



**How far-red light enrichment
modulates tomato resistance
towards *Botrytis cinerea***

Sarah Coubier

**How far-red light enrichment
modulates tomato resistance
towards *Botrytis cinerea***

Sarah Courbier

Reading committee :

Prof. dr. Yuling Bai

Prof. dr. Leo F.M. Marcelis

Prof. dr. Han A.B. Wösten

Prof. dr. Corné M.J. Pieterse

Dr. Charlotte M.M. Gommers

**How far-red light enrichment modulates tomato resistance
towards *Botrytis cinerea***

Copyright © 2020 Sarah Courbier

All rights reserved. No part of this thesis may be reproduced, stored or transmitted in any way or by any means without the prior permission of the author, or when applicable, of the publishers of the scientific papers.

Utrecht University, Plant Ecophysiology.

ISBN: 978-90-393-7288-3

Cover design: Dr. Maud Combier

Layout and design: Mirelle van Tulder, persoonlijkproefschrift.nl

Printing: Ridderprint | www.ridderprint.nl

How far-red light enrichment modulates tomato resistance towards *Botrytis cinerea*

Hoe ver-rood licht de resistentie van tomaat tegen *Botrytis cinerea* beïnvloedt
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

woensdag 28 oktober 2020 des ochtends te 9.15 uur

door

Sarah Courbier

geboren op 11 mei 1991
te Brive-la-Gaillarde, Frankrijk

Promotor:

Prof. dr. R. Pierik

Copromotor:

Dr. A.C.M. Van Wees

Contents

Chapter 1	7
General Introduction	
Chapter 2	21
Far-Red light enrichment induces changes in tomato architecture and immunity towards <i>Botrytis cinerea</i>	
Chapter 3	43
Transcriptome changes of tomato induced by <i>Botrytis cinerea</i> infection are modulated by far-red light	
Chapter 4	75
Far-red light modulates tomato defenses induced by <i>Botrytis cinerea</i> by influencing synergism between jasmonic acid and ethylene pathways	
Chapter 5	101
Host sweet host: Far-red light increases susceptibility by changing the sugar pool in tomato	
Chapter 6	123
General Discussion	
References	136
Summary	148
Résumé	151
Samenvatting	154
Acknowledgements	157
About the author	163
List of publications	164



Chapter

General Introduction

1

Sarah Courbier and Ronald Pierik

*Plant Ecophysiology, Institute of Environmental Biology, Utrecht
University, the Netherlands*

A modified version of this chapter has been published as :

Courbier, S., Pierik, R. 2019. *Canopy light quality modulates stress responses in plants. iScience* **22**: 441-452.

Plants growing at high density are in constant competition for light with each other. The shade avoidance syndrome (SAS) is an effective strategy for plants to escape neighboring vegetation. Even though the molecular mechanisms regulating SAS have been long studied, interactions between light and other environmental signaling pathways have only recently received attention. Under natural conditions, plants deal with multiple stresses simultaneously. It is key to identify commonalities, distinctions and interactions between plant responses to different environmental cues to understand plant growth and development under such complex conditions. Here, we outline the current understanding of the interplay between canopy light signaling and biotic stresses. Understanding plant responses to multiple stimuli, factoring in the dominance of light for plant life, is essential to generate crops with increased resilience against climate change.

Should I stay or should I grow ?

Global warming and overall climate change are major factors influencing plant life and fitness on the planet. Plants are increasingly challenged by their environment and they are forced to acclimate to sustain their survival. Also, as world population increases, food security is threatened by limited and continuously deteriorated arable land areas. The need for more resilient plants is, therefore, indispensable to feed the population in the future and one option is to grow plants at higher densities whilst maintaining individual plant productivity. The latter is not always straightforward since with increasing plant density, competition for light between individuals increases as well, leading to growth reductions. Plants employ a suite of developmental adjustments to increase their competitive ability, known as the “shade avoidance syndrome (SAS)” (Ballaré and Pierik, 2017; Casal, 2013). SAS response is constituted of rapid hypocotyl, internode and petiole elongation as well as upward movement of leaves (hyponasty), apical dominance and early flowering in *Arabidopsis thaliana* (Arabidopsis) (Casal, 2012; Roig-Villanova and Martínez-García, 2016). Although immense progress has been made in unraveling the molecular mechanisms underpinning different SAS components, the impacts of density light cues and SAS responses on the ability of plants to deal with other challenging conditions such as biotic stresses has not been very intensively studied in crop systems.

Shade avoidance syndrome

Plants need light to grow and have evolved elaborate light quality radars referred to as photoreceptors. Phytochromes (PHY) are sensitive to red (R) and far-red (FR) light; cryptochromes (CRY) and phototropins absorb UV-A and blue (B) light and these together are the long-established photoreceptors used by plants to sense their light environment (Quail, 1994). In addition, plants express the zeitelupe family of blue light receptors: ZTL, FKF1 and LKP2 (Demarsy and Fankhauser, 2009) and the UVR8 receptor for UV-B light detection (Rizzini et al., 2011). Plants absorb R and B light with chlorophyll in their chloroplasts for photosynthesis and reflect or transmit FR light. At high planting density, the absorption of R light and reflection of FR by plants lead to low red: far-red (R:FR) light conditions within the canopy (fig. 1.1). Changes in the R:FR ratio are sensed by phytochromes where phyB plays a major role in triggering shade avoidance in *Arabidopsis* (Franklin, 2008). Upon approximately equal fluence rates of R and FR (high R:FR), such as in direct sunlight, the majority of the phyB pool is activated and active phyB (Pfr) translocates to the nucleus. In the nucleus phyB physically interacts with the bHLH transcription factors Phytochrome Interacting Factors (PIFs) and promotes their inactivation and degradation by the 26S proteasome (Li et al., 2016). On the contrary, as planting density increases, R:FR decreases and under such low R:FR, phytochrome is photoconverted into its inactive form (Pr). This inactivation disables PHY interaction with PIFs. Subsequently, active PIFs can accumulate and bind G and E boxes in the promoters of their target genes to regulate their expression (Casal, 2013; Franklin, 2008; Franklin and Quail, 2010; Lorrain et al., 2008). Core targets for PIFs are genes associated with auxin homeostasis and cell wall remodeling to control growth (fig. 1.1) (Hornitschek et al., 2012; Li et al., 2012; Pedmale et al., 2016). Even though several auxin biosynthetic routes have been discovered in plants, the best characterized is based on the conversion of tryptophan (trp) into indole-3-acetic acid (IAA) by TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) and YUCCA flavin monooxygenase enzymes (YUC). Upon low R:FR perception, the release of PIFs (notably PIF4, PIF5 and PIF7) upon phyB inactivation promotes the expression of *TAA1* involved in the conversion of trp into indole-3-pyruvic acid (IPA) (Franklin et al., 2011). PIFs are also able to induce *YUC2*, *YUC5*, *YUC8* and *YUC9* leading to the conversion of IPA into IAA which promotes elongation growth (Franklin et al., 2011; Hornitschek et al., 2012; Li et al., 2012). IAA has been described to be mobile and its transport during light responses is highly coordinated in plants via the PIN-FORMED proteins PIN3, PIN4 and PIN7 (Keuskamp et al., 2010; Kohnen et al., 2016). Also, auxin

biosynthesis and transport allows for coordination of growth responses across an organ or even organism in response to heterogeneous light conditions (Küpers et al., 2018; Pantazopoulou et al., 2017). In *Arabidopsis* seedlings, auxin is synthesized upon low R:FR in the cotyledons and shoot apical meristem and subsequently transported to the hypocotyls where it stimulates cell elongation (Küpers et al., 2018). Elongation growth depends on a key regulatory hub between brassinosteroids (BR), auxin and gibberellin (GA) signaling pathways known as the BAP/D module that has PIFs at the core (Franciosini et al., 2017). This module is composed of the three transcription factor groups BRASSINAZOLE-RESISTANT1 (BZR1), AUXIN RESPONSE FACTOR6 (ARF6) and PHYTOCHROME INTERACTING FACTOR4 (PIF4) that have been shown to physically bind each other, promote elongation growth and share half of their downstream targets (Oh et al., 2014). Growth promotion through the BAP/D module is inhibited by DELLA proteins, negative growth regulators. DELLAs have been shown to compete with the other transcription factors to bind ARF6 and to interact with PIFs and prevent their binding to target sequences on the DNA (Oh et al., 2014). However, upon low R:FR conditions, GA signaling is upregulated resulting in enhanced levels of bioactive GA which subsequently lead to degradation of DELLAs, thus de-repressing PIFs (Li et al., 2016). Also, the inhibition of *phyB* by low R:FR contributes to releasing more PIFs to activate downstream growth-related gene expression (fig. 1.1).

Phytochrome signaling has also been shown to regulate the plant metabolic state associated with biomass production in *Arabidopsis*. Mutant plants lacking *phyB* showed a reduced chlorophyll content associated with lower CO₂ uptake in leaves compared to the wild-type (WT) and pointing towards an impairment of photosynthesis (Yang et al., 2016). Even though photosynthesis might be impaired, *phyB* mutants accumulate more sucrose and starch in daytime. Nevertheless, these mutants still have reduced biomass compared to WT, indicating that phytochrome signaling plays a key role in biomass production and carbon resource allocation in *Arabidopsis* (De Wit et al., 2018; Yang et al., 2016). The exact mechanisms responsible for increased sugar levels in *phyB* mutants and its physiological consequences are yet unknown. Interestingly, even though most plant species show a shade avoidance behavior (Roig-Villanova and Martínez-García, 2016), accelerated shoot elongation is not always an option. For example plant species growing under tree canopies can obviously not outgrow the trees (Gommers et al., 2013).

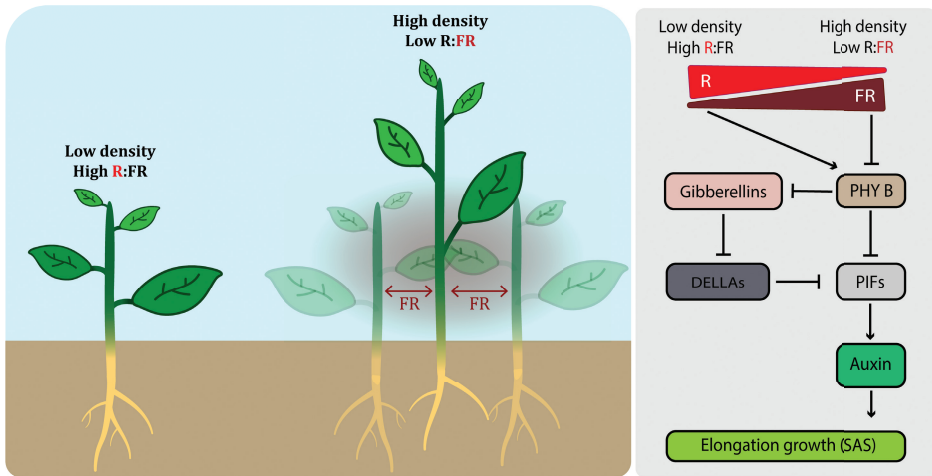


Figure 1.1: Simplified overview of the shade avoidance syndrome. High planting densities lead to a reduction of the ratio between red and far-red light (R:FR) resulting in shoot elongation growth and slightly reduced root development. At the molecular level, low R:FR conditions result in reduced activity of phytochrome B (PHYB). Inactive PHYB allows Phytochrome interacting factors (PIFs) to induce shoot elongation in an auxin-dependent manner. In parallel, GA levels are increased leading to the inhibition of the growth repressors DELLA proteins also leading to shoot elongation.

Recent studies in shade tolerant and shade avoiding *Geranium* species showed that shoot elongation upon low R:FR exposure only occurs in the grassland species *Geranium pyrenaicum* and not in *G. robertianum* mainly found in forest understories (Gommers et al., 2017). The mechanism behind shade tolerance is still poorly understood but could be of great importance in crop systems where an excessive elongation growth due to high plant density impairs yield and stem sturdiness in maize for example (Wang et al., 2016). Indeed, modification of the core, PIF-dependent SAS pathway seems to act in SAS suppression in a shade tolerant plant (Gommers et al., 2018, 2017). In addition to affecting shoot elongation, phyB signaling has also been reported to influence root development in an auxin-dependent manner (fig. 1.1) (Morelli and Ruberti, 2000). Low R:FR conditions sensed by phyB mainly in the shoot is sufficient to modulate auxin flow and gradients in the roots and control root growth in *Arabidopsis* (Salisbury et al., 2007). Upon additional FR radiation on *Arabidopsis* shoots, plants exhibit pronounced reductions in main root length and lateral root formation (fig. 1.1) (van Gelderen et al., 2018). Low R:FR exposure in shoots stimulates the expression and stability of the transcription factor HY5 which can translocate from the shoot to the root system and controls lateral root formation (van Gelderen et al., 2018). Even though shade avoidance is well understood in *Arabidopsis*, some

aspects like shade tolerance or shoot to root signaling have only recently been studied at mechanistic levels and are therefore still rather poorly understood.

Canopy light shapes plant-biotic interactions

Plants carry an ever-evolving arsenal of defense mechanisms in order to restrict pathogens from colonizing (Jones and Dangl, 2006). Plants can recognize signatures from the attackers' surface such as Microbe-, Herbivore- or Damage- associated molecular patterns (MAMPs, HAMPs and DAMPs) and these patterns induce downstream defense signaling that is known as “pattern-triggered immunity” (PTI) (Hogenhout and Bos, 2011; Hou et al., 2019; Zipfel, 2014). The suppression of PTI by pathogen effectors is referred to as “effector triggered susceptibility” (ETS), which is a sign of pathogen success that can subsequently be counteracted by plants upon recognizing those effectors and inducing a response known as “effector triggered immunity” (ETI) (Cui et al., 2015; Peng et al., 2018). Plant defense responses against biotrophic pathogens, i.e. pathogens feeding on living plant cells, are mainly regulated by salicylic acid (SA) while plant responses against necrotrophic pathogens, i.e. pathogens feeding on dead plant cells, are regulated by jasmonic acid (JA) in combination with ethylene (ET) (Glazebrook, 2005).

Low R:FR light inhibits plant immunity

Jasmonic acid pathway

The negative effect of low R:FR conditions on plant immunity has been in the spotlight for the last decade and clear evidence of crosslinks between growth and defense mechanisms have been elucidated. JA is a core regulatory hormone orchestrating defense responses against insects and necrotrophic pathogens (Turner et al., 2013). Bioactive JA is derived from galactolipids at the membrane of chloroplast forming α -linolenic acid (C18:3). After several oxygenation steps, α -linolenic acid is transformed into 13-hydroperoxyoctadecatrienoic acid (HPOT), epoxy-octadecatrienoic acid (EOT) and then into the JA precursor OPDA (Oxaphytodienic acid) that can be translocated to the peroxisome for maturation. OPDA is converted into (+)-7-iso-JA via a succession of oxidation/reduction reactions. One last enzymatic step is needed for the formation of bioactive JA via JAR1 that allows the conjugation of JA with amino acids such as isoleucine (Ile) leading to the production of JA-Ile. Bioactive JA is recognized by

the SCF-COI1 complex in the nucleus leading to the ubiquitination and subsequent degradation of the negative defense regulator JASMONATE ZIM domain proteins (JAZ) in turn promoting JA-mediated defense (Pandey et al., 2016; Wasternack and Hause, 2013; Wasternack and Strnad, 2018). Pioneering work demonstrated that plants lacking phyB or exposed to low R:FR conditions had decreased levels of resistance towards herbivores associated with reduced sensitivity to JAs (fig. 1.2) (Izaguirre et al., 2006; McGuire and Agrawal, 2005; Moreno et al., 2009). In *Arabidopsis*, low R:FR conditions reduce the emissions of most volatile organic compounds (VOCs) and suppress methyl-jasmonate (MeJA)-induced gene expression upon herbivore attack (Kegge et al., 2013). Studies in crop systems like tomato confirmed that low R:FR drastically modifies the MeJA-regulated VOCs composition and showed that this impacted indirect defenses by attracting predatory insects (fig. 1.2) (Cortés et al., 2016). In barley, constitutive VOCs emissions were also altered in response to low R:FR and this was found to modulate plant-plant interactions between VOC emitters and VOC receivers in experimental setups (Kegge et al., 2015).

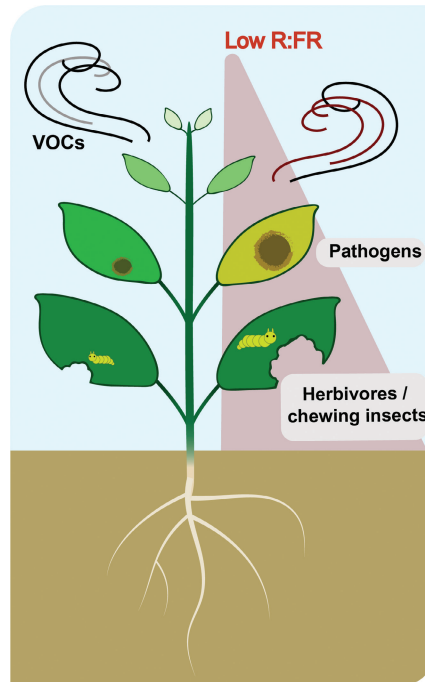


Figure 1.2 : Low red : far-red modulates plant immunity. Low R:FR increases susceptibility to pathogens and to chewing insects and shapes the plant volatiles organic compounds (VOCs) composition and become more attractive to herbivores.

Several studies have investigated the mechanisms through which phytochromes would then modulate JA responses. Part of the mechanism appears to involve the interaction between the growth repressing DELLA proteins and the defense repressing JAZ proteins that are degraded upon JA signaling (Ballaré, 2014; Pieterse et al., 2014). During shade avoidance, DELLAs are degraded upon increased gibberellin levels and this relieves their sequestration of JAZs. The release of JAZ proteins upon DELLA degradation can lead to the inhibition of the MYC2 transcription factor, which would otherwise activate downstream defense responses (Hou et al., 2010). Consistently, increased stability of JAZ10 was observed in the *phyB* mutant background in Arabidopsis, possibly following from DELLA degradation (Leone et al., 2014). Consistent with the importance of JAZ10, the hypersensitivity to *Botrytis cinerea* in the *phyB* mutant was partially overcome in the *jaz10 phyB* double mutant (Cerrudo et al., 2017).

Ethylene pathway

Ethylene has been studied in many different contexts, including stress-induced shoot elongation, similar to that occurring in shade. When terrestrial plants get flooded, e.g. through heavy rains, CO₂ and O₂ availability become limited leading to an energy crisis (Sasidharan et al., 2018). Ethylene accumulates in such plants and can elicit different responses: priming of upcoming hypoxia resistance (Hartman et al., 2019) or accelerated shoot elongation to reach the air. The latter response acts through light signaling components such as *COP1*, *PIF* and *HY5* orthologues in *Rumex palustris*. Consistently, a study on hypocotyl elongation in Arabidopsis seedlings exposed to either shade or elevated ethylene, confirmed shade and ethylene to converge on a shared downstream module to control elongation growth (Das et al., 2016; van Veen et al., 2013).

Besides being an important plant growth regulator, ethylene is also a major regulator of plant defense against necrotrophic pathogen attacks (Han et al., 2010). Ethylene acts together with JA in promoting defense response against necrotrophic pathogens (Chang et al., 2013; Zhu et al., 2011). Plant defense responses against *B. cinerea* require the coaction of JA and ethylene, and the production of both these hormones is stimulated in plants exposed to *B. cinerea*. The exact mechanism through which this coaction of JA and ethylene occurs is not fully understood yet. However, it is of interest that low R:FR conditions stimulate ethylene synthesis, even though ethylene

plays only a modest role in the shade avoidance response to this cue (Pierik et al., 2009, 2004). It would, therefore, be very interesting to study the regulation of both hormones in the context of plant disease resistance at high density.

Salicylic acid pathway

Upon biotrophic pathogen attack, different defense responses need to be activated compared to infection with a necrotrophic pathogen. Indeed these are also orchestrated by a different plant hormone: SA. Upon infection, SA production is initiated and leads to redox changes in the attacked plant cells. The most studied regulator of SA signaling is NPR1 which is found as oligomers in the cytoplasm of untouched plants but translocated into the nucleus as monomers upon SA accumulation (Mou et al., 2003; Tada et al., 2008). Once in the nucleus, NPR1 allows the activation of SA-induced resistance genes. Low R:FR conditions have been shown to downregulate both JA- but also SA-mediated defenses upon pathogen attack, coinciding with inhibition of NPR1 phosphorylation leading to poor downstream defense gene induction (De Wit et al., 2013). SA-deficient mutants were not able to fully exhibit petiole elongation response upon FR exposure suggesting a role of SA in growth response in *Arabidopsis* (Nozue et al., 2018) that would need further studies to unravel the mechanisms. Interestingly, BR signaling that mostly regulates growth and developmental responses, has been shown to be associated with flagellin (a well-known MAMP) recognition and signaling upon pathogen challenge via the interaction between the BR receptor kinase BRI1 and its coreceptor BAK1 (Chinchilla et al., 2007). BR is thought to inhibit defense responses through the induction of the positive growth regulator *BRASSINAZOLE-RESISTANT 1* gene *BZR1* (Lozano-Durán et al., 2013; Lozano-Durán and Zipfel, 2015). As mentioned earlier, *BZR1* is a key component of the BAP/D module involved in growth regulation. Upon low R:FR conditions, plants might prioritize growth-mediated BR responses via the BAP/D module over flagellin-mediated defenses in case of a pathogen attack.

As previously mentioned soluble sugar levels are strongly affected by low R:FR (De Wit et al., 2018; Yang et al., 2016). Carbohydrates are the main carbon source targeted by pathogens upon infection. The increased susceptibility observed in low R:FR or phytochrome mutant plants might also be associated with increased levels of accessible carbohydrates for the pathogen in plant tissue. High sugar levels in plants usually correlates with the production of secondary metabolites and/or

defense gene induction such as MAPK, PR genes or phenylpropanoid metabolic pathway (reviewed in Bolouri Moghaddam and Van den Ende, 2012). In low R:FR, lower plant defense towards *B. cinerea* was associated with lower metabolite production and lower defense genes activation (Cargnel et al., 2014). Low R:FR, thus seems to enhance soluble sugars and reduce defense-related processes that together would potentially increase lesion development in pathogen-infected plant tissue. Although growth responses to shade cues seem to typically dominate investments into immune responses, the interactions are probably more complicated. It will be important to investigate if environmental conditions and developmental stage of the plant impact on this crosstalk, and perhaps even drive the direction of the interactions observed.

Perspectives

The well-established tradeoff between growth and defense is not so much an ON/OFF switch, but appears to be a highly fine-tuned process (Ballaré and Austin, 2019). In crops, high density-associated low R:FR conditions will suppress the JA pathways leading to a weakened defense pathway (fig. 1.2). It is paramount to understand how pathogenic interactions are affected by high density in order to create resilient plant systems in the future. Detailed studies on the molecular mechanisms of these interactions are required for this. There is indeed precedent that the pathways can be uncoupled, shown by specific mutants in Arabidopsis such as *sav3-2*, which lacks an elongation response, but still shows FR-mediated defense suppression (Cerrudo et al., 2012). Shade avoidance induction by blue light depletion rather than low R:FR on the other hand gives elongation without a measurable effect on resistance (Cerrudo et al., 2012). Forest understory plants are typically shade tolerant and show no shade avoidance responses to low R:FR (Gommers et al., 2013). Interestingly, one such species, *Geranium robertianum* does also not show downregulation by low R:FR of its JA-dependent defenses, and even slightly increases its resistance against *B. cinerea* (Gommers et al., 2017). Studies into the mechanisms of these shade-defense interactions have focused successfully on defense hormone signal transduction. An interesting novel avenue might be related to plant metabolism. Plants experiencing low R:FR or high density growth conditions accumulate more soluble sugars during the day and synthesize fewer defense-associated secondary metabolites, possibly becoming better targets for pathogens. So far, it is unknown how changes in primary metabolism under low R:FR could affect plant disease resistance and/or shade avoidance. Unravelling the molecular mechanisms underpinning interactions between

photoreceptor signaling and biotic stress pathways in a crop system like tomato will provide the knowledge needed to optimize stress resilience at the common-practice high planting densities. Although world-wide most crops are grown outdoors, the Netherlands are world-renowned for their technologically advanced greenhouse cropping systems. High value crops that are grown in greenhouses include cucumber, sweet pepper and tomato. In order to grow these throughout the year, supplemental lighting is essential. The development of LED lighting technology has potential to revolutionize greenhouse cropping systems since it is not only energy-efficient, but also offers the opportunity to fine tune the light quality. Understanding trade-offs between for example growth and defense in detail, and understanding which light qualities may ameliorate potentially negative impacts in greenhouse crops will be key to optimal use of these lighting technologies at a commercial scale. The study in this thesis was conducted within the multi-partner research program “LED it Be 50%” in which partners from academia and industry collaborate to generate the knowledge required to transition from conventional artificial lighting to LED lighting of tomato production in greenhouses.

Thesis outline

Far-red-enriched light conditions are known to increase plant susceptibility towards pathogens (Cargnel et al., 2014; De Wit et al., 2013). In **Chapter 1**, we briefly summarized and discussed what is known about the interaction between growth and defense mechanisms in plants. Building on the knowledge generated from the model plant *Arabidopsis*, we investigated this phenomenon in the highly cultivