density adjustments are used as cultural controls for grey mold within CEs as both factors can contribute to air movement and inoculum dispersal. It is commonly accepted that an integrated approach utilizing multiple methods is the most effective for disease suppression rather than individual treatments. With the development and integration of LED lighting systems in CEs, the ability to adjust the lighting spectral composition has become of increasing interest as a plausible IPM strategy.

2.6. Strawberry-*Botrytis* pathosystem

The incidence of grey mold in strawberries begins with the completion of several processes (conidiation, dispersal, and infection) which are influenced by various host-phenological stages, as well as biological and environmental factors (Carisse, 2016; Jarvis, 1994). A key aspect of the pathosystem, prior to the differentiation between host susceptible tissues, is the consideration of the inoculum (source, amount, and type) being primary or secondary. Primary inocula are propagules that have carried over from previous cropping cycles to begin new epidemics whereas secondary inocula are produced after the primary infection-conidiation cycle (**Error! Reference s**

ource not found.). Primary inocula can result in the infection of young strawberry leaves early in the cropping season, which will then senesce and die, providing the opportunity for tissue colonization and conidiation, producing secondary inoculum. Furthermore, after subsequent secondary inoculum dispersal, Petrasch et al. (2019) suggests that when dispersed conidia come into contact with host inflorescence there are two infection periods, primary (inflorescent component infection) and secondary (fruit receptacle tissue infection). In this context, it is important to distinguish that during secondary infection, the pathogen is in a latency period, and can exist in a prolonged quiescent stage which holds important implications in the timing of prophylaxis (Jarvis, 1994). This demonstration of quiescence is not unique to *Botrytis spp.*, yet the mechanisms that govern quiescence have yet to be entirely elucidated.

Quiescent infection of strawberry fruit refers to an asymptomatic state with the arrestment of hyphal growth, typically breaking with fruit maturation, wounding, or senescence, and is followed by a transition into aggressive hyphal growth that can lead to conidiation within 24 – 48 hours (Jarvis, 1977; Prusky and Lichter, 2007). Jersch et al. (1989) suggested that, although ripe and unripe fruits have similar proanthocyanidins (PAs) contents, the polymerization of PAs (leading to the subsequent loss of biological activity) is the basis for the prolongation of quiescence in immature fruit. This was then attributed to the heightened resistance in *Fragaria* × *ananassa* Duch. cv. 'Elvira', which is a variety that has white, compacted receptacle tissues post-maturation with an observed heightening in resistance (Jersch et al., 1989). These findings support suggestions made by Prusky (1996) that the three modes for inducing quiescent inductions are: (1) a deficiency in pathogen-required host nutritional resources, (2) the presence of antifungal compounds in unripe fruits, and (3) the inhibition of fungal pathogenicity factors due to an unsuitable environment. The dynamic of these factors influences the pathogen's behavior within the fruit. Furthering our

understanding of the breaking of quiescence is critical for developing targeted approaches to safeguard crops and minimize losses that result from disease.

When considering inflorescent tissue susceptibility, work by Jarvis and Borecka (1968) demonstrates that white flower buds or fully opened flowers are the most susceptible, followed by calyx and corolla structures, then finally receptacle tissue. Further examination of strawberry floral structures by Bristow et al. (1986) described that germinated conidia on stigmas took 4-6 weeks to reach the base of styles, and suggested that stamens are significantly more susceptible to hyphal intrusion than styles. In addition to this work, Jung (1956) found that in an abundance of samples, there were no cases of the fungus penetrating the style as far as the ovary. Although, in some species, such as pear (*Pyrus communis* L.), it has been demonstrated that conidia can germinate on the stigma with hyphal growth extending down the style into the ovule (Zeller, 1926). Yet, in the case of strawberry inflorescence, it seems that infection via the receptacle is primarily through perigonium tissues. For instance, petal retention until fruit set was found by Powelson (1960) and Bristow et al. (1986) to increase the chance of fruit infection considerably compared to fruits where petals were removed or had naturally dropped.

2.6.1 Relevance of the fruit ripening process coinciding with infection

The fruit ripening process of strawberries significantly influences the disease progression of *B. cinerea*. Ripening fruits undergo four processes that can be correlated to disease progression, which plays a key role in fruit transition from resistant to susceptible (**Figure 3**). These include (1) cell wall modifications, (2) cuticle changes, (3) sugar accumulation, and (4) hormone biosynthesis and signaling (Petrasch et al., 2019). It is important to note that not all four processes must coincide, but rather aspects of one process are sufficient in the breaking of quiescence.

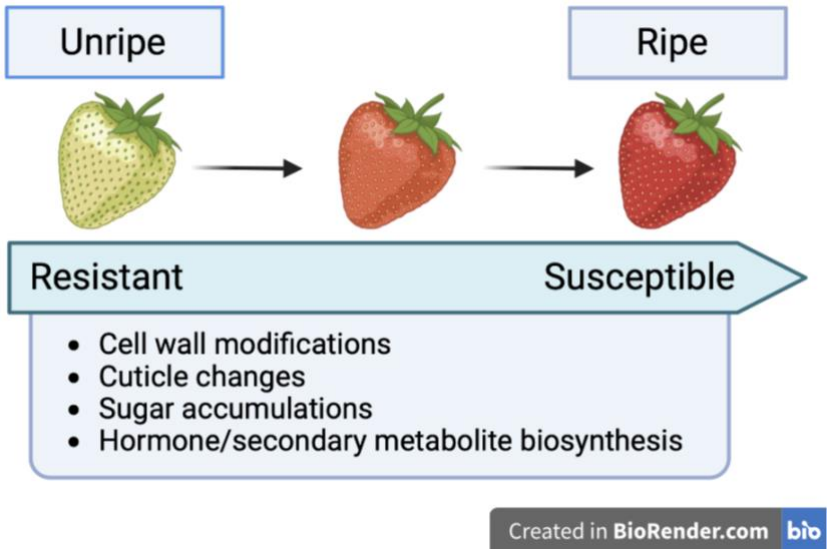

Figure 3. Ripening process of strawberry fruit and the four processes that promote susceptibility of infection to *Botrytis cinerea.* Adapted from Pritts et al. (1998). Image created with BioRender (https://www.biorender.com).

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Cell wall modifications, specifically the reduction of pectin content and depolymerization of PAs during ripening, influence fruit susceptibility to infection (Jersch et al., 1989; Silva et al., 2023). This change results in a softer and more permeable fruit, making it easier for the fungus to penetrate and initiate infection. It has also been demonstrated that in grapes (*Vitis vinifera* L.) changes in cuticle density and permeability are important factors regarding penetration via the appressorium (Commenil et al., 1997; Herzog et al., 2015). Interestingly, Jarvis (1962) notes that when scouting for infected strawberries, only 1% of infections surveyed could be attributed to cuticle penetration, as the remainder resulted from the proximal end, customary to a saprophytically based mycelial infection.

Sugar accumulation is closely related to the early stages of disease development when symptoms are not visible (Hu et al., 2019). This increase in sugar content may serve as a signal to the pathogen that ripening has begun. It has been found that sucrose regulates abscisic acid (ABA) levels in strawberries (Jia et al., 2013). Therefore, sugar accumulation during initial infection may serve as a primary inhibitor for the growth of *B. cinerea*, however as fruit succumbs to aggressive infection these sugars may eventually serve the pathogen as a substantial source of carbon. Hormone biosynthesis (4) and signaling also plays a critical role in the ripening and infection process of strawberry fruit. Ethylene is a plant hormone that is essential for fruit ripening and senescence, and its production increases during the ripening process (Durán-Soria et al., 2020). The production of ethylene is often attributed to one of the earliest detectable events in plant-pathogen interactions. As strawberry is a non-climacteric fruit, basal attachment is a necessary catalyst for fruit ripening, thus the production of ethylene is unavoidable. Ethylene is also responsible for the expression of disease-related plant defense genes (Ecker and Davis, 1987). However, in strawberries, ABA modulates growth and softening as well as the accumulation of volatile organic compounds

(VOCs) and anthocyanins, whereas the role of ethylene is limited (2016; Jia et al., 2011). This suggests that ABA may play a larger role in combating *B. cinerea* infection than ethylene.

The ripening process is primarily driven by the light-induced transcription factor FaMYB10 (Lin-Wang et al., 2010). Initially, the expression of FaMYB10 is low during early developmental stages, but as the fruit ripens, expression levels gradually increase coinciding with color development and the production of VOCs. Transcriptome analysis of *Fragaria vesca* L. under constant dark and light conditions further confirmed that the R2R3 MYB transcription factor plays an important role in further light signaling pathways, and revealed that the induction of FvMYB10 is essential for anthocyanin and sugar accumulation (Xu et al., 2018). Although it is proposed that light serves as the initial signal for fruit maturation, and therefore a key determinant in susceptibility, various lighting qualities have been shown to induce different effects in hormone and secondary metabolite synthesis, sugar accumulation, and the morphology of ripening fruits (**Table 1**) (Choi et al., 2015; Díaz-Galián et al., 2021; Hidaka et al., 2013; Kadomura-Ishikawa et al., 2013; Miao et al., 2016; Naznin, 2016; Xu, Charles, et al., 2017). Notably, work by Zhang et al. (2018) has demonstrated that this variance can occur even at the varietal level by finding a significant difference in the biosynthesis of anthocyanins and proanthocyanins in the ripening fruit of two distinct *Fragaria x ananassa* spp. genotypes. The effect of lighting spectra on the four previously mentioned fruit ripening processes should be further studied to gain insight into how lighting spectra might be manipulated to mitigate the breaking of quiescent infection.

Table 1. Overview of strawberry plant defense responses (including phytochemical production, and light regulated transcription/translation) under various lighting spectra in controlled environment plant production settings.

Light Spectrum	Wavelength (nm)	Species & Variety	Plant Response[z]	Reference
BLUE (280 – 500 nm)	448	*Fragaria* x *ananassa* Duch. var. 'Daewang'	↑ anthocyanin (fruit) ↓ sucrose (fruit)	Choi et al. (2015)
	450	*Fragaria* x *ananassa* Duch. var. 'Toyonaka'	↑ expression of *LAR* and *ANR* ↑ anthocyanin/proanthocyanidin (fruit)	Zhang et al. (2018)
	450	*Fragaria* x *ananassa* Duch. var. 'Tokun'	🕒 induction of *MYB10* 🕒 anthocyanin biosynthesis upregulation of *MYB1* at late stages of fruit maturation, slowing anthocyanin accumulation	Zhang et al. (2018)
	465	*Fragaria* x *ananassa* Duch. var. 'Sachinoka'	Induction of *FaPHOT* Induced pigmentation ↑ anthocyanin	Kadomura-Ishikawa et al. (2013)
	470	*Fragaria* x *ananassa* Duch. var. 'Pechka'	↑ gibberellic acid ↑ cytokinin	Magar et al. (2018)
RED (630 – 700 nm)	661	*Fragaria* x *ananassa* Duch. var. 'Daewang'	↑ chlorophyll (leaves) ↑ oxalic, citric, malic acid (fruit) ↑ TPC (fruit) ↓ glucose (fruit) delayed maturation (fruit)	Choi et al. (2015)
	640	*Fragaria* x *ananassa* Duch. var. 'Pechka'	↓ inflorescence	Magar (2018)
FAR-RED (730 – 750 nm)	730	*Fragaria* x *ananassa* Duch. var. 'Toyonaka'	↑ expression of *LAR* and *ANR* ↑ anthocyanin/proanthocyanidin (fruit)	Zhang et al. (2018)
	730	*Fragaria* x *ananassa* Duch. var. 'Tokun'	🕒 induction of *MYB10* 🕒 anthocyanin biosynthesis upregulation of *MYB1* at late stages of fruit maturation, slowing anthocyanin accumulation	Zhang et al. (2018)
	450 + 730 (Ratio 1:1)	*Fragaria* x *ananassa* Duch. var. 'Toyonaka'	↑ proanthocyanidin (fruit)	Zhang et al. (2018)

PURPLE (Red + Blue)	450 + 730 (Ratio 1:1)	*Fragaria* x *ananassa* Duch. var. 'Tokun'	↑ expression of *LAR* and *ANR* ↑ anthocyanin/proanthocyanidin (fruit) ↑ expression of *LAR* and *ANR* 🕒 induction of *MYB10* 🕒 anthocyanin biosynthesis upregulation of *MYB1* at late stages of fruit maturation, slowing anthocyanin accumulation	Zhang et al. (2018)
	448 + 661 (Ratio 3:7)	*Fragaria* x *ananassa* Duch. var. 'Daewang'	↑ fruit production ↑ anthocyanin production	Choi et al. (2015)
	449 + 661 (Ratio 1:19)	*Fragaria* x *ananassa* Duch. var. 'Albion'	↑ inflorescence ↑ crown development	Naznin (2016)
FULL SPECTRUM	450/470 + 620/660/730 (Ratio 3:7, 1:7)	*Fragaria* x *ananassa* Duch. var. 'Fortuna'	↓ second category fruit ↑ fruit weight	Díaz-Galián et al. (2021)
	White LED	*Fragaria* x *ananassa* Duch. var. 'Fukuoka S6'	↑ fruit weight ↑ fruit set ↑ marketable yield ↑ dry matter	Hidaka et al. (2013)
	Continuous Light	*Fragaria vesca* L. var. 'Ruegen'	↑ anthocyanin and sugar accumulation regulates *FvMYB10* transcription and post-translation	Xu et al. (2018)

[z] ↑ increase, ↓ decrease, 🕒 early/premature.

2.7. Manipulating light for survival: unraveling photomorphogenesis, tropism, and protection strategies in plant-*Botrytis* interactions

In the battle against plant pathogens such as *Botrytis cinerea*, the manipulation of light emerges as a powerful tool for plant survival. The interplay between photomorphogenesis, tropism, and protection strategies becomes paramount in understanding how plants navigate and respond to the challenges posed by this notorious fungal pathogen, yet they also provide insight into how the pathogen utilizes light for survival and proliferation. It is important to acknowledge that light-induced effects can vary among different plant species. Therefore, the subsequent subsections will provide insights into photomorphogenesis, tropism, and protection strategies in a general plant context while referring to the studied crop of interest, strawberry. **Table 1** offers more specific insights into strawberry's responses under various LED lamp spectra, while **Table 3** examines the effects of similar spectra on *B. cinerea,* providing pertinent details within the context of this review and contributing to a deeper understanding of the study's objectives. Additionally, the following sections will predominantly focus on the qualities of Blue (B), Red (R), and Far-red (FR) light. These particular spectral qualities are representative of the study's objectives and integral to the development and response mechanisms of both plants and *B. cinerea*.

2.7.1 Photomorphogenesis

Both plants and *B. cinerea* undergo photomorphogenesis, a process in which light influences the transition between key stages of their physiological development. Light, or its absence, plays a crucial role in regulating various aspects of their life cycles, including germination (*via* seed or spore), vegetative growth, the production of survival structures (such as seeds or sclerotia), and the induction of asexual or sexual reproductive structures (**Table 2**). The spectral composition of

light, particularly B, R, and FR light, in terms of quality and quantity, plays a pivotal role in regulating these processes.

Table 2. The influence of the lighting spectra on the photomorphogenic responses of both plants and *Botrytis cinerea.* Spectra are identified by bandwidth ranges given in nanometers (nm).

Spectra	Wavelength (nm)	Plant Photomorphogenic Responses	*Botrytis cinerea* Photomorphogenic Responses
UV	200 - 400 nm	De-etiolation	Promotes conidiation
BLUE	400 - 425 nm	Inflorescence	Represses conidiation and sclerotial development
GREEN	425 - 490 nm	Leaf flattening and stomatal opening, tropism	
GREEN	490 - 550 nm	De-etiolation, leaf expansion, inflorescence	Represses mycelial growth and conidial germination. Deformation of hyphae intracellular ultrastructure
RED	620 - 700 nm	Germination, de-etiolation, shade avoidance, leaf flattening + expansion, inflorescence, branching	Represses conidiation
FAR-RED	700 - 740 nm		Promotes conidiation

In the case of strawberries, the ratio of B to R light has significant implications for the formation of asexual and sexual structures, such as inflorescence, fruit formation, or runner initiation (Choi et al., 2015; Naznin and Lefsrud, 2017; Warner et al., 2021; Wu et al., 2011). Interestingly, it has been observed that R light suppresses conidiation of *B. cinerea*, while it can induce runner formation in strawberries, indicating opposing responses for the induction of asexual reproductive structures (Li et al., 2021; Tan and Epton, 1973).

Understanding the intricate role of light in the photomorphogenesis of plants and fungi, particularly in relation to key developmental stages, is crucial for optimizing (or inhibiting) their growth and reproductive processes. As research in this field progresses, a deeper understanding of photomorphogenesis will contribute to the development of targeted lighting strategies and cultivation practices that can enhance the productivity and resiliency of plants with possible implications for controlling certain fungal species, such as *B. cinerea*, within various agricultural and horticultural contexts.

2.7.2 Tropism

Plants and the pathogen *B. cinerea* exhibit phototropic responses, with some interesting differences in their behaviors. Positive phototropism, in which the organism grows toward the light, is observed in both cases. However, *B. cinerea* also displays negative phototropic growth during infection. This is evident during the initial stages of infection when conidial germ tubes emerge on the unilluminated sides of conidia and grow away from daylight (Gettkandt, 1963; Jaffe and Etzold, 1962). In contrast, the formation of conidiophores and apothecia in *B. cinerea* is light-dependent and exhibits positive phototropic responses, likely due to evolutionary adaptations for effective inoculum dispersal and survival. Schumacher (2017) suggests that positive phototropic growth is induced at an early stage during structure differentiation to establish a direction for

polarized growth. Where B light primarily regulates this positive phototropic behavior (Corrochano, 2019; Schumacher, 2017).

On the other hand, near-UV and FR light have been found to reduce germ tube length and induce a negative phototropic response in *B. cinerea*, while R light promotes elongation and positive phototropic responses (Islam et al., 1998). This complements the work of Jaffe and Etzold (1962) and Gettkandt (1963), who demonstrated increased germ tube penetration under near-UV and B light on plant epidermal tissues, while R light suppressed penetration and promoted germ tube growth perpendicular to the surface of plant tissue.

In plants, positive phototropic growth is similarly employed to enhance survivability, particularly through shade-avoidance responses. These responses are regulated by phytochrome photoreceptors, which sense the ratio of R and FR light in the ambient environment (Folta and Childers, 2008). Interestingly, shade-avoidance responses in plants have been shown to interact with defense mechanisms by inhibiting the synthesis of jasmonic acid (JA), a phytohormone involved in defense-related metabolite accumulation but also a potent growth inhibitor (Ballaré and Austin, 2019; Pierik and Ballaré, 2021). This link between etiolation or shade-avoidance and the inhibition of JA synthesis can be further attributed to fluctuations in carbon availability, establishing the basis for growth vs. defense trade-offs (Guo et al., 2018; Havko et al., 2016).

Regarding infection by conidial germ tubes, R light induces positive phototropic growth and decreases the likelihood of penetration, while FR light induces a negative phototropic response. This may be a strategic adaptation of *B. cinerea* to infect the host during shade-avoidance, taking advantage of the limitations in the host's synthesis of defense compounds. As previously mentioned, positive phototropic growth is observed in both organisms, promoting survival, and influencing important developmental processes. However, the specific behaviors and interactions

differ between organisms, highlighting the complexity of light-mediated responses in plant-pathogen interactions. Further research in this area will deepen our understanding of how tropism shapes the growth and defense strategies of plants and the infection mechanisms of pathogens, ultimately leading to improved strategies for managing and controlling plant diseases.

2.7.3 Protection Strategies

Light is a crucial environmental factor that plants utilize to regulate their defense against biotic stressors, including infection by necrotrophic pathogens. Research has demonstrated that light plays a significant role in modifying the strategies employed by plants to protect themselves. It can greatly influence the production of important phytohormones, such as salicylic acid (SA) and JA, which are pivotal in defense mechanisms. SA pathways are typically activated in response to biotrophic pathogens, leading to the expression of defense genes and the production of pathogenesis-related proteins. In contrast, JA pathways are predominantly activated in response to necrotrophic pathogens and contribute to the synthesis of defense-related compounds like lignin, phenolics, and terpenes (Pieterse et al., 2012).

In the face of biotic attack, plants allocate resources to the production of JA in a light-dependent manner, often at the expense of downregulating genes related to photosynthesis or reducing their photosynthetic capacity (Bilgin et al., 2010). Exploring the role of spectral composition in strawberries' production of JA in response to *B. cinerea* infection may offer valuable insights for developing lighting regimes that can induce effective protection mechanisms. Furthermore, organic acids and phenolic compounds are crucial components of strawberries' defense against *B. cinerea*. Choi et al. (2015) discovered that both R light and a high R:B light ratio significantly increased the levels of oxalic, citric, and malic acid, as well as total phenolics (**Table 1**). Notably, R light was also found to delay fruit maturation, potentially serving as a protective mechanism to

restrict quiescent infections in the host's receptacle (Choi et al., 2015). Continued research in this field will enhance our understanding of the interplay between light, phytohormones, and metabolites in plant protection mechanisms, paving the way for improved strategies to combat pathogens and enhance crop resilience. Assessment of light-based management practices of *Botrytis cinerea* in CE production settings

Studies investigating the influence of light on the growth and development of *B. cinerea* can be traced back to over a century ago, providing valuable fundamental research on how light affects this organism (Bewley, 1923; Brierley, 1921; Godfrey, 1923; Klein, 1885; Peltier, 1912; Robinson, 1914). However, these reports exhibit significant variations, likely due to the genetic diversity and phenotypic variation among isolates. These early accounts were the first to propose that light, or its absence, acts as a fundamental factor triggering distinct morphological responses in *B. cinerea*. Paul (1929) initially suggested the existence of three distinct morphological types (*sclerotial, spore,* and *mycelia* forming) based on observations of natural isolates that predominantly formed sclerotia, spores, or mycelia. Canessa et al. (2013) later proposed further classification based on the differentiation of *light-responsive* strains, which undergo photomorphogenesis, and *blind* strains, which exhibit a standard phenotype regardless of various lighting spectra (or constant darkness). The blind strains can be further categorized as *always* sclerotial, spore, or mycelia forming, while light-responsive strains demonstrate differentiation between structures.

Further studies dating back to the 1970s explored the effects of narrow-bandwidth spectra on *B. cinerea*, providing initial insights into the genetic basis of light responsiveness (referred to as *mycochromes* by Honda and Yunoki (1978)) (Tan and Epton, 1973, 1974a). These studies were particularly significant as they demonstrated that modified spectral compositions could induce differentiation and de-differentiation of morphological structures, with specific wavelengths

attributed to various inductions. For instance, it was observed that exposure to near-UV (ultraviolet) light could counteract the inhibitory effects of B light (300-420nm) on sporulation (Tan, 1974a, 1974b). However, this reversal was subsequently nullified when the same samples were exposed to B light again. Tan (1974a) further demonstrated that spore formation under B light not only ceased but also resulted in the dedifferentiation of conidiophores into sterile hyphae.

As the influence of spectral composition on morphological development (and its reversal) became evident, subsequent studies focused on investigating the effects of spectral composition on the *B. cinerea* pathosystem and its interactions with various hosts within CEs (**Table 3**). Particularly noteworthy preliminary findings were reported by Elad (1997), who demonstrated that screening visible spectra ranging from 540 – 800 nm using polyethylene sheeting led to a significant reduction of 35% – 75% in conidial load and grey mold incidence in low-tunnels. Additionally, Paul et al. (2005) found that polyethylene sheeting with increased UV transmittance effectively reduced crop susceptibility to *B. cinerea*-induced disease incidence. Previously mentioned initial studies relied on fluorescent lamps with chromatic filters to manipulate spectral composition (Tan, 1974a, 1974b). However, with the introduction of Blue LEDs in the 1990s and their subsequent development and commercialization, it became apparent that spectral composition in CEs could be precisely controlled through narrow bandwidth emissions and adjustable spectral distribution, unlike previous methods that produced wide bandwidth emissions.

Using LEDs, Zhu et al. (2013) discovered that exposing *B. cinerea* to B and R light effectively suppressed both mycelial growth and conidial germination in the B05.10 isolate as well as three wild-type isolates. The complete inhibition of sporulation by Blue LEDs observed in all tested isolates aligned with previous findings, but interestingly, the inhibition of germination varied significantly among the isolates. More recent studies have focused on investigating the impact of narrow-bandwidth spectral emissions on disease incidence. Xu, Fu, et al. (2017) demonstrated that

both R and purple (R+B) light inhibited disease development. Whereas R light was found to enhance tomato basal resistance, possibly through the accumulation of anthocyanins, R+B light primarily inhibited the pathogens morphological development. Therefore, the use of narrow bandwidths to control disease development can be attributed to the photoinhibition of the pathogen, the regulation of host defense mechanisms, or a combination of both. Furthermore, Meng, Van Labeke, et al. (2020) showed that 12 hours of R light exposure effectively enhanced strawberry leaf resistance to *B. cinerea*, while 12 hours of B light exposure increased leaf susceptibility. This particular study also revealed that 12 hours of R light exposure inhibited spore germination on leaf surfaces, further indicating an interplay between pathogen development and host resistance.

While this section primarily focuses on mixed narrow-bandwidth emissions provided by LED lamps, it is important to mention the effectiveness of far-UV light. Recently developed GDLs utilizing Krypton-Chlorine gas offer emissions of narrow bandwidth far-UV light at 222 nm. Janisiewicz et al. (2021) demonstrated that treating strawberry crops with these lamps proved highly efficient, being 3 – 10 times more effective than UV-C light in sterilizing conidia, while also having the advantage of being applicable at any time of day without requiring a dark period (Takeda et al., 2021). However, the incorporation of far-UV lights as a treatment option is less desirable for growers due to their prohibitive costs. In comparison, LED lamps provide numerous benefits for overall plant health and can be implemented as a supplemental lighting source in CE production settings.

Table 3. Overview of light responses of *Botrytis cinerea* under various lighting spectra within controlled environment production settings.

Light Spectrum	Wavelength (nm)	Photoperiod/ Exposure Timing	Isolate (Host Plant/Location)	Response	Reference
NUV/UV (200 – 400 nm)	253.7 nm	3 minutes/ 35 minutes	*B. cinerea* (Rose)	Single spore sterilization. Spore groupings were still able to germinate. Survival when protected within host tissue.	J. Rotem and H.J. Aust (1991)
	275 – 375 nm	12 hours	*B. cinerea* (83 isolates from locations across the UK)	Increased sporulation 54-fold when exposed to NUV light.	West et al. (2000)
	UVA content % (0,0.175, 0.375) Blue 480 nm/UV 310 nm	16 hours	*B. cinerea*	Blue:UV 1.1:1, 2.3:1, 4.2:1 = 100/20/13 sporulation %, respectively.	Reuveni et al. (1989)
	Alternating sequence of near UV and Blue, ending with near UV	×	*B. cinerea*	Conidiation promoted.	Tan & Epton (1974); Tan (I974)
	×	×	*B. cinerea*	Conidiation promoted.	Hite (1973), Honda and Tunoki (1978), Peterson et al. (1988)
BLUE (280 – 500 nm)	Alternating sequence of near UV and Blue, ending with Blue	×	*B. cinerea*	Conidiation repressed & sclerotial development initiated (de-differentiation).	Tan & Epton (1974); Tan, (I974)
	400 – 500 nm	15 umol m^2s^1 (4d constant for growth rate/8h for germination)	B0023 & B0024 (grapes) B05.10 (reference) B0025 (nectarines)	0/3.25/45.37 germination inhibition %, respective of isolates listed. Conidiation completely inhibited for all isolates.	Zhu et al. (2013)
GREEN (500 – 570 nm)	500 – 575 nm	15 umol m^2s^1 (4d constant for growth rate/8h for germination)	B0023 & B0024 (grapes) B05.10 (reference) B0025 (nectarines)	Repression of mycelial growth & conidial germination. Deformation & enlargement of intracellular structures. Reduction of fungal biomass in fruit tissues.	Zhu et al. (2013)
RED (630 – 700 nm)	×	×	*B. cinerea*	Conidiation repressed.	Tan & Epton (1974); Tan, (I974)
	600 – 700 nm	15 umol m^2s^1 (4d constant for growth rate/8h for germination)	B05.10 (reference) B0025 (nectarines)	Inhibited growth, 3.24/45.37 germination %, respective of isolates listed.	Zhu et al. (2013)
FAR-RED (700 – 740 nm)	×	×	*B. cinerea*	Conidiation promoted.	Tan & Epton (1974); Tan, (I974)

FULL SPECTRUM	Pink pigmented polyethene sheet (visible light screened from 470 – 650 nm)	Short day	Four isolates of *B. cinerea* (16, 181 from cucumber and 204, 206 from tomato)	Reduced conidiation and grey mold incidence in commercial greenhouses like complete dark treatment. Isolate 181 was not inhibited.	Elad (1997)
	Green pigmented polyethylene sheet (visible light screened from 560 – 800 nm)	Short day	Four isolates of *B. cinerea* (16, 181 from cucumber and 204, 206 from tomato)	Reduced conidiation and grey mold incidence in commercial greenhouses by 35-75 % for all isolates listed.	Elad (1997)
	Continuous Darkness	16 hours	*B. cinerea*	Conidiation repressed >3.8 % compared to controls of white light).	Reuveni et al (1989)

Information regarding wavelength or photoperiod that was not explicit in-text is signified using an ×. Days and Hours are abbreviated as “d” and “h”. Isolates are referred to as per the author, if no isolate identifier was given it is denoted as *B. cinerea.*

Connecting Statement to Chapter 3

The following chapter focuses on characterizing and examining the photomorphogenesis of a wild-type isolate of *B. cinerea* when exposed to three ratios of Far-red:Blue LED lighting (5:1, 1:1, 1:5) *in vitro* within a controlled environment. The growth, sporulation, and germination of the pathogen is shown in the results and the development of the pathogen is characterized and discussed.

Chapter 3: A photomorphogenic study of *Botrytis cinerea* Pers. under Far-red:Blue LEDs

3.1. Abstract

Light-emitting diode (LED) technology has become an indispensable tool in controlled environment (CE) plant production settings for supplemental plant lighting. The ubiquitous hemibiotrophic fungal pathogen *Botrytis cinerea* is a common pest within CE and utilizes light as an environmental cue to regulate morphogenesis, a key factor influencing asexual development and thus the dissemination of infectious propagule. The effects of various wavelength-specific lighting qualities on the photomorphogenesis of *B. cinerea* has been well-documented. However, the impact of mixed narrow-bandwidth LED emissions on its development remains unclear. The aim of this study was to evaluate the effect of Far-red:Blue LED lighting diode ratios (1:5, 5:1, 1:1) on the photomorphogenesis of *B. cinerea in-vitro* within a CE. Results show that a Blue-dominant diode ratio of 1:5 was the most effective treatment for reducing hyphal growth and sporulation of *B. cinerea* compared to ratios of 5:1 and 1:1, although all LED treatments were effective at inhibiting morphogenesis when compared to controls treatments of White (high-pressure sodium lamps (HPSL) + ambient light) and Dark (complete darkness). Colonies exposed to LED treatments were observed to produce sterile arial hyphae, absent of conidia, with intermittent gaps or breaks in circular mycelial banding, indicating a possible disruption in entrainment. Additionally, all colonies exposed to LED treatments showed signs of positive phototropic growth. Interestingly, when examining the effect of treatments on conidial germination, no difference was found between LED treatments and controls. Furthermore, this study demonstrates that incorporating Blue light into a Far-red:Blue diode ratio of 5:1 was effective in counteracting the conidiation-promoting effects previously reported for Far-red light. Together, these findings have important implications regarding the asexual proliferation of *B. cinerea* in CE plant production under LED lighting.

3.2. Introduction

Spectral irradiance by the sun, or the emission of light, is crucial for the existence and sustainment of all life forms on earth. Phytopathogenic fungi, like all sessile eukaryotic organisms, must sense and interpret their ambient environment and adapt accordingly for survival (i.e., to infect and proliferate). Thus, light or its absence acts as the primary signaling mechanism that these organisms use to interpret their environment and infect a host. A complex regulatory network of light-sensitive proteins (photoreceptors) and signal transduction pathways allows for the induction of these adaptive responses by regulating developmental stages (morphogenesis) and ensuring proper circadian rhythm synchronization (entrainment) (Herrera-Estrella and Horwitz, 2007; Rodriguez-Romero et al., 2010; Schumacher, 2017). Accordingly, the possible outcomes of plant-pathogen interactions are dictated in a light-dependent manner (Roden and Ingle, 2009).

The ascomycete *Botrytis cinerea* has long been known for its absolute responses in the presence or absence of lighting conditions (Bewley, 1923; Brierley, 1921; Godfrey, 1923; Klein, 1885; Peltier, 1912; Robinson, 1914). Light is essential in the production of inoculum, or conidiation, and on the contrary, the formation of sclerotia is exclusive to complete darkness. Furthermore, numerous works have documented the specific morphological changes that are induced by various lighting qualities of the visible spectra, including non-visible near-UV emissions (**Table 4**). These light responses can be characterized as induced differentiation between morphological structures such as hyphae, conidiophores, and sclerotia. However, they have also been described as photoreversible, which means that certain wavelengths can de-differentiate the morphological structures induced by other wavelengths, as described by Tan (1974b). The basis of propagule proliferation and dissemination, be it sexual or asexual, can therefore be attributed to light, or photomorphogenesis.

Table 4. The influence of the lighting spectra on the photomorphogenic responses of *Botrytis cinerea.* Spectra are identified by bandwidth ranges given in nanometers (nm).

Light Quality	Response	Wavelength (nm)	Source
NEAR-UV	Promotes conidiation.	200 – 240	(Honda and Yunoki, 1978; Peterson et al., 1988; Tan, 1974b; Tan and Epton, 1974a, 1974b)
BLUE	Represses conidiation & sclerotial development (*de-differentiation*).	280 – 500	(Tan, 1974a; Tan and Epton, 1974a; Zhu et al., 2013)
GREEN	Represses mycelial growth, and conidial germination. Deformation of intracellular structures.	500 – 575	(Zhu et al., 2013)
RED	Represses conidiation.	600 – 700	(Tan and Epton, 1974a)
FAR-RED	Promotes conidiation.	700 – 800	(Tan and Epton, 1974a)

It was first suggested by Paul (1929) that isolates can be grouped into three distinct morphological types (*sclerotia, spore*, and *mycelia* forming) based on natural isolates that were observed to predominantly form one of these structures. Recently, a further method of characterization was proposed by Canessa et al. (2013), which can be associated with an isolate's ability to perceive light (*light-responsive*) or not (*blind*). Additional study of light-induced photomorphological responses and characterization of isolates using accurate terminology is important in *B. cinerea* due to its genetic plasticity and high phenotypic variation. Studies that incorporate mixed narrow-bandwidth emissions are particularly relevant, as the composition of these mixed emissions and their effect on morphological development and entrainment are not fully understood. In this regard, LED technology has provided the potential to control the spectral distribution of a CE (Nakamura and Fasol, 1997). Therefore, LEDs can not only be used to alter varying aspects of fungal photomorphological responses but have also furthered our ability to study the potential effects of mixed narrow-bandwidth emissions on photomorphogenesis. This is of particular interest in CE plant production, as manipulating the spectral composition could prove to be a novel method of cultural control or disease mitigation.

The use of spectral composition as a method of cultural control is a complex issue because host responses to spectral composition vary widely, and different stages of the pathosystem may affect the outcome. One way to validate this approach is by examining whether spectral composition can interrupt the morphogenesis of the pathogen, particularly by reducing the number of infectious propagules. While previous works have explored the effect of spectral composition on *B. cinerea* within CEs (Elad, 1997; Meng, Mestdagh, et al., 2020; Meng, Van Labeke, et al., 2020; Reuveni R, 1989; West et al., 2000; Xu, Fu, et al., 2017), these works typically focus on specific bandwidths. In CEs, light sources with broad-bandwidth emissions, such as natural sunlight or gas-discharge lamps (GDLs), often provide significant emissions in regions of the spectra that are not

required by plants. However, with the increasing use of LEDs in CEs, lamps with mixed narrow-bandwidth spectral qualities have become more common. These lamps mainly consist of Blue and Far-red diodes, which correspond to the peaks of the quantum yield curve for photosynthetically active radiation (McCree, 1971). Because a mixed narrow-bandwidth spectral composition of Far-red:Blue lighting can benefit overall plant health, it is important to investigate the effects that either a Far-red or Blue dominant lighting source has on the photomorphogenesis of *B. cinerea*. Therefore, evaluating a dominant bandwidth ratio is the primary step toward proving the usefulness of spectral composition as a cultural control method, and can contribute to the direction of further works.

3.3. Materials and Methods

3.3.1 Strain, storage, and inoculation techniques

The wild-type strain of *B. cinerea* used in subsequent experiments was isolated from naturally infected flower buds of cannabis plants (*Cannabis sativa* L.) grown in CE production facilities in British Columbia, Canada (Punja, 2018). The isolate was identified using PCR with the primers *ITS1*F-*ITS4* (*ITS1*-F 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and *ITS4* 5′-TCCTCCGCTTATTGATATGC-3′) and confirmed to be above 99% identical to the NCBI GenBank database sequence of the B05.10 strain (Punja et al., 2019).

Mycelia plugs taken from initial isolates were cultured on Nutriselect® potato dextrose agar (PDA) (Sigma Aldrich, CA) plates. Methods for preservation and subsequent morphological assays have been adapted from Delcán et al. (2002) and De Miccolis Angelini et al. (2010). Initial colonies were cut into strips, placed into sterile screw-top gestation tubes, and lyophilized over four days for bulk, long-term preservation. This allowed for the preservation of shelf-stable samples in the form of dried mycelial strips. All further colonies were started by rehydrating lyophilized material in sterile distilled water (dH_2O). For subsequent growth and sporulation assays, 5 mm mycelial plugs were taken from the edge of a one-week-old re-hydrated colony and placed in the center of a PDA plate.

To ensure the viability of spores, some of the initial colonies intended for lyophilization were grown in an incubator at 24 ºC for 2 weeks to allow for sporulation. These sporulated colonies were then preserved as spore solutions. For spore collection, plates were first flooded with 10 ml of a sterile dH_2O + 1% (v/v) Tween-20 solution. A rounded L-shaped scraper was used to gently rub the surface of each flooded plate to dislodge conidia. Solutions were poured into 15 ml conical Eppendorf tubes and vortexed at medium power for 1 minute. Spore solutions were then filtered

through a double layer of cheesecloth to trap mycelial debris. Glycerol was then added to the solution to achieve a total glycerol concentration of 20% (v/v). The solution was collected and then counted with a hematocytometer to quantify spores/ml of solution. The solution was then dispersed between 2 ml Eppendorf tubes and stored at 4 ºC for semi-long-term preservation (< 1 year).

3.3.2 LED & CE lighting configuration

In this study, three LED lamp prototypes (U Technology Corporation, CA) were used to provide varying peak emissions of Far-red (725 nm) and Blue (455 nm) wavelengths. The lamps were installed 1.5 m above a greenhouse bench and separated with a double layer of landscaping fabric to isolate treatments, with the tops left uncovered to allow for heat dissipation and ambient light to enter. Each lamp represented a different Far-red:Blue light ratio (5:1, 1:1, 1:5) based on the number of diodes in the array (1,729 diodes per array, divided into the respective ratios of Far-red:Blue). Experimental controls within the CE were treatments of White (HPSLs + ambient light) and Dark (samples covered with aluminum foil). The lamps were controlled with an automated timer to replicate photoperiods in conjunction with the greenhouse lighting regime. Three photoperiods were tested; Photoperiod A: 24-hour light treatment, Photoperiod B: 16-hour light treatment + 8 hours complete darkness, and Photoperiod C: 12 hours White + 4-hour light treatment + 8 hours complete darkness (**Figure 4**). Spectral output and light intensity were measured with a spectroradiometer (Apogee Instruments Inc., model PS-300, USA) and a light meter (Digi-Sense, model 00653-22, CA), respectively, and recorded accordingly as: 182 ± 40 kLux (5:1), 230 ± 40 kLux (1:1), and 325.6 ± 40 kLux (1:5). A control treatment of White within the CE was recorded at an intensity of 418.8 ± 80 kLux. Through the course of the experiment, data was collected periodically and is reported here as mean (±) standard deviation of intensity (kLux).

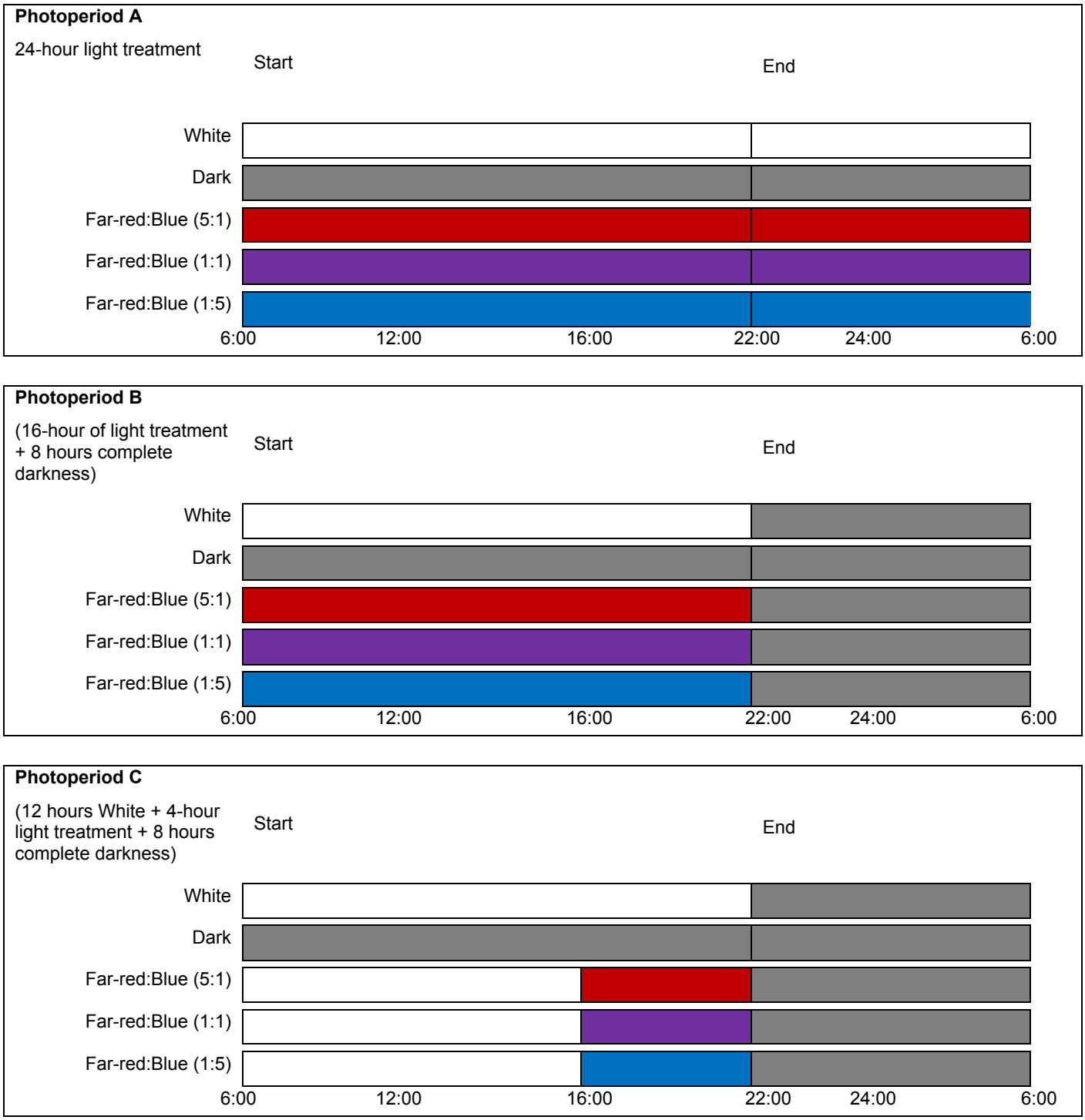

Figure 4. Experimental photoperiods and treatment exposure timings used in this study. Light treatments according to day-length are shown. The (1) mycelial growth assays and (2) sporulation assays utilized Photoperiods (1)-ABC and (2)-AB, respectively. In all experiments, regardless of photoperiod, sample colonies of *Botrytis cinerea* were placed under lighting treatments beginning at 12:00 hours. As photoperiod fluctuated slightly over the course of these experiments, photoperiod 'Start' and 'End' times tended to vary, because of this, HPSL and LED exposure was adjusted as needed to complement a 16-hour/8-hour light/dark cycle. Photoperiod A: 24-hour light

treatment, Photoperiod B: 16-hour light treatment + 8 hours complete darkness, and Photoperiod C: 12 hours White + 4-hour light treatment + 8 hours complete darkness

3.3.3 Mycelial growth assays

Using a cork borer, 5 mm mycelial plugs were taken from the edge of a one-week-old, rehydrated colony and placed in the center of each PDA plate for inoculation. Each plate was then scored on the underside with two perpendicular lines of different colors to take radial measurements each day over the course of 7 days. Each plate was then sealed using parafilm to secure the cover and to limit humidity exchange with the ambient environment. Inoculated plates were then placed under respective light treatments for the duration of the experiment within the CE. Temperatures were monitored with the use of a Traceable® Logger-Trac™ (VWR, CA) and recorded as daily means ± standard deviation. Throughout the course of a trial, all seven days were recorded to have daily temperature means between 24-28 ºC. If the mean temperature was recorded to be outside of these conditions, the trial was omitted from data analysis. Trials were performed as a factorial experiment using a completely randomized design (CRD) with the corresponding 5 treatments, 7 daily measurements, 5 biological replicates, and 3 temporal replicates.

3.3.4 Sporulation assays

Colonies were started similarly to those of mycelial growth assays but were grown under respective light treatments within the CE for 14 days. Spores were collected from plates as previously described in **Section 3.3.1**. The solution was then counted with a hematocytometer to quantify spores/ml of solution and reported as spores/ml averaged among biological replicates. Trials were performed as a factorial experiment using a CRD with 5 treatments, 3/4 biological replicates for Photoperiods A/B, respectively, and 3 temporal replicates.

3.3.5 Spore germination assays

Glass microscope slides were sublimated with molten PDA using a 10 ml glass pipette and placed into an empty petri dish containing damp sterile filter paper to ensure high relative humidity. A

frozen spore solution with a concentration of $1x10^6$ spores/ml containing 20% glycerol was thawed and placed in a swing rotor centrifuge for 8 minutes at 1000 rpm. The supernatant was then discarded and replaced with sterile Gambour's B5 solution (10 mM K_2HPO_4, 20 mM Glucose, and 3.2 g/l Gambour B5 powder, adjusted to a pH of 6.0). Three 5 µl aliquots of a spore solution were pipetted along the center of each slide, spaced evenly. Each droplet was then covered with a glass coverslip. Petri dishes were then sealed with parafilm and placed under respective lighting treatments for 11 hours. For every measurement timing (at 0, 3, 5, 7, 9, and 11 hours) a new set of three biological replicates was used and then discarded. To quantify sporulation, the total number of spores was counted through a randomly chosen field of vision in one microscope visual field at ×100 magnification and the number of germinated and non-germinated spores was determined. This was done three times for each slide, corresponding to one image per coverslip per slide. All three measurements were averaged together as one biological replicate. This trial was performed as a factorial experiment using a CRD with 5 treatments, 5 measurements, 3 biological replicates, and 1 temporal replicate.

3.3.6 Statistical analysis

Statistical analyses for all morphological assays (mycelial growth, sporulation, and germination) were performed using JMP®, Version 17 (SAS Institute Inc., USA). For these assays, each dataset underwent assessment for normality and equal variances using Levene's tests and skewness. Data sets meeting these assumptions underwent one-way analysis of variances (ANOVAs) to identify significant differences. Subsequently, significant differences between treatment groups were isolated using Post-hoc Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$).

Datasets violating ANOVA assumptions, with either unequal variances according to Levene's Test or skewness outside the range of -1 to 1, underwent the Kruskal-Wallis method to determine significant differences among treatment groups. An all-pairs comparison using the Steel-Dwass method, a conservative test controlling family-wise error rate in multiple comparisons and reducing false positives, further identified specific group differences ($p < 0.05$). Levene's Test *p* values and skewness are shown in such cases.

3.4. Results

3.4.1 Phenotypic characterization of isolate under lighting treatments

The isolate exhibited a preference for sporulation in both control treatments of White and Dark, suggesting an adaptive inclination towards asexual dissemination, being absent of sclerotial production in Dark treatment. However, samples subjected to complete darkness in an incubator (24 ºC, constant temperature) were found to seldomly form sclerotia with no observable pattern, suggesting the ability of the pathogen to exhibit adaptive responses to environmental cues. Therefore, based on terminology utilized for phenotypic characterization (**Section 3.2**), the isolate having a stronger preference for sporulation can be categorized as always sporulating (**Figure 5**). In addition to being always sporulating, the isolates ability to form sclerotia in a light-dependent manner indicates the isolate is also light-responsive (Canessa et al., 2013). Additional physiological observations were made regarding a possible disruption in entrainment among colonies grown under Photoperiod B, consisting of 16-hour light treatment + 8 hours complete darkness, in which colonies exposed to all Far-red:Blue treatments exhibited intermittent breaks or gaps in banding when compared to those grown under a control of White (**Figure 6**).

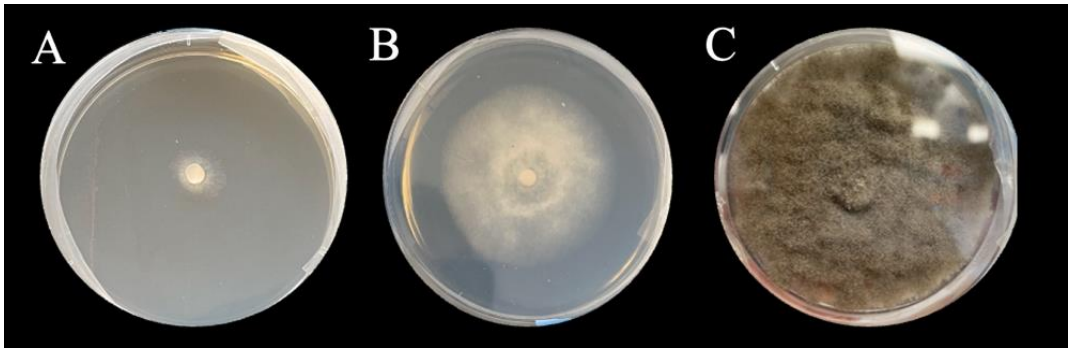

Figure 5. Growth and sporulation of a wild-type isolate of *Botrytis cinerea* over the course of 2 weeks. The colony in this figure was grown in complete dark conditions in an incubator at 24 ºC. (A) Mycelial plug with initial growth at 24 hours post-placement. (B) Mycelial growth at 7 days. (C) Fully sporulated plate at 14 days.

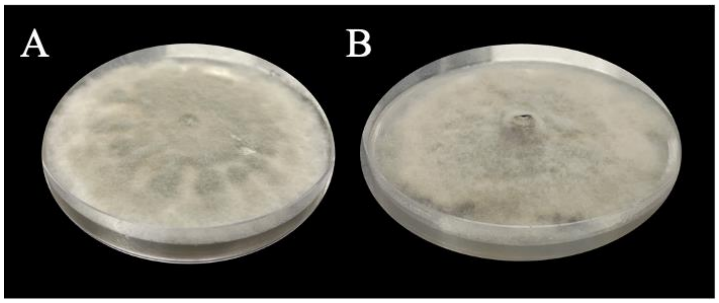

Figure 6. Banding phenotypes of a wild-type isolate of *Botrytis cinerea* grown in a controlled environment for 2 weeks under Photoperiod B, 16-hour light treatment + 8 hours complete darkness. (A) Colony grown under Far-red:Blue 1:5. (B) Colony grown under White.

3.4.2 Influence of lighting quality on mycelial growth

To visualize the data, a line plot for Days 2, 3, 5, and 7 was plotted for each treatment and photoperiod (**Figure 7**). Compared to the observed logarithmic growth of *B. cinerea* under Dark and White control treatments, all Far-red:Blue light treatments induced stationary growth of radially growing mycelia, regardless of photoperiod. The induction of stationarity was observed earliest under Photoperiod B, whereas stationarity can be observed at Day 5 onward for both Photoperiods A and C. Among the Far-red:Blue treatments, a Far-red:Blue ratio of 1:5 was found to be the most effective in inhibiting the radial growth of *B. cinerea* for each photoperiod tested.

For a more comprehensive analysis, the data were classified by day for both treatment and trial groupings. Subsequently, normality and variance tests were conducted to assess the distribution of treatment data in the absence of trial effects, as well as the distribution of trial data without the influence of treatment effects, specifically focusing on Days 2, 3, 5, and 7 (**Table 5**). Notably, across all photoperiods, both evaluations of trial and treatment means over the four isolated days revealed instances of unsuccessful Levene's tests ($p < 0.05$) or skewness beyond the acceptable range of -1 to 1, with the exception being trial means on Day 7 in Photoperiod C. Subsequently, the Kruskal-Wallis method was employed to ascertain noteworthy distinctions among treatment and trial groupings for each specific day/photoperiod pairing. This indicated that despite the violation of the assumption of equal variances, the divergence in group means could still be considered as statistically significant. In the scenario of trial means on Day 7 in Photoperiod C, the validation of significance was achieved through ANOVA, followed by a subsequent post-hoc Tukey HSD test to distinguish differences. These methodologies revealed that on Day 7, across all photoperiods, all Far-red:Blue treatment groups exhibited significant divergence in means from the Dark and White control groups (apart from the 5:1 treatment in Photoperiod A). Furthermore, when examining trial groupings by day, statistical differences were observed within all days across

trials, underscoring an important source of non-parametricity within the dataset. While this approach was largely undertaken to account for the variability witnessed among trials, it effectively substantiated the significant distinctions on Day 7 between all Far-red:Blue treatments and controls. Therefore, establishing a statistical foundation for the efficacy of Far-red:Blue treatments in impeding mycelial growth as compared to controls, irrespective of photoperiod and irrespective of trial conditions (**Table 5**).

Continuing the analysis of each photoperiod's data, similar procedures were carried out for treatments categorized both by day and by trial. This additional step was essential to further confirm the earlier observations, particularly given the significant variation observed in trial group means as outlined previously. Across distinct trials, all treatment groups exhibited a normal distribution. However, differences in variances between group means were found to be non-uniform, primarily due to the greater standard error observed among the control groups (**Figure 7**). Notably, in the context of Photoperiods B and C, the Dark control group displayed minimal variation. This is attributed to the majority of colony margins extending to the edges of the petri dish for all samples within these specific photoperiods (**Error! Reference source not found.**).

Regarding plausibility for the causation for trial variability, it is important to acknowledge the significant influence of temperature on the growth and development of *B. cinerea*. Hence, it is probable that the variations observed within photoperiods between trials can be attributed to the fluctuations in ambient temperature within the CE. Where the average 24-hour day/night temperature was recorded as 20 ºC ± 8 in Photoperiod A, the ambient temperature fluctuated within the CE as well during Photoperiods B and C, similar to A, with a 24-hour day/night average temperature of 28 ºC ± 10 being recorded. Additionally, treatment means of Dark and White within Photoperiod B and C vary significantly from each other at Day 7 ($p < 0.05$), as compared to Photoperiod A where controls of Dark and White are not significantly different, which can likely

be attributed to the greater temperature fluctuation in Photoperiods B and C or possibly a higher average maximum temperature, recorded at a peak of 38 ºC.

Upon closer examination of the treatment effects on Day 7 across all photoperiods, it was observed that all Far-red:Blue treatments exhibited significant differences compared to the control groups. However, it is important to note that significant differences between the Far-red:Blue treatments and controls were only observed consistently throughout the entire 7-day period in Photoperiod B. Within Photoperiod B, a Far-red:Blue ratio of 1:5 proved to be the most effective in inhibiting growth, as it displayed a significant difference compared to the Far-red:Blue ratio of 5:1 on Day 7. Therefore, the treatment/photoperiod combination that yielded the highest inhibition of mycelial growth is observed with a Far-red:Blue ratio of 1:5 under Photoperiod B, exhibiting a mean growth area inhibition of 84.57%, the highest among all the treatment/photoperiod combinations tested (data not shown).

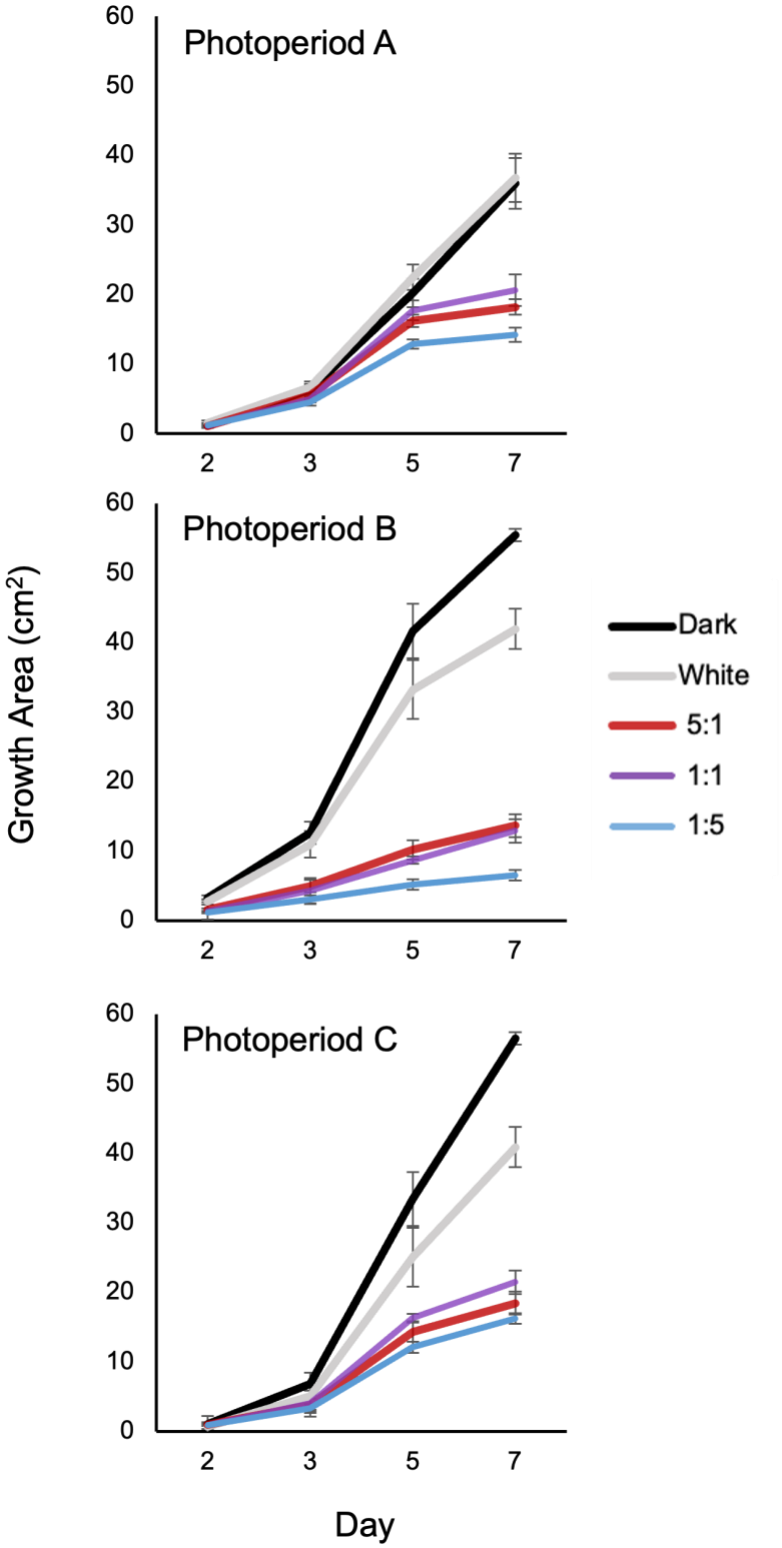

Figure 7. Line graphs comparing the effect of five lighting qualities on the mean radial mycelial growth area (cm^2) of *Botrytis cinerea in vitro* within a CE exposed to three photoperiods. Displayed as cm^2 of radial growth by day with error bars demonstrating sample variability. Photoperiod A: 24-hour light treatment, Photoperiod B: 16-hour light

treatment + 8 hours complete darkness, and Photoperiod C: 12 hours White + 4-hour light treatment + 8 hours complete darkness. (n = 15)

Table 5. Mean radial growth area of *Botrytis cinerea* over a 7-day period under respective light treatments during Photoperiods A, B, and C

	Growth Area (cm^2)											
	Photoperiod A				Photoperiod B				Photoperiod C			
	Day 2	Day 3	Day 5	Day 7	Day 2	Day 3	Day 5	Day 7	Day 2	Day 3	Day 5	Day 7
Light Treatment												
Dark	1.16	6.29	20.23 ^{ab}	35.99 ^{ab}	3.27 ^{a}	13.03 ^{a}	41.61 ^{a}	55.43 ^{a}	1.04	6.86 ^{a}	33.53 ^{a}	56.51 ^{a}
White	1.50	6.68	22.47 ^{a}	36.79 ^{a}	2.70 ^{ab}	10.94 ^{ab}	21.08 ^{a}	41.90 ^{b}	0.69	4.99 ^{ab}	25.05 ^{ab}	40.87 ^{b}
5:1	1.09	5.55	16.20 ^{ab}	18.17 ^{c}	1.56 ^{bc}	4.92 ^{bc}	8.06 ^{b}	13.64 ^{c}	0.96	3.76 ^{ab}	14.26 ^{c}	18.41 ^{c}
1:1	1.08	4.91	17.70 ^{ab}	20.58 ^{bc}	1.36 ^{c}	4.26 ^{c}	6.48 ^{bc}	12.91 ^{cd}	0.90	3.97 ^{ab}	16.31 ^{bc}	21.43 ^{c}
1:5	1.22	4.45	12.87 ^{b}	14.21 ^{c}	1.19 ^{c}	2.98 ^{c}	4.29 ^{c}	6.49 ^{d}	0.91	3.25 ^{b}	12.06 ^{c}	16.24 ^{c}
***p* value**												
Skewness	1.03	1.44	1.17	0.85	1.33	1.11	1.12	0.53	1.44	0.82	0.75	0.50
Levene's Test	0.19	0.07	0.0013	<.0001	<.0001	<.0001	<.0001	<.0001	0.0033	0.0099	0.0366	<.0001
Kuskall-Wallace	0.11	0.18	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	0.1777	0.0106	<.0001	<.0001
ANOVA	-	-	-	-	-	-	-	-	-	-	-	-
Trials												
1	2.86 ^{a}	8.39 ^{a}	22.27 ^{a}	32.21 ^{a}	2.86 ^{a}	12.42 ^{a}	28.14 ^{a}	31.83 ^{a}	1.56 ^{a}	7.09 ^{a}	28.74 ^{a}	36.48 ^{a}
2	0.43 ^{b}	3.68 ^{b}	14.86 ^{b}	24.14 ^{b}	1.86 ^{b}	6.75 ^{b}	19.89 ^{ab}	21.50 ^{b}	0.68 ^{b}	4.68 ^{b}	19.16 ^{b}	26.71 ^{b}
3	0.35 ^{b}	4.67 ^{c}	16.55 ^{b}	19.08 ^{b}	1.33 ^{b}	1.33 ^{c}	11.24 ^{b}	24.89 ^{ab}	0.46 ^{c}	1.92 ^{c}	11.97 ^{c}	27.73 ^{b}
***p* value**												
Skewness	1.03	1.44	1.17	0.85	1.33	1.11	1.12	0.53	1.44	0.82	0.75	0.50
Levene's Test	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	0.0381	<.0001	0.0007	0.0133	0.0634
Kruskall-Wallace	<.0001	<.0001	0.0011	0.0105	<.0001	<.0001	0.0032	0.0271	<.0001	<.0001	<.0001	-
ANOVA	-	-	-	-	-	-	-	-	-	-	-	<.0001
Trial 1												
Dark	2.65	10.09	30.26	50.27	3.49	17.71	45.91	56.75	1.38	8.40	42.21 ^{a}	56.74 ^{a}
White	3.60	10.56	30.87	50.27	4.82	21.01	54.95	56.75	0.94	7.28	33.64 ^{ab}	44.41 ^{b}
5:1	2.71	7.99	17.38	18.90	2.33	9.16	17.07	19.48	2.05	7.28	22.96 ^{c}	27.33 ^{c}
1:1	2.60	6.76	18.79	24.78	1.79	7.91	13.51	16.39	1.66	6.69	25.36 ^{bc}	29.51 ^{c}
1:5	2.72	6.52	14.09	16.82	1.85	6.29	9.28	9.77	1.79	5.80	19.51 ^{c}	24.40 ^{c}
***p* value**												
Skewness	0.55	0.76	0.50	0.15	0.79	0.54	0.53	0.29	0.36	1.34	0.68	0.50
Levene's Test	0.0444	0.0034	0.0400	0.0128	0.0351	0.0033	0.0231	0.0054	0.0229	0.0308	0.2630	0.1622
Kuskall-Wallace	0.2134	0.0765	0.0007	0.0006	0.0039	0.0002	0.0002	0.0002	0.0506	0.5766	-	-
ANOVA	-	-	-	-	-	-	-	-	-	-	<.0001	<.0001
Trial 2												
Dark	0.40 ^{ab}	4.59 ^{ab}	18.64 ^{a}	40.48 ^{a}	4.27	16.84	56.75	56.75	1.16	9.56	36.96 ^{a}	56.09
White	0.49 ^{ab}	4.90 ^{a}	20.61 ^{a}	40.81 ^{a}	1.61	8.19	27.99	35.03	0.67	4.78	22.94 ^{b}	36.24
5:1	0.28 ^{b}	3.15 ^{ab}	12.79 ^{ab}	15.39 ^{b}	1.41	3.94	7.14	7.88	0.46	2.61	11.57 ^{c}	12.59
1:1	0.36 ^{ab}	3.02 ^{ab}	12.59 ^{ab}	13.97 ^{b}	1.05	3.03	4.58	4.97	0.57	3.73	13.97 ^{c}	16.81
1:5	0.61 ^{a}	2.74 ^{b}	9.66 ^{b}	10.05 ^{b}	0.97	1.77	3.02	2.90	0.55	2.72	10.35 ^{c}	11.79
***p* value**												
Skewness	1.51	0.54	0.49	0.45	1.36	1.03	0.97	0.74	1.35	1.24	0.85	0.78
Levene's Test	0.0516	0.5315	0.0179	0.2233	0.0225	0.0082	0.0254	0.0005	0.2155	0.7148	0.8255	<.0001
Kuskall-Wallace	0.0084	-	0.0004	-	0.0011	0.0002	0.0002	0.0002	0.0157	0.0013	-	0.0005
ANOVA	-	0.0003	-	<.0001	-	-	-	-	-	-	<.0001	-
Trial 3												
Dark	0.42	4.19 ^{b}	11.78	17.23	2.05	4.54	22.17	52.79	0.58 ^{a}	2.61 ^{a}	18.39 ^{a}	56.74
White	0.42	4.57 ^{ab}	15.94	19.21	1.68	3.61	16.58	33.93	0.48 ^{b}	2.93 ^{a}	18.56 ^{a}	41.95
5:1	0.29	5.52 ^{a}	18.43	20.21	0.94	1.66	6.32	13.55	0.38 ^{c}	1.38 ^{b}	8.25 ^{bc}	15.29
1:1	0.28	4.96 ^{ab}	21.73	22.98	1.23	1.85	7.93	17.36	0.48 ^{b}	1.48 ^{b}	9.63 ^{b}	17.96
1:5	0.35	4.09 ^{b}	14.87	15.75	0.76	0.89	3.19	6.81	0.39 ^{bc}	1.21 ^{b}	6.32 ^{c}	12.53
***p* value**												
Skewness	0.23	0.35	1.83	1.50	0.64	0.54	0.54	0.75	0.30	0.43	0.53	0.89
Levene's Test	0.0004	0.6912	0.0139	0.0446	0.0085	0.0478	0.0005	0.0005	0.8771	0.6801	0.0707	<.0001
Kuskall-Wallace	0.0015	-	0.0011	0.1536	0.0002	0.0002	0.0002	0.0001	-	-	-	0.0005
ANOVA	-	0.0004	-	-	-	-	-	-	<.0001	<.0001	<.0001	-

Normality and variance were analyzed by skewness and Levene's tests. Parametric data sets was then analyzed by ANOVA with a post-hoc Tukey HSD test to assign significant differences, while nonparametric data sets were analyzed by the Kruskall-Wallace method, followed by a post-hoc Steel-Dwass comparison for all pairs ($p \leq 0.0$). Lower-case superscript letters indicate statistically significant differences. Photoperiod A: 24-hour light treatment, Photoperiod B: 16-hour light treatment + 8 hours complete darkness, and Photoperiod C: 12 hours White + 4-hour light treatment + 8 hours complete darkness. Sample size (n) was 15, 175, 5 for Light Treatment, Trials, and Trail 1, 2, 3, respectively.

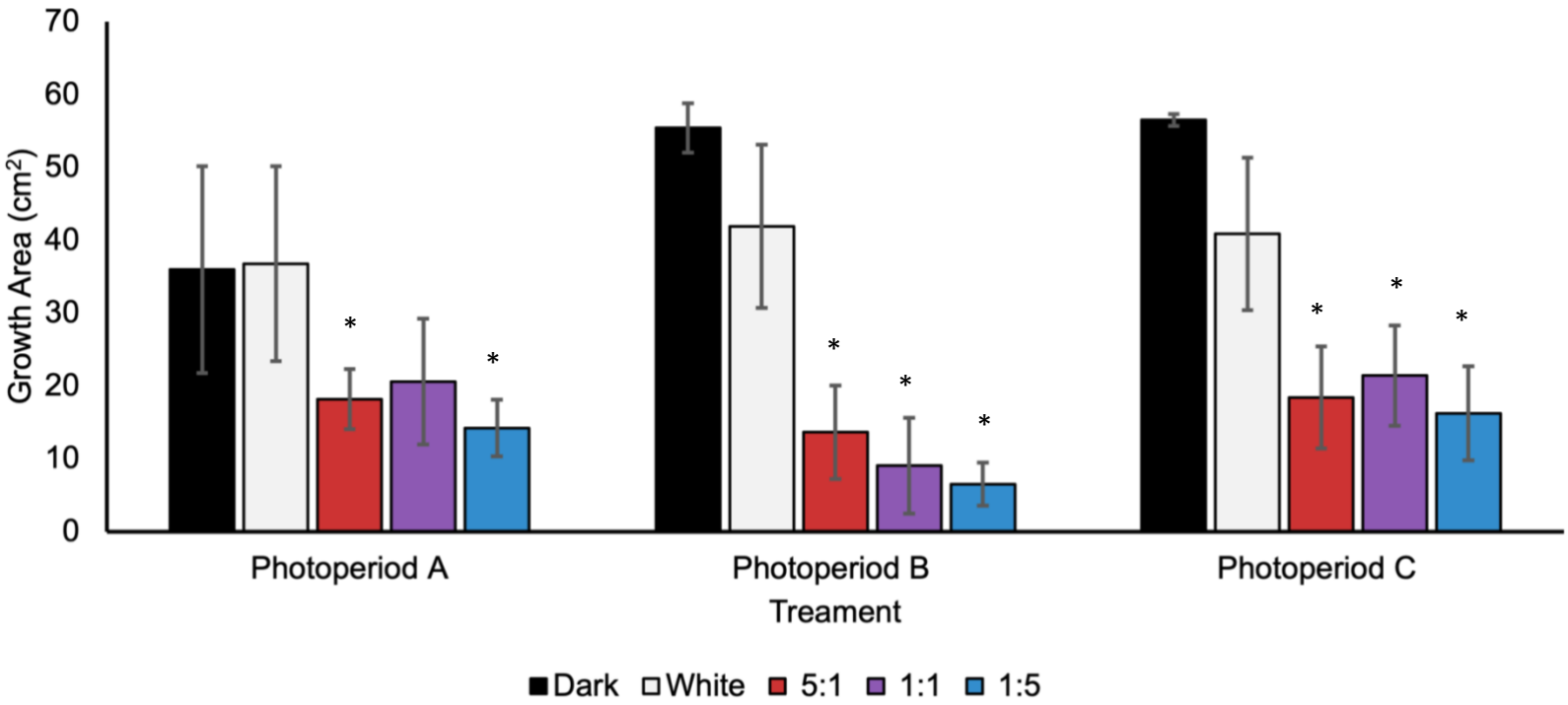

Figure 8. Bar graph comparing the effect of five lighting qualities on the mean radial mycelial growth area (cm^2) of *Botrytis cinerea* at Day 7 *in vitro* within a CE exposed to three photoperiods with error bars demonstrating sample variability. (*) denotes statistically significant differences among Far-red:Blue light treatments (5:1, 1:1, 1:5) from controls (Dark and White) determined by a post-hoc Steel-Dwass comparison for all pairs, ($p < 0.05$). Photoperiod A: 24-hour light treatment, Photoperiod B: 16-hour light treatment + 8 hours complete darkness, and Photoperiod C: 12 hours White + 4-hour light treatment + 8 hours complete darkness. ($n = 15$)

3.4.3 Influence of lighting quality on sporulation

The two photoperiods tested (A and B) had significantly different sample sizes (n = 40 and 45 for Photoperiods A and B, respectively), making a direct comparison of treatment means inappropriate. However, statistical tests were used to determine whether there was a significant difference between the respective treatments within each photoperiod. Although the data was found to be independent, a non-normal distribution was noted (Skewness = 3.12 and 4.09 for Photoperiods A and B, respectively) as well as unequal variances between treatments validated by Levene's tests, therefore a non-parametric test was considered the most appropriate for analyzing the treatment effects (**Table 6**). To determine if significant differences existed among treatments within each photoperiod, the Kruskal-Wallis test was employed, which revealed a significant difference among treatments ($p < 0.03$ and 0.001 for Photoperiods A and B, respectively).

To determine which groups were significantly different from each other, analysis using an all-pairs comparison using the Steel-Dwass method was conducted for each photoperiod. The results showed that in Photoperiod A all treatments were considered non-significant, which can be primarily attributed to sample variance. Whereas, in Photoperiod B, a treatment of Far-red:Blue ratio of 1:5 had significantly lower sporulation when compared to both Dark ($p < 0.008$) and White treatments ($p < 0.001$). Interestingly, a treatment of Far-red:Blue ratio of 1:1 also had significantly lower sporulation compared to both Dark ($p < 0.03$) and White ($p < 0.001$) treatments in Photoperiod B. To further explore the variation within photoperiod trials, a one-way ANOVA was performed, given that trials within photoperiod groupings revealed instances of successful Levene's tests ($p > 0.05$) and skewness within the acceptable range of -1 to 1 (data not shown). Although the assumptions for normality and homoscedasticity were not met to distinguish

significance between both photoperiods and individual treatments, the analysis did confirm that trials within photoperiodic groups were not significantly different from each other ($p > 0.05$).

Interestingly, when comparing treatments means between Photoperiod A and B, a noticeable decrease in spore concentration (spores/ml) between all treatments can be observed (**Figure 9**). This is not necessarily due to the effect of photoperiod but is likely influenced by culture viability or other experimental factors that can vary within CE's (i.e., daily temperature, relative humidity, or fluctuations in ambient lighting intensity).

The results suggest that different light treatments and photoperiods do have significant effects on the sporulation of *B. cinerea* within a CE, with Far-red:Blue ratios of 1:5 and 1:1 showing promising inhibition. However, due to the significant variation between samples and limitations in the sampling design, making direct comparisons between photoperiods and light treatments is currently speculative. Consequently, further studies incorporating improved sampling designs and careful consideration of environmental factors are warranted to validate and confirm these findings with greater confidence.

Table 6. Mean sporulation of *Botrytis cinerea* under respective light treatments separated by photoperiod.

	Mean Sporulation (spores/ml)	
Treatment	**Photoperiod A**	**Photoperiod B**
Dark	2,816,111	81,875[a]
White	3,517,556	1,026,625[a]
5:1	439,333	18,625[ab]
1:1	528,111	375[b]
1:5	335,111	0[b]

	Photoperiod A	**Levene's Test *p* value**
Levene's Test	0.030	0.001
Kruskall-Wallace	<.0001	<.0001

Significance was determined by the Kruskall-Wallis method followed by a post-hoc Steel-Dwass comparison for all pairs, ($p < 0.05$). Levene's test p values are included to display non-normal data. Different lower-case letters indicate statistically significant differences among treatments. Photoperiod A: 24-h light treatment, Photoperiod B: 16-hour light treatment + 8 hours complete darkness. The sample size (n) for Photoperiod A and Photoperiod B was 40 and 45, respectively.

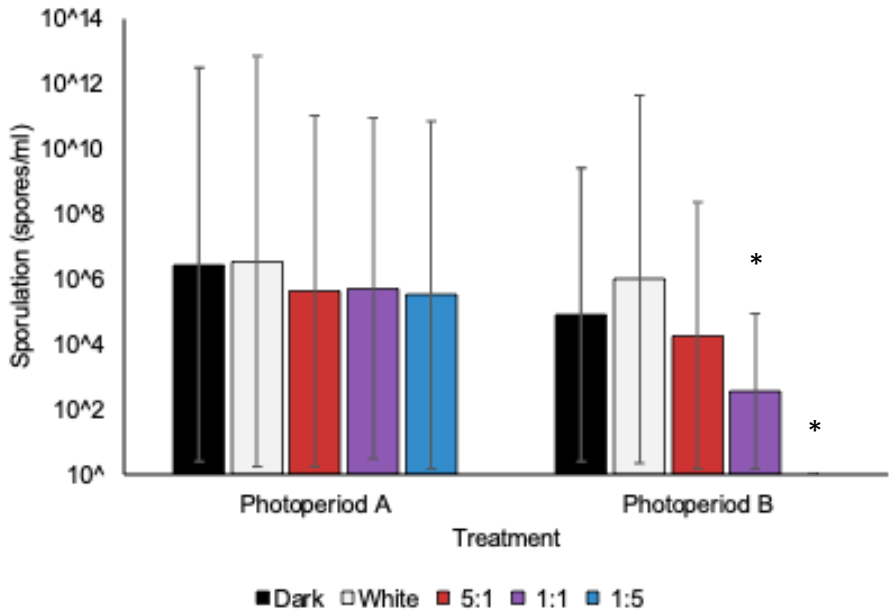

Figure 9. Bar graph comparing the effect of five lighting qualities on the mean sporulation (spores/ml) of *Botrytis cinerea* at two weeks *in vitro* within a CE exposed to two photoperiods with error bars demonstrating sample variability. (*) denotes statistically significant differences among Far-red:Blue light treatments (5:1, 1:1, 1:5) from controls (Dark and White) determined by a post-hoc Steel-Dwass comparison for all pairs, ($p \leq 0.05$). Photoperiod A: 24-h light treatment, Photoperiod B: 16-hour light treatment + 8 hours complete darkness. The sample size (n) for Photoperiod A and B was 40 and 45, respectively.

3.4.4 Influence of lighting quality on spore germination

The data was separated based on hourly intervals, and treatment means underwent assessment for homogeneity. Levene's tests revealed unequal variances ($p < 0.05$) and skewness exceeded an acceptable range of -1 to 1 (data not shown). Consequently, the Kruskal-Wallis test was employed to examine significance among groups ($p < 0.05$), followed by an all-pairs comparison utilizing the Steel-Dwass method for each hour. The outcomes did not indicate noteworthy differences among treatments across all exposure timings. Notably, at 9 hours, although a Kruskal-Wallace test displayed significance ($p = 0.0308$), the Steel-Dwass method did not identify significant distinctions between pairs (**Table *7***). Nonetheless, the Far-red:Blue ratio of 1:5 treatment demonstrated potential biological significance in inhibiting germination compared to controls at 9 hours after exposure, with mean percentage reductions of 56.3% and 57.1% for Dark and White respectively. Conversely, no significant treatment differences were evident at 11 hours (**Table *7***).

Table 7. Mean germination (%) of *Botrytis cinerea* over an 11-hour period exposed to different light treatments (Dark, White, and Far-red:Blue 5:1, 1:1, 1:5).

	Germination (%)			
Light Treatment	**5 hours**	**7 hours**	**9 hours**	**11 hours**
Dark	10.93	38.67	42.40	42.57
White	5.10	38.47	43.20	44.60
5:1	5.53	29.50	31.97	36.57
1:1	5.33	29.37	32.73	35.40
1:5	5.30	16.40	18.53	36.80
***p* value**				
Levene's Test	0.0402	0.3892	0.0214	0.0468
Kruskall Wallace	0.1246	0.0255	0.0308	0.1436

Significance was determined by the Kruskall-Wallis method followed by a post-hoc Steel-Dwass comparison for all pairs, ($p < 0.05$). Levene's test p values are included to display non-normal data. Time points included 0, 3, 5, 7, 9, 11 hours after exposure (0 and 3 hours, not shown here, showed < 1% germination for all treatments). ($n = 15$)

3.5. Discussion

Phenotypic observations of *B. cinerea* play a crucial role in understanding its proliferation and adaptability within CE settings. This study investigated the effects of Far-red:Blue LED lighting treatments on the phenotypic traits of *B. cinerea*, including its growth, sporulation, germination, and sclerotization abilities, aiming to assess the potential for cultural control using mixed narrow-bandwidth spectral compositions within a CE in an *in vitro* setting.

Regarding the spectral composition of the tested treatments and their effects on photomorphology, all Far-red:Blue treatments effectively inhibited radial hyphal growth and sporulation compared to the control treatments of White and Dark. Continuous exposure (24-hour light treatment) and a photoperiod of 16-hour light treatment + 8 hours complete darkness significantly reduced sporulation while inducing the formation of sterile areal hyphae, indicating disruptions in the photomorphogenesis of the isolate. Among the tested photoperiods, the 16-hour light treatment + 8 hours complete darkness was the most effective, aligning with practical plant cultivation lighting regimes. Interestingly, spore germination rates did not significantly differ across all treatments at 11 hours post-exposure, warranting further investigation into the relationship between lighting treatments and the pathogen's post-germination physiological development. Preliminary observations of germination and subsequent mycelial growth revealed increased internodal spacing between hyphal branches under a Far-red diode dominant treatment. However, technical restraints prevented the measurement and quantification of internodal spacing, suggesting the possibility of uncovering interesting results in the initial development of colonies generated from germinated spores, potentially revealing additional phenotypic variations.

Intraspecies phenotypic variation in *B. cinerea* is well-documented and influenced by factors such as temperature, culturing substrate, and lighting quality (Martinez et al., 2003; Meng, Mestdagh,

et al., 2020; Mirzaei et al., 2009; Notte et al., 2021). Interestingly, phenotypic observations of the studied isolate indicated its responsiveness to light, as evidenced by its ability to sclerotize. This suggests potential adaptability of the isolate. Overall, the results indicate that Far-red:Blue light treatments effectively inhibit the growth, proliferation, and dissemination of the isolate, while its ability to sclerotize suggests potential adaptive behavior under treatment conditions. Further validation should assess the impact of these treatments on *in-planta* infection incidence.

Previous studies by Meng, Mestdagh, et al. (2020) reported significant phenotypic variation among *B. cinerea* isolates exposed to Red and Blue LED light treatments. This contrasts earlier findings by Tan and Epton (1973) and Zhu et al. (2013), who reported the inhibitory effects of Red and Blue light on sporulation. Meng, Mestdagh, et al. (2020) found that the inhibitory effects varied among tested isolates, with some showing no inhibition. In line with the initial findings of Tan and Epton (1973) and Zhu et al. (2013), the wild-type isolate in this study exhibited sporulation inhibition under all treatments that incorporated Blue light. This suggests that a Far-red:Blue ratio of 5:1 was sufficient to inhibit sporulation, despite the previously reported promotion of sporulation by Far-red light. To gain a better understanding of the impact of Far-red:Blue lighting on isolates within a CE, future studies should incorporate a wider range of isolates from various environments to explore phenotypic variabilities, as observed by Meng, Mestdagh, et al. (2020). Notably, these results are the first to report the inhibitory effects of a mixture of Blue light in a Far-red dominant diode lamp on sporulation.

Canessa et al. (2013) reported that Red light (wavelength 540 nm) promoted a banding phenotype in the model B05.10 isolate. Disrupted banding growth patterns are likely due to disruption of the pathogen's internal biological clock (Baker et al., 2012; Hurley et al., 2015). In addition to significantly reducing sporulation, Far-red:Blue treatments were found to cause breaks in darker

bands, potentially disrupting the pathogen's circadian rhythm. Further characterization of transcription factors associated with the pathogen's biological clock, as seen in previous studies, would help validate these observations and elucidate the mechanisms underlying the disruptions in photomorphogenesis.

In this study, all LED treatments had a lower lighting intensity than the control treatment of White, suggesting a potentially greater role of lighting intensity in sporulation and growth than previously considered. Lighting intensity is reported here in the unit kLux, although reporting lighting intensity as photosynthetic photon flux density (PPFD) measured in µmol/s/m² is essential in future studies for consistency in the literature. Moreover, notable disparities were observed in mycelial growth and sporulation assays among trials, likely stemming from environmental fluctuations within the CE, especially in maximum daytime temperature means. Considering the variations in environmental conditions, every photoperiod involved a minimum of five trials, but only those which had daily temperature means ranging from 24 – 28 ºC were selected for statistical examination. All additional trials were not factored into analysis due to the homogeneity between treatment means (growth area < 20 cm^2 at Day 3 onward for all treatments). This was observed as induced stationarity in growth, likely due to excessive temperatures. These fluctuations had a significant impact on trial reproducibility. Given the potential influence of these factors on the phenotypic variation between isolates, future studies incorporating CEs should not only consider using a larger number of isolates but should also account for considerable fluctuations in factors such as lighting intensity and temperature during experimental design.

This study provides valuable insights into the effects of Far-red:Blue LED lighting treatments on the phenotypic traits of *B. cinerea*. The findings convincingly demonstrate the potential of these lighting treatments to effectively inhibit the growth, proliferation, and dissemination of the

pathogen. Notably, the results indicate that a Blue diode dominant lamp composition emerged as the most effective lighting treatment in inhibiting the growth and replication of the pathogen. These findings hold promise for the development of targeted lighting strategies in managing *B. cinerea* and highlight the importance of considering specific spectral compositions when exploring novel cultural control methods.

3.6. Conclusions

B. cinerea utilizes light to sense and interpret its ambient environment which enables it to adapt accordingly for survival (i.e., to infect and proliferate). Thus, light or its absence acts as a primary signaling mechanism regulating asexual development and dissemination. A Blue-dominant Far-red:Blue ratio of 1:5 proved to be the most effective treatment in inhibiting the hyphal growth and sporulation of *B. cinerea*. These findings have important implications for controlling the asexual proliferation of *B. cinerea* in CE plant production, primarily through the disruption of morphogenesis. To gain a deeper understanding of how *B. cinerea* can adapt to lighting quality and develop post-germination, future studies should include a variety of isolates isolated from CEs, similar to the wild-type isolate investigated in this study, while taking into account the various factors that can fluctuate within a CE.

Connecting Statement to Chapter 4

The following chapter focuses on examining the development of lesions on strawberry plants infected with a wild-type isolate of *B. cinerea* when exposed to three ratios of Far-red:Blue LED lighting (5:1, 1:1, 1:5) *in vitro* within a controlled environment. The total phenolic content of young strawberry plants (*Fragaria* × *ananassa* Duch.) is then examined in both infected and noninfected plant materials. This chapter serves to investigate the efficacy of the previously discussed lighting treatments to inhibit disease *in planta*.

Chapter 4: Exploring the effect of Far-red:Blue LED ratios on *Botrytis cinerea* Pers. infection in strawberry plants (*Fragaria* × *ananassa* Duch.) within a controlled environment

4.1. Abstract

The absolute morphological responses of the hemibiotrophic pathogen *B. cinerea* to lighting conditions have long been recognized. Light plays a crucial role in the induction of asexual replication (conidiation) and hyphal growth, which are fundamental processes for the proliferation and dissemination of infectious propagules. Additionally, studies have reported the ability of light-emitting diodes (LEDs) to promote plant defense responses and the accumulation of phytochemicals. Therefore, the incorporation of LED lights can influence the development and possibly the infection process of the pathogen in controlled environments (CEs). This study investigates the correlation between lighting treatments using Far-red:Blue LED lighting ratios of 5:1, 1:1, and 1:1 on lesion development caused by *B. cinerea* on detached strawberry leaves (*Fragaria* × *ananassa* Duch. var. Seascape), as well as plant immune response through *in planta* infections. Comparing diseased leaf area and making qualitative observations of lesion development and surrounding tissues provided valuable insights. At 7 days post-inoculation (DPI), leaf samples exposed to the Dark control (complete darkness) exhibited advanced stages of disease, with considerable variation in lesion size despite optimal conditions for disease progression. In contrast, samples under the respective LED treatments showed limited lesion formation, with the 1:1 and 1:5 ratios significantly inhibiting lesions compared to the White control (high-pressure sodium lamps (HPSLs) + ambient light), as well as demonstrating lower sample variability. These findings support the crucial role of light exposure in mitigating disease progression, emphasizing a variation in progression dependent on spectral composition. Further

observations were made regarding the tissues surrounding the lesions under different lighting treatments. Interestingly, leaf samples treated with White and Far-red:Blue ratios of 5:1 and 1:5 exhibited chlorosis around the lesion margins, which was absent in the Dark treatment and the Far-red:Blue 1:1 treatment. The absence of chlorosis in the latter treatment suggests either an induced plant defense response or physiological inhibition of the pathogen, limiting its impact on the host and potential subsequent defense response. The lack of chlorotic tissue response in the Dark treatment, as well as in the 1:1 treatment, indicates a possible downregulation of the light-induced defense response via 1:1 treatment, thereby influencing lesion development. However, further investigation is required to fully elucidate the underlying mechanisms. In examining plant defense response, the 1:5 treatment was found to decrease total phenolic content (TPC), while the 5:1 treatment increased TPC in non-infected plants compared to a control of White. Interestingly, the Far-red:Blue 1:5 treatment increased TPC in infected plants, suggesting a possible enhancement of plant defense response. In contrast, the Far-red:Blue 1:1 treatment showed no significant changes in TPC between infected and non-infected plants. This study provides insights into the effects of lighting treatments on lesion development and plant defense responses of strawberry plants against *B. cinerea*. The findings emphasize the role of light in disease mitigation and defense modulation, contributing to the development of novel cultural control strategies in CE production settings by utilizing specific lighting compositions.

4.2. Introduction

The hemibiotrophic fungal pathogen *B. cinerea* is frequently encountered in CE production settings, primarily due to the ubiquity of its asexually produced propagules, or spores. Interestingly, the pathogen has long been acknowledged for its distinct morphological responses to varying lighting conditions, initially documented by several studies (Bewley, 1923; Brierley, 1921; Godfrey, 1923; Klein, 1885; Peltier, 1912; Robinson, 1914). Light plays a pivotal role in regulating the induction of asexual replication, known as conidiation, therefore impacting the dissemination of infectious propagules and disease incidence. Previous research indicates that near-UV and Far-red wavelengths facilitate or promote conidiation (Honda and Yunoki, 1978; Peterson et al., 1988; Tan, 1974b; Tan and Epton, 1974a, 1974b), whereas Blue and Red light wavelengths inhibit this process (Tan, 1974a; Tan and Epton, 1974a; Zhu et al., 2013) producing sterile, arial hyphae absent of conidia. These findings highlight the potential influence of LED lights on the development and infection process of the pathogen within CEs and provide context for studies that seek to validate the efficacy of spectral composition as a method of cultural control.

When studying the impact of spectral composition on the infection process and host defense, assessing the accumulation of secondary metabolites can serve as an initial key indicator for efficacy. Phenolic compounds represent a diverse array of secondary metabolites that encompass various subclasses, such as flavonoids, phenolic acids, and tannins, and are considered the most expansive group of secondary metabolites in the plant kingdom (Boudet, 2007). Anthocyanins, a flavonoid derivative, are responsible for the vivid red, purple, and blue pigmentation observed in numerous fruits, flowers, and leaves. Notably, anthocyanins possess antioxidative properties and thereby are critical in protecting plants against (a)biotic stressors. Certain phenolic compounds are constitutively expressed, including anthocyanins in flower and fruit tissues, whereas other phenolic

metabolites are induced during a biotic stress repones pathway or during specific development stages (Warner et al., 2021). Specifically, upon exposure to stress or pathogen invasion, phenolics tend to accumulate in the subepidermal layers of plant tissues (Schmitz-Hoerner and Weissenböck, 2003). The synthesis and accumulation of phenolics in response to pathogen attacks can directly influence a plant's ability to defend against subsequent infections (Kefeli et al., 2003). Numerous studies have demonstrated the capacity of LED lighting spectra to enhance the production of phenolic compounds in strawberry plants (*Fragaria* × *ananassa* Duch.) grown within CEs, as provided in **Table 8**. Compared to control conditions utilizing full spectrum White light (via HPSLs), both Blue and Far-red light have been reported to promote anthocyanin biosynthesis in both fruits and leaves. However, research by Zhang et al. (2018) reveals there are significant variations in the accumulation of phenolics that can be seen within the varietal level.

Table 8. Overview of strawberry plant defense responses (including phytochemical production, and light regulated transcription/translation) under various lighting spectra in controlled environment plant production settings.

Light Spectrum	Wavelength (nm)	Species & Variety	Plant Response[z]	Reference
BLUE (280 – 500 nm)	448	*Fragaria* x *ananassa* Duch. var. 'Daewang'	↑ anthocyanin (fruit)	Choi et al. (2015)
	450	*Fragaria* x *ananassa* Duch. var. 'Toyonaka'	↑ anthocyanin/proanthocyanidin (fruit)	Zhang et al. (2018)
	450	*Fragaria* x *ananassa* Duch. var. 'Tokun'	🕒 anthocyanin biosynthesis and accumulation	Zhang et al. (2018)
	465	*Fragaria* x *ananassa* Duch. var. 'Sachinoka'	↑ anthocyanin	Kadomura-Ishikawa et al. (2013)
FAR-RED (730 – 750 nm)	730	*Fragaria* x *ananassa* Duch. var. 'Toyonaka'	↑ anthocyanin/proanthocyanidin (fruit)	Zhang et al. (2018)
	730	*Fragaria* x *ananassa* Duch. var. 'Tokun'	🕒 anthocyanin biosynthesis and accumulation	Zhang et al. (2018)
PURPLE (Far-red + Blue Light)	450 + 730 (Ratio 1:1)	*Fragaria* x *ananassa* Duch. var. 'Toyonaka'	↑ proanthocyanidin (fruit)	Zhang et al. (2018)
	450 + 730 (Ratio 1:1)	*Fragaria* x *ananassa* Duch. var. 'Tokun'	↑ anthocyanin/proanthocyanidin (fruit) 🕒 anthocyanin biosynthesis and accumulation	Zhang et al. (2018)
	448 + 661 (Ratio 3:7)	*Fragaria* x *ananassa* Duch. var. 'Daewang'	↑ anthocyanin production	Choi et al. (2015)

[z] ↑ increase, 🕒 early/premature.

Further studies of *in planta* infection conducted by Meng, Van Labeke, et al. (2020) demonstrate that Blue LED light increases the susceptibility of detached strawberry leaves to *B. cinerea* infection, correlating with a decrease in TPC, while Red LED light has the opposite effect. These findings align with the work of Jersch et al. (1989), who suggested that proanthocyanidins serve as the foundation for inducing quiescent (or non-symptomatic) infections in immature strawberry fruits. Collectively, these results imply that the ability of spectral composition to enhance phenolic compound levels may underpin a reduction in susceptibility to *B. cinerea*.

The impact of spectral composition on the morphogenesis of *B. cinerea*, along with the reported effects of LED lights on the synthesis and accumulation of phenolic compounds, which play a pivotal role in plant defense responses, suggests that manipulating spectral composition through LED lights holds promise as a novel cultural control method within CEs. Considering the influence of mixed narrow-bandwidth spectral compositions, particularly Far-red:Blue lighting, on phenolic compound production as part of the plant defense response, it becomes imperative to explore the effects of dominant Far-red or Blue lighting sources on the infection process of *B. cinerea* and to establish the effectiveness of spectral composition as a cultural control technique.

4.3. Materials and Methods

4.3.1 Plant material & lighting configuration

All experimental plant material was collected from mature strawberry plants (*Fragaria* × *ananassa* 'Seascape') grown within a CE. Initially, refrigerated bare-root plants (Production Lareault Inc., CA) were potted directly into 6-inch pots using a saturated Agro Mix® G6 media (Fafard, CA) and grown under a 16-hour photoperiod with a daily average temperature of 25 ºC ± 10. Supplemental lighting was provided with HPSLs at 100μmol $m^{-2}s^{-1}$. Plants were fertigated twice daily for 15 minutes using an automated drip irrigation system equipped with a D14MZ10 fertilizer injector (Dosatron®, USA), delivering a total of 247.1 ml of water and 123.5 mg of 20-20-20 N-P-K fertilizer per plant at each irrigation event.

Three lamp prototypes (U Technology Corporation, CA) were used for LED treatments. Lamps were installed 1.5 m above a greenhouse bench and separated with a double layer of landscaping fabric for isolation. The upper portion of the suspension structure was left uncovered to allow for heat dissipation and the ingress of ambient light. Each lamp consisted of two combined spectral peak emissions of Far-red (725 nm) and Blue (455nm) wavelengths represented as different ratios of Far-red:Blue light (5:1, 1:1, 1:5) based on the number of diodes in the array (i.e., 1,729 diodes per array, divided into the respective ratios of Far-red:Blue diodes). Experimental control treatments within the CE consisted of Dark (samples covered with aluminum foil) and White (HPSLs + ambient light).

Both the HPSLs and LED lamps were automated with timers to replicate a photoperiod consisting of 16-hour light treatment + 8 hours complete darkness (**Figure 10**). Spectral output and light intensity were measured with a spectroradiometer (Apogee Instruments Inc., model PS-300, USA) and a light meter (Digi-Sense, model 00653-22, CA), respectively, and recorded accordingly as:

182 ± 40 kLux (5:1), 230 ± 40 kLux (1:1), and 325.6 ± 40 kLux (1:5). A control treatment of White within the CE was recorded at an intensity of 418.8 ± 80 kLux. Through the course of the experiment, data was collected periodically and is reported here as mean (±) standard deviation of intensity (kLux).

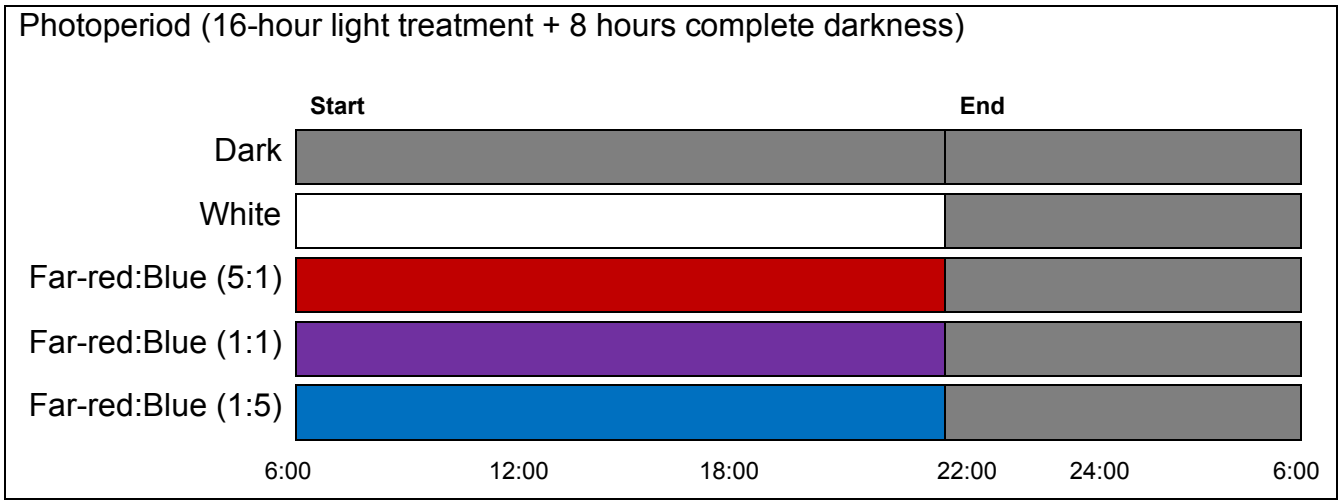

Figure 10. Experimental lighting treatments used in this study are shown according to day-length. In all experiments, samples were placed under the respective lighting treatments beginning at 12:00 hours. As the natural photoperiod fluctuated slightly over the course of these experiments, lamps were adjusted as needed to complement a 16-hour light/8-hour dark cycle.

4.3.2 Isolate, storage, and inoculation techniques

The wild-type strain of *Botrytis cinerea* used in all subsequent experiments was initially isolated from naturally infected flower buds of cannabis plants (*Cannabis sativa* L.) grown in CE production facilities in British Columbia, Canada, and identified as B05.10 strain (> 99% identical) (Punja, 2018).

To test for the pathogenicity of the isolate on strawberry, 5 mm mycelial plugs were taken and used to inoculate surface sterilized strawberries placed in Fisherbrand™ DuoClick specimen containers (Thermo Fisher Scientific, CA). Inoculated fruits were then placed in an incubator at 22 ºC in complete darkness and allowed to develop visible symptoms of the disease. Infected plant tissue was then dissected and cultured on Nutriselect® Sabouraud Agar (SBA) (Sigma Aldrich, CA) containing 0.50 mg/l chloramphenicol (Sigma Aldrich) as described by Hare (2013) for re-isolation. Visual identification methods using a compound microscope were used to validate the reisolated specimen as *B. cinerea* according to morphological characterizations described by Jarvis (1977) by examining both mycelial fragments and the structure of conidiophores.

Mycelia plugs taken from subsequent SBA plates were then re-cultured on Nutriselect® potato dextrose agar (PDA) (Sigma Aldrich) plates. Colonies were incubated for one week, cut into strips, and placed into Eisco™ glass test tubes with screw caps (Thermo Fisher Scientific). Samples were then lyophilized with a Heto PowerDry freeze dryer (Thermo Fisher Scientifc) over four days to allow for long-term and shelf-stable culture preservation. To generate additional colonies on PDA, the process involved rehydrating 1 cm cuttings obtained from lyophilized strips in sterile distilled water (dH2O) for 30 seconds and subsequently placing them onto PDA plates. For both leaf and live plant infection assays, 5 mm mycelial plugs were taken from the edge of one-week-old re-hydrated colonies and placed directly onto plant material.

4.3.3 Leaf disease progression assays

Strawberry leaves taken from mature plants (see **Section 4.3.1**) were harvested and placed in water at room temperature to prevent premature senescence. For surface sterilization, Dawn soap (Procter & Gamble, USA) containing surfactant compounds such as sodium lauryl sulfate and cocamidopropyl betaine was used to remove any pests or debris, taking care to keep trifoliate structures intact. In a sterile environment, the leaves were immersed in a 7.5% bleach solution for 30 seconds, followed by rinsing with sterilized dH2O. This process was repeated twice. Afterwards, leaflets were separated keeping as much of the petiole intact as possible. Leaflets were then placed adaxial side up on sterile foil and left to air dry for 15 – 30 minutes. Dry leaflets were then fixed onto water-agar plates, with the petiole inserted into the media for stability as adapted from Lian et al. (2018). Leaflet margins were then gently pressed into the media to stabilize the sample. For inoculation, two 5 mm mycelial plugs were taken from the edge of a one-week-old colony and placed upside down on opposite sides of the midrib. Samples were incubated for 24 hours at 22 ºC in complete darkness to induce infection, after which samples were then placed within the CE under the respective lighting treatments. Temperature was monitored with the use of a Traceable® Logger-Trac™ (VWR, CA) and recorded as daily means ± standard deviation. Throughout the course of a 7-day trial, daily temperature means were recorded. If the temperature was recorded to be outside of the 24-28 ºC range with a standard deviation < 10 ºC, the trial was omitted from data analysis.

Over the course of 7 days, samples were removed and photographed daily inside of a biological safety cabinet, then replaced under their respective lighting treatments to monitor the progression of lesions. Images were batch processed using the ImageJ Fiji plugin (National Institute of Health, USA) to calculate the lesion area of each sample, recorded as percentage of diseased tissue

(Schindelin et al., 2012). Trials were performed as a completely randomized design (CRD) with 5 treatments, 5 biological replicates, and 3 temporal replicates.

4.3.4 Generation of plant material for *in planta* assays

Mature strawberry plants were monitored daily for stolon initiation and runner development. When stolon tips were observed to produce true leaves, they were marked and dated. Marked runners were harvested after approximately 1 week, having produced 2-3 petioles on average. Runners were then placed into magenta boxes filled with saturated Agro Mix® G6 media (Fafard, CA) (200 ml dH_2O/40 g media) (v/w) with an average soil moisture content recorded as 85% ± 5%, calculated using an oven-drying technique. An additional 10 ml of water was then added to the magenta box to retain a high relative humidity to promote rooting. Magenta boxes were placed within the CE for two weeks to allow for further plant development. After one-week, magenta boxes were opened and 1 ml of a 20-20-20 N-P-K nutrient solution (0.05 g/ml) (w/v) was pipetted into the root zone to encourage further root development. After an additional week, plants with runners that showed no discoloration in leaves, and extensively developed roots (flush with the bottom of the magenta box) were chosen to evaluate the effect of lighting treatment on TPC.

An assay using non-infected plant material was conducted first. Non-infected plants were placed under the respective lighting treatments of White and Far-red:Blue ratios of 5:1, 1:1, 1:5 at the start of the photoperiod. Samples were collected at the time of placement as well as 6 hours post-lighting exposure. This assay was performed as a completely randomized design (CRD) with 5 treatments, and 4 biological replicates.

Assays using infected plant material were then conducted to determine plant response under the respective lighting treatments 3 DPI. Infected plants were inoculated by placing a 5 mm mycelial plug on the apical side of the crown. Plants were then placed under the same lighting treatments

as described previously. For a negative control, an equal sample size of non-infected plants were also placed under each treatment. This assay was performed as a completely randomized design with 4 light treatments and 3 biological replicates for both infected and non-infected plants.

4.3.5 Total phenolic content determination

To determine the TPC of plant samples, a colorimetric method adapted from Kupina et al. (2019) was used. Initially plant tissue samples were harvested, placed in 2 ml Eppendorf tubes, and flash frozen using liquid nitrogen. Frozen samples were then ground using 5 mm metallic beads with a Spex Geno Grinder tissue homogenizer (SpexSamplePrep, USA) and 100 mg were weighed, taking care to record final weights to the nearest 0.1 mg. Samples was then suspended in 1.8 ml of methanol and sonicated in an ice water bath for 20 minutes. The final sample weight and adjusted final solution volume where then used to calculate individual sample extract concentrations (mg/ml). Samples were then spun in a microcentrifuge (Eppendorf, USA) at 1200 rpm for 5 minutes and the supernatant was collected for further analysis. The sample supernatant, with a volume of 200 ml, was then diluted 5 times with dH2O to achieve a final volume of 1 ml. A standard curve was prepared using a gallic acid stock solution of 1000 mg/l. The concentrations used for calibration were 0, 40, 80, 120, 160, 200 mg/l of gallic acid.

To prepare the colorimetric reaction, 25 ml Eppendorf tubes were filled with 15 ml dH_2O, 1 ml Folin & Ciocalteu's phenol reagent (Sigma Aldrich) and one of the following: (*1*) 1.00 ml diluted sample test solution; (*2*) 1.00 ml calibration standard solution 1-5; or (*3*) 1.00 ml dH_2O (blank). The contents of each tube were vortexed and allowed to sit for 6 minutes, after which 3 ml of 20% sodium carbonate was added to each tube and vortexed. Tubes were then allowed to rest in an incubator at 30 ºC for 120 minutes in complete darkness. In triplicates, 200 µl of each solution was pipetted into a microplate and analyzed with a FLUOstar Omega spectrophotometer (BMG Lab

Tech, DE) at 765 nm. Recorded values of sample test solutions were adjusted using the calibration curve with the mean of these adjusted triplicate values being used for data analysis. Calculations used to determine the total phenols (% w/w, fresh weight) in gallic acid equivalents (GAE) were adapted from Kupina et al. (2019) and are reported here on a fresh weight basis. Final concentrations were adjusted according to individual sample extract concentrations (mg/ml) to equalize for the variances in initial concentrations.

4.3.6 Statistical analysis

The data were analyzed using Statistical Analysis Software (SAS) 9.4 version (Analytics Software and Solutions, USA). Normality of group means was checked using a Shapiro-Wilk's test and homogeneity of variances was compared using a Levene's test for all experiments. Leaf disease progression assays were analyzed using a two-way analysis of variance (ANOVA) to determine any significant interaction between trial and treatment, with the data separated by day. Trial means were assessed for normality and homogeneity and found to be valid. However, the treatment means were not normally distributed or homogenous. Further exploration of the data was done using a square root transformation, but unequal group variances were still observed within treatment groups. Therefore, a non-parametric Kruskal-Wallis test was performed to rank the treatment means, primarily due to variance between treatment effects rather than trial effects. To analyze the TPC of samples in both *in planta* assays, a one-way ANOVA test was conducted to determine any significant differences between treatments in assays without infection, whereas a two-way ANOVA was conducted for significance in assays that incorporated infection. The treatment means in both trials were validated as normally distributed and homogenous. A significance level of $p < 0.05$ was considered to be significant for all statistical tests.

4.4. Results

4.4.1 Influence of lighting quality on disease progression

Over a period of 168 hours, samples exposed to complete darkness exhibited characteristics of advanced disease development. This was observed as the formation of blackened, necrotic tissue, typically reaching the leaf margin, which produced a brown exudate, characteristic of necrotrophic fungal infections. Conversely, leaf samples exposed to all lighting treatments exhibited lighter brown lesions with a reduction in exudate. Interestingly, light treatments of White and Far-red:Blue ratios of 5:1 and 1:5 displayed yellowing around the margin of lesions, which was absent in treatments of Dark and Far-red:Blue 1:1 (**Figure 11**). Throughout all trials, leaf samples remained in a state of prolonged senescence over 168 hours without showing any signs of wilting due to environmental stressors, regardless of the treatment they received. Therefore, the observed lesion development and coloration can be primarily attributed to infection.

Figure 11. Effect of five light qualities (Dark, White, and Far-red:Blue 5:1, 1:1, 1:5) on *Botrytis cinerea* lesions on detached strawberry leaves within a controlled environment. Samples shown were photographed at 48- and 168-hours post-inoculation (HPI).

In all conducted trials, none of the respective lighting treatments resulted in disease development beyond 10% of the total leaf area until 144 HPI (**Figure 12**). However, starting in as little as 96 HPI, significant differences in the percentage of diseased leaf area were observed between leaves exposed to lighting treatments, irrespective of treatment, and leaves in complete darkness ($p < 0.05$). As the lesion area increased logarithmically among samples in complete darkness, a significant variability was observed between samples. At 168 HPI, the range of diseased leaf area reached as high as 64%. However, when leaves were exposed to lighting treatments, the variability in lesion area was comparatively lower. Specifically, the Far-red:Blue 5:1 treatment exhibited a maximum range of 20% diseased leaf area at 168 HPI, while all other light treatments resulted in less variability.

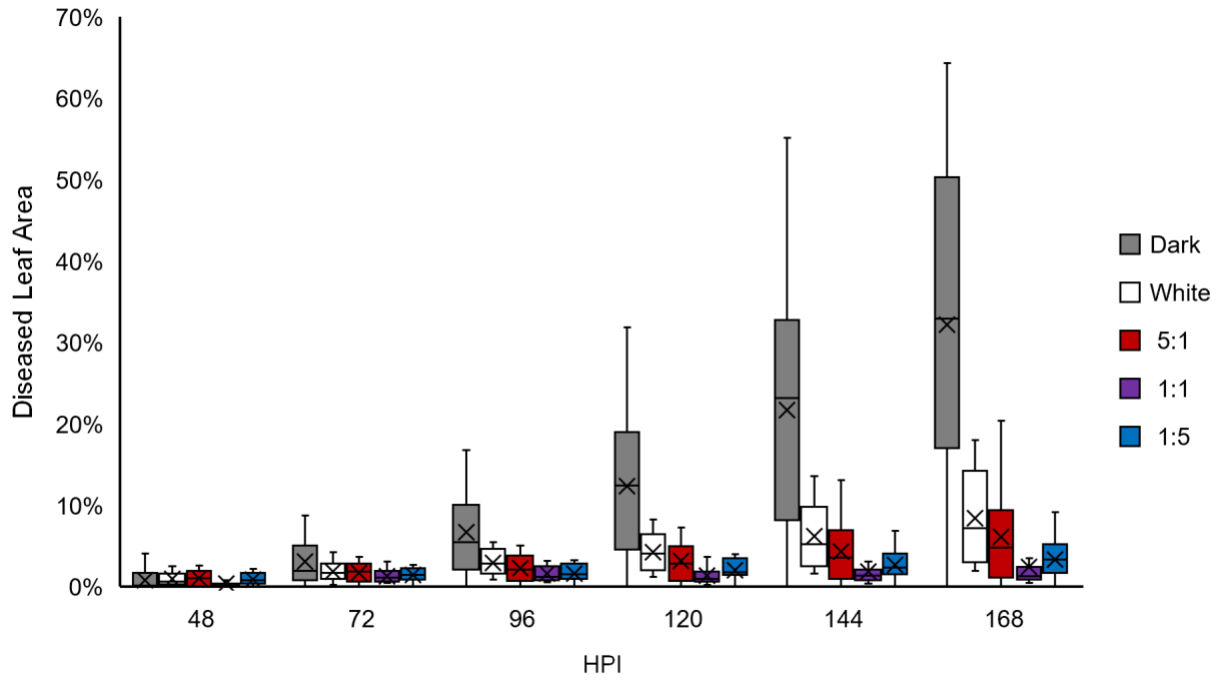

Figure 12. Effect of five light qualities (Dark, White, and Far-red:Blue 5:1, 1:1, 1:5) on *Botrytis cinerea* lesion development (shown as percentage of diseased leaf area) on detached strawberry leaves over a 7-day period under respective light treatments with a 16-hour photoperiod. The sample size (*n*) was 15. Significance was determined using a non-parametric Tukey-Kramer test to adjust for multiple comparisons using least squares means. Hours post-infection is indicated as HPI.

When assessing for normality within treatment groups, only the 1:1 Far-red:Blue treatment was found to be non-normal ($p < 0.001$). Although, as the data set was observed to be non-homogenous due to the differences in variability between the Dark treatment and all other lighting treatments ($p < 0.001$), the Dark treatment was then excluded for further analysis (**Table 9**). Consequently, the assumption of homogeneity between group means was met ($p > 0.05$), but normality was still violated for the Far-red:Blue 1:1 treatment. Therefore, a Kruskal-Wallis test was used to rank means to determine significant differences. This showed that the 1:1 and 1:5 Far-red:Blue treatments were the most effective treatments in inhibiting the spread of lesions compared to the White control group with respective means of 1.27% and 3.20% diseased leaf area.

Table 9. *Botrytis cinerea* lesion development on detached strawberry leaves over a 7-day period under respective light treatments with a 16-hour photoperiod exposed to different light treatments (Dark, White, and Far-red:Blue 5:1, 1:1, 1:5).

Mean Lesion Area (cm^2)			
Source	***p* values**		
Treatment	<.0001		
Trial	0.5668		
Treatment*Trial (7 DPI)	<.0001		
Treatment (7 DPI)		**Shapiro-Wilk's Test**	**Levene's Test**
Dark	32.07^{a}	0.6153	<0.001
White	8.47^{b}	0.0798	
5:1	6.00^{b}	0.0903	
1:1	1.27^{c}	0.0183	
1:5	3.20^{b}	0.3070	
***p* value**	0.0350		
Treatment (7 DPI)		**Shapiro-Wilk's Test**	**Levene's Test**
White	8.47^{a}	0.0798	0.0875
5:1	6.00^{ab}	0.0903	
1:1	1.27^{c}	0.0183	
1:5	3.20^{b}	0.3070	
***p* value**	0.0022		

The results of a two-way ANOVA are shown, with isolation of 7 days post-infection (DPI) with and without Dark treatment. Shapiro-Wilk's scores as well as Levene's scores are given to indicate invalid assumptions of normality and homogeneity. Therefore, significance was determined using a non-parametric Tukey-Kramer test to adjust for multiple comparisons using least squares means. Different lower-case letters indicate statistically significant differences among treatments ($p < 0.05$). Sample size (n) was 15.

4.4.2 Influence of lighting quality on total phenolic content

The TPC assays of uninfected, rooted strawberry plantlets revealed no significant difference between the treatment group sampled at the beginning of the photoperiod and those sampled 6 hours after the start of the photoperiod, as illustrated in **Figure 13**. However, treatment groups of Far-red:Blue 5:1 and 1:5 were shown to be significantly different from each other ($p < 0.05$). Furthermore, the Far-red:Blue 5:1 treatment exhibited the highest TPC among all treatments with a recorded mean of 1.23 ± 0.22 GAE mg/g (% w/w, fresh), whereas a treatment of 1:5 had lowest observed mean of 0.87 ± 0.09 GAE mg/g (% w/w, fresh). Interestingly, the Far-red:Blue 1:5 and 1:1 treatments were observed to have less variability between samples when compared to other groups, with standard deviations of 0.09 and 0.08 GAE mg/g (% w/w, fresh), respectively.

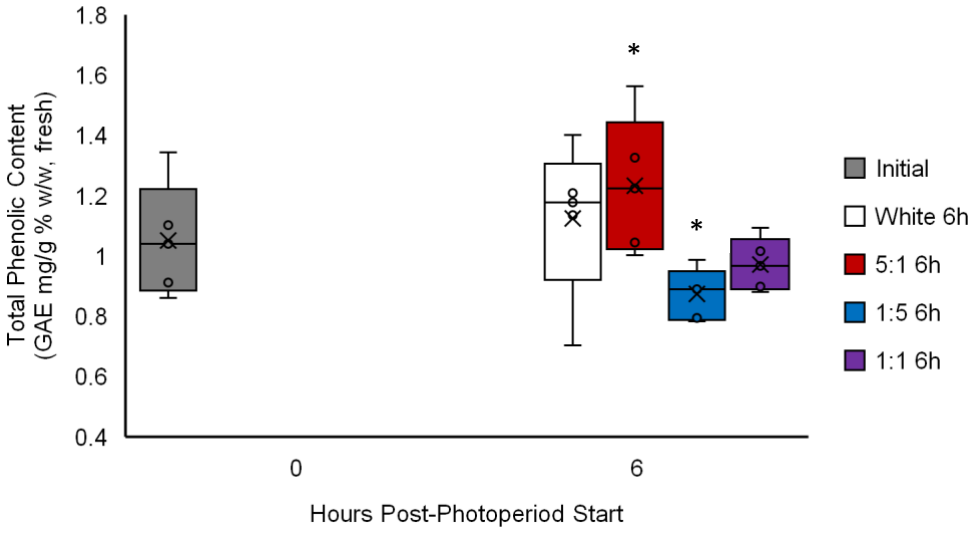

Figure 13. Effect of four lighting qualities (White, and Far-red:Blue 5:1, 1:1, 1:5) on the total phenolic content (TPC) of strawberry. Tissue samples were taken at the initial time point of 0 hours and 6 hours (6h) post-photoperiod start. The sample size (*n*) was 4. Significance was determined using a non-parametric Tukey-Kramer test to adjust for multiple comparisons using least squares means. (*) denotes significant differences ($p < 0.05$)

The TPC of rooted strawberry plants with and without infection was determined 3 DPI (**Figure 14**). Initially, a significant difference was observed only between treatments of Far-red:Blue 5:1 and 1:1 within the control treatment group ($p > 0.05$). Additionally, the recorded Far-red:Blue 1:5 treatment mean of 0.7 ± 0.15 GAE mg/g (% w/w, fresh) was observed to be lowest of all treatments. These results further validated observations made in the previous trial of the TPC of uninfected plants. The fact that the Far-red:Blue 1:5 control group showed the lowest treatment mean but displayed a significant response to infection compared to controls suggests a potential plant defense response in relation to the lighting treatment. Interestingly, no significant difference was found in all other lighting treatment groups between infected and non-infected plants.

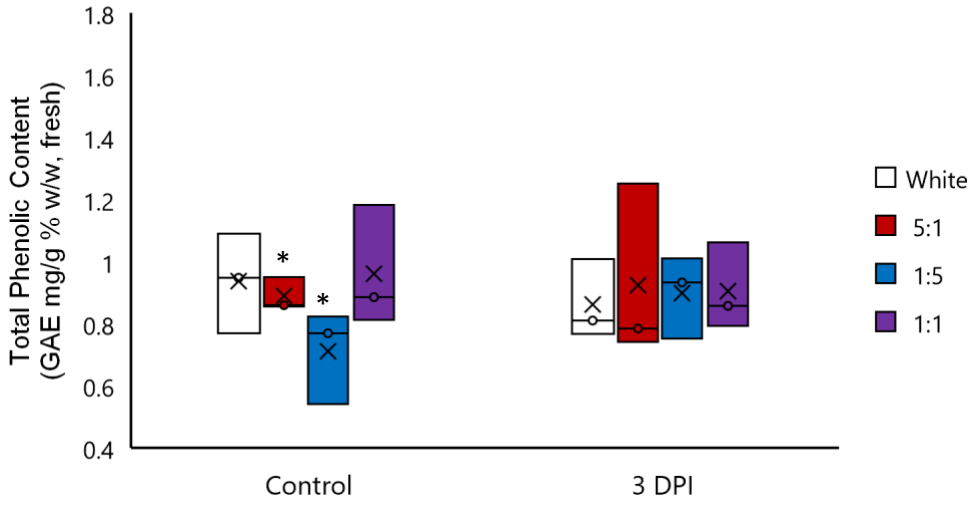

Figure 14. Effect of four lighting qualities (White, and Far-red:Blue 5:1, 1:1, 1:5) on the total phenolic content (TPC) of strawberry plants infected and not infected with *Botrytis cinerea*. Tissue samples were taken 3 days post-infection (DPI) at 6 hours post-photoperiod start. The sample size (*n*) was 3. Significance was determined using a non-parametric Tukey-Kramer test to adjust for multiple comparisons using least squares means. (*) denotes significant differences ($p < 0.05$)

4.5. Discussion

This first objective of this study focused on investigating the correlation between lighting treatment and the advancement of lesion development caused by *B. cinerea* on detached strawberry leaves. Valuable insights were gained through a comparison of diseased leaf area and making qualitative observations from the developed lesions and surrounding tissues. As anticipated, leaf samples exposed to complete darkness were indicative of advanced stages of disease development. In contrast, samples exposed to all respective lighting treatments displayed lesions limited in formation. These findings support the understanding that light exposure plays a crucial role in mitigating disease progression and influencing a plant's defense response.

Further observations were made when comparing the tissues surrounding the lesions under the respective lighting treatments. Leaf samples treated with White and Far-red:Blue ratios of 5:1 and 1:5 exhibited signs of chlorosis around the lesion margins, which was absent in the Dark treatment and the 1:1 treatment. Given the observed effectiveness of the 1:1 treatment on lesion reduction, it is hypothesized this absence of coloration could either indicate an induced plant defense response or possible physiological inhibition of the pathogen, thereby limiting its impact on the host and any subsequent host induced response (Pavicic et al., 2021; Williamson et al., 2007). Further investigation is required to elucidate the underlying mechanisms behind these observations. Moreover, additional studies are necessary to explore the causation of chlorosis observed on leaves treated with White and ratios of 5:1 and 1:5, as well as the absence of this phenomenon under complete darkness. Understanding the factors driving this differential response will provide valuable insight into the interplay between lighting treatment and a plant's defense mechanisms.

When examining the results of the 1:1 treatment at 168 HPI, a significant reduction in diseased leaf area was observed when compared to the controls of White or complete darkness, with a

recorded mean of 1.27% diseased leaf area. Interestingly, at 168 HPI this treatment also exhibited the lowest recorded sample range of 1%, demonstrating the ability of this treatment to significantly reduce the variability between treated samples as compared to all other treatments, further underscoring its effectiveness. Conversely, when examining the distribution of samples at 168 HPI exposed to complete darkness an unexpected pattern was observed. Despite conditions favoring disease development, approximately 20% of the observed lesions in the complete dark treatment remained below 10% of the total leaf area. This revealed considerable variation in the pathogen's virulence on strawberry leaves, considering that these were ideal environmental conditions for disease progression with some lesions exceeding 60% of the total leaf area.

It has been reported that low Red:Far-red ratios induce shade avoidance responses and downregulate plant defense hormones like jasmonic acid, while high Red:Far-red ratios promote plant defense (Pierik and Ballaré, 2021). In support of this, Xu, Fu, et al. (2017) found that Red LED light (650-660 nm) significantly inhibited lesion formation. Similarly, Meng, Van Labeke, et al. (2020) reported that exposing infected strawberry leaves to Red LED light was found to reduce development. Additionally, Courbier et al. (2021) demonstrated that supplemental Far-red lighting increased tomato leaf susceptibility to *B. cinerea*, attributing this response to a growth-immunity trade-off. In this study, a Far-red:Blue ratio of 1:5, with dominant Blue light, significantly inhibited disease development compared to the control group. This contrasted with previous reports that Blue LED light was ineffective at inhibiting disease development (Meng, Van Labeke, et al., 2020; Xu, Fu, et al., 2017). It was also found that a Far-red:Blue ratio of 5:1 promoted disease development, supporting the suggestion of a defense-growth trade-off reported by Courbier et al. (2021). Moreover, these findings suggest that incorporating Far-red light in a Far-red:Blue ratio of

1:5 ratio effectively counteracted the reduced leaf resistance which was previously associated with Blue LED light.

The latter part of this study aimed to investigate the defense response of infected and non-infected strawberry plants under the respective lighting treatments. Previous research has indicated that plants undergo diurnal variations in their synthesis of metabolic compounds, driven by their circadian clock and associated machinery, allowing them to anticipate environmental or conditional changes (Venkat and Muneer, 2022). In a previous study, Meng, Van Labeke, et al. (2020) reported that non-infected plants exhibited fluctuations in TPC throughout the photoperiod regardless of lighting treatment, yet plant TPC significantly differed when exposed to Blue or Red LED lighting treatments, with Blue LED light decreasing the overall TPC throughout the entire photoperiod and Red LED light increasing TPC.

This study revealed that, at 6 hours after the start of the photoperiod, both 5:1 and 1:5 treatments had contrasting effects on the TPC of non-infected strawberry plants. Specifically, the 5:1 treatment resulted in a significantly higher TPC compared to the 1:5 treatment. However, neither group showed a significant difference from the TPC of plants recorded at the beginning of the photoperiod. These results further support the findings of Meng, Van Labeke, et al. (2020), who suggested that Blue LED light decreases the production of phenolic compounds in strawberry plants compared to a control treatment of White. It is important to note that although these observations were further validated in the control group of the infected plant assay, which was sampled three days after exposure, the results should be considered as preliminary findings.

In the infected plant assay, no significant differences were observed between infected plant and non-infected plants within the control group of White. However, when comparing the uninfected plants exposed to two different treatments, namely Far-red:Blue light at a ratio of 1:5 and White,

it was observed that the TPC decreased in the 1:5 treatment group. On the other hand, the TPC increased in the 1:5 treatment group of infected plants. These findings suggest that the 1:5 treatment might have stimulated a stronger defense response in the plants, particularly in the infected ones. Furthermore, when examining the Far-red:Blue 1:1 treatment in both the non-infected and infected groupings, no significant changes were observed, which was similar to the control group of White. This could be indicative that a ratio of 1:1 is sufficient in regulating a plants production of phenolic compounds similar to that of White. Although, given that a limiting variable of this study is the amount of time points in which samples were collected at, and the previously reported diurnal fluctuations in plant TPC, it is necessary to expand sampling timepoints in future studies to adequately assess a plant response to lighting treatment coupled and uncoupled with infection.

4.6. Conclusion

In conclusion, this study provides valuable insights into the relationship between lighting treatment and the progression of *B. cinerea* infection on strawberry plant tissue and subsequent host response. The presented findings highlight the significant role that lighting treatments have in mitigating disease progression and influencing plant defense responses. A Far-red:Blue ratio of 1:1 was the most effective at inhibiting the spread of lesions, while a ratio of 1:5 showed promising results that indicated the induction of a plants defense response. Furthermore, these results complement those of previous studies, regarding the addition of Far-red LED light to Blue LED light to counteract the previously reported effects of a Blue LED light treatment, underscoring the complexity of pathogen-host interactions under supplemental lighting conditions.

Chapter 5: General Discussion

In northern regions of North America, CE strawberry production is being expanded to meet the year-round demand of consumers. This is particularly seen through the use of raised lateral trough systems that incorporate soilless substrates (McCartney and Lefsrud, 2018). The cultural conditions that growers strive to achieve for production in these environments can be conducive to the hemi-biotrophic fungal pathogen *B. cinerea*, the causal agent of grey mold. Disease incidence of grey mold can be economically destructive both pre and post-harvest, given the pathogen's ability to remain in a quiescent state within the hosts receptacle tissues (Petrasch et al., 2019). As supplementary lighting by LEDs is being incorporated into these production settings more often, it is important to evaluate the effects that mixed-narrow bandwidth LED lamps can have on the strawberry-*Botrytis* pathosystem. *B. cinerea* is an ideal pathogen to study when examining photoresponse given the wide availability of literature regarding the pathogen's photomorphogenesis under various narrow bandwidth lighting qualities (**Table 4, Section 3.2**).

This study aimed to characterize key aspects of the strawberry-*Botrytis* pathosystem by incorporating mixed narrow-bandwidth LED lighting treatments. Initially, the first objective was to investigate the photomorphogenesis of a *B. cinerea* isolate *in vitro* within a CE, using three diode ratios of Far-red:Blue light (5:1, 1:1, 1:5). This involved examining propagule formation, appressorium potential, mycelial growth, sporulation, and germination. It has been previously observed that Blue light inhibits sporulation of *B. cinerea*, while Far-red light promotes sporulation (Tan and Epton, 1974a). By studying these initial stages, the primary aim was to understand how the tested mixed narrow-bandwidth LED lamps could influence the pathogen's development before disease incidence occurs.

Beginning with mycelial growth assays, results indicated that all tested LED lamps, regardless of the ratio, significantly reduced the radial mycelial growth of *B. cinerea* colonies compared to the control treatments of White (ambient light + HPS lamps) and complete darkness. These results contrast with those of Meng, Mestdagh, et al. (2020), who studied the phenotypic variation of 15 *B. cinerea* isolates under Red, Blue, and Red:Blue LED lamps and found no observable effect on mycelial growth regardless of photoperiod or exposure timing. This suggests that either spectral intensity or the incorporation of Far-red light might have played a key role in mycelial growth inhibition. However, it is worth noting that in the conducted mycelial growth assays, all LED lamps had lower irradiance (measured as kLux) than the control treatment of White, suggesting that intensity was insignificant. Additionally, the tested treatments included wavelengths of Far-red light rather than Red light. If irradiance did not contribute to growth inhibition, it is possible that a higher wavelength (or amplitude) of Far-red light compared to Red light was a significant factor in inhibiting growth. Notably, Meng, Mestdagh, et al. (2020) tested 15 isolates, making it unlikely that the observed growth inhibition on the tested isolate in this study was isolate-specific. For future studies, it is crucial to adhere to the standard practice of recent publications and report lighting intensity as photosynthetic photon flux density (PPFD) measured as micromole per second per m^2 ($\mu mol/s/m^2$) to create consistency in the literature. Additionally, incorporating multiple isolates for phenotypic characterization holds promise in providing a more comprehensive understanding of the impact of lighting treatments.

It was observed that the isolate used could sometimes form sclerotia in the presence of complete darkness. As such, it was thus characterized as light-responsive, having been noted to have a strong preference for sporulation in light or dark conditions (Canessa et al., 2013). Therefore, the next assays consisted of evaluating the sporulation potential in regard to the previously tested lighting

treatments. The results showed that the pathogen exhibited a significant decrease in sporulation under all LED lighting treatments compared to controls, suggesting that these lighting qualities reduced the reproductive capacity of the pathogen. Previously, it was reported by Tan and Epton (1974a) that Far-red light promoted the sporulation of *B. cinerea* while Blue light was found to be inhibiting. Interestingly, these findings indicate that the addition of Blue light emitting diodes into a Far-red diode dominant lamp in a ratio of 5:1 was sufficient enough to counteract the reported promotional effect. Given the reduced area of the fungal colony and ability of the fungus to generate radially growing mycelia, this could have contributed to the observed decrease in sporulation. Although, many of the spores solutions collected from samples cultured under LED treatments were simply too dilute to effectively count spores, with most solutions collected absent of spore pellets after rotary centrifugation.

Sporulation assays were then conducted under exposure of two photoperiods: 24 hours of exposure, and 16 hours of exposure + 8 hours of darkness. There was an observed decrease in the sporulation ability of the isolate between the two, with a photoperiod of 16 hours of exposure + 8 hours of darkness appearing to be the most effective. This could be due to external factors, such as a loss of viability of the isolate or possible temperature fluctuations within the CE. Additionally, control treatments of White and complete darkness were also found to have a significant reduction in sporulation between the two photoperiods. Further validation is required to confirm these findings, taking into consideration temperature as an experimental factor. Also, the viability of the culture should be evaluated between trials to ensure it is maintained.

The next assays consisted of evaluating the germination of spores over 11 hours of treatment exposure. It was determined that this specific exposure timing resulted in the highest germination percentage. Subsequent increases in germination rates were only marginal, potentially attributed

to the solution concentration. Initially, a reduction in germination was observed under a Blue diode dominant treatment of 1:5. Although, at 11 hours there was no significant differences in germination between treatments. Preliminary observations of germination and subsequent mycelial growth were made after 11 hours of germination and noted (data not presented). It was found that initial mycelial growth under a Far-red diode dominant treatment 5:1 displayed increased internodal spacing between hyphal branches. Although, given technical restraints, measuring and quantifying internodal spacing was not achieved. Even though treatments seemed to have no effect on the germination of spores, future studies might find interesting results in the initial development of colonies generated from germinated spores, with possible variation in phenotypic characteristics.

Taken together, results from these assays confirm that the spectral composition of supplementary lighting can influence the development and asexual replication of *B. cinerea in vitro* within a CE. The LED treatments tested were effective at reducing both the mycelial growth of the pathogen, but more notably its ability to sporulate and therefore replicate, a crucial step in the proliferation of infectious propagules. Given the dispersal of conidia to typically serve as the initial source of primary inoculum with CE, a plausible interruption in this key stage of development would imply a plausible reduction in disease incidence through preventative measures and not necessarily through complete interruption of plant-host interactions.

The presented results from the initial study on the photomorphogenesis of the pathogen are promising, but further investigation of a host's response via infection assays was necessary to determine any significant differences between the LED treatment ratios *in planta*. This led to the second objective, which was to investigate the defense response of strawberry plants (*Fragaria* x *ananassa* Duch. var. Seascape) during infection by *B. cinerea* through measuring the progression

of lesion development on detached leaves, as well as comparing changes in total phenolic content (TPC) pre and post-infection.

Initially, when infecting detached strawberry leaves via mycelial plugs, it was found that a ratio of 1:1 or 1:5, with an equal or dominant Blue diode composition, was more effective than a dominant Far-red diode composition, at inhibiting the development of necrotic lesions (**Figure 12, Section 4.4.1**). In regard to previous reports, it has been suggested that narrow-bandwidth Red light promotes strawberry leaf basal resistance, while Blue light decreases resistance compared to a control treatment of HPSLs (Meng, Van Labeke, et al., 2020). Our findings complement this study by indicating an interplay between both tested lighting qualities of Far-red and Blue light and their direct effects on each other. Notably, in the treatment utilizing a Blue diode dominant lamp the addition of Far-red diodes was efficient enough to counteract the previously reported decrease in susceptibility which was observed by a narrow-bandwidth Blue lighting treatment. It is worth noting that inoculation via actively growing mycelial plugs was found to be necessary in facilitating consistent formation of necrotic lesions as opposed to previous attempts using spore solutions. Therefore, these results should be interpreted accordingly, and further studies should be conscientious of the difficulties associated with the inoculation of strawberry leaves via spore solutions.

Additionally, infected leaves exposed to both White and Far-red:Blue ratios of 5:1 and 1:5 exhibited signs of chlorosis surrounding the lesion margins, whereas leaves exposed to complete darkness and a Far-red:Blue ratio of 1:1 and were absent of such discoloration. It has been reported that fungal pathogens have the ability to produce diverse secondary metabolites, often considered as either general toxins or host-specific toxins, such as mycotoxins and secretory proteinaceous toxins (Doehlemann et al., 2017). These substances play a key role in triggering host-cell death

and enhancing the pathogenicity of the fungi on their host (Howlett, 2006). Therefore, concerning the inconsistencies in chlorosis observed between lighting treatments, it is suggested that future works investigate the accumulation of general toxins and HSTs associated with *B. cinerea*, such as botrydial (Howlett, 2006) or CFEM1 (Zhu et al., 2017). These specific toxins have been linked to the induction of chlorosis and studying their abundance surrounding infected tissues could provide valuable insights into the mechanisms behind the observed differences.

Continuing with the second objective of this study, the focus was on quantifying and comparing changes in the TPC between infected and non-infected immature strawberry plants. The aim was to gain insights into the plant defense response to the lighting treatment. This investigation rested on the existing knowledge that plants exhibit diurnal variations in the synthesis of metabolic compounds (Venkat and Muneer, 2022). Confirming previous findings by Meng, Van Labeke, et al. (2020), it was observed that fluctuations in TPC occurred throughout the photoperiod in the non-infected strawberry plants, regardless of the lighting treatment, between 0 and 6 hours post-start of the photoperiod. Interestingly, the exposure to either Blue or Far-red lighting treatments resulted in significant differences in TPC, with reported increases and decreases under Blue and Far-red light, respectively. The distinct effects of the Far-red diode dominant treatment and the Blue diode dominant treatment supported earlier research by Meng, Van Labeke, et al. (2020), ultimately affirming that Blue light reduces phenolic compound production in strawberry plants compared to white light.

The next focus was to compare infected and non-infected assays, which suggested that the 1:5 treatment potentially stimulated a more robust defense response in the plants, particularly evident among those subjected to infection. Conversely, when examining the equal diode ratio treatment of Far-red:Blue 1:1 in both non-infected and infected groups, no significant changes were found,

akin to the plants exposed to the White control. This outcome indicates that a ratio of 1:1 is likely sufficient in regulating phenolic compound production in plants, similar to the effects of the White control.

However, it is important to acknowledge that these results represent preliminary findings, contingent upon further temporal replication. The limited number of time points for sample collection and the influence of diurnal fluctuations in plant TPC emphasize areas for consideration. Consequently, future studies should strive to expand the sampling time points to achieve a more comprehensive understanding of the plant response to lighting treatments, both in the presence and absence of infection.

Chapter 6: General Conclusion

This study thoroughly investigated the impact of Far-red:Blue lighting ratios (5:1, 1:1, and 1:5) on the photomorphogenesis of *B. cinerea in vitro* within a controlled environment (CE). Additionally, it explored the effect of these lighting treatments on the pathogenicity of *B. cinerea* on immature strawberry plants. The presented results revealed that all ratios effectively disrupted the pathogen's asexual reproduction, growth, and development, however, when treatments were applied *in planta* it was found that a balanced ratio of 1:1 or a Blue diode dominant ratio of 1:5 demonstrated the greatest efficacy in reducing necrotic lesion development. Furthermore, a Blue diode dominant ratio of 1:5 is thought to enhance plant defense responses, which was observed as a significant increase in the total phenolic content of leaf tissue.

While earlier studies have concentrated on the effects of different narrow-bandwidth lighting sources on strawberry-*Botrytis* interactions, this research represents the first investigation into the effectiveness of Far-red:Blue mixed narrow-bandwidth lighting. In doing so, this study places emphasis on characterizing the phenotypic responses of the isolate used by examining photomorphogenesis and structural development, key indicators of viability. As the integration of multiple spectral qualities becomes more common in CEs, this study provides valuable insights into the effect that supplemental lighting has on this respective pathosystem. It concludes that a Blue dominant diode ratio composition appears to be more effective in inhibiting the photomorphogenesis and pathogenicity of *B cinerea* as well as potentially enhancing strawberry plant defense responses. These findings contribute to advancing our understanding of lighting strategies for disease management and plant protection.

6.1. Future Directions

I. Investigation of Mechanisms: To gain deeper insights into the underlying mechanisms behind the observed effects, future research should focus on elucidating the molecular and physiological processes involved. Examining rhythmic gene expression patterns and metabolic changes in response to different lighting treatments can shed light on the specific mechanisms by which light influences the growth and defense responses of both the pathogen and the plant.

II. Field Validation and Application: To translate the findings of this controlled environment study into practical applications, future research should include field validation experiments. Assessing the efficacy of optimized lighting strategies in real-world agricultural settings, such as commercial greenhouse equipped with supplemental LED lighting, is a suggested expansion of this work.

III. Exploring Pathotype Variation: Future works could explore pathotype variation by analyzing isolates obtained from production systems that employ different lighting regimes. By focusing on single-nucleotide polymorphism regions associated with light-responsive genes, valuable insights could be discovered into how specific lighting conditions influence the genetic makeup and characteristics of *Botrytis cinerea* pathotypes.

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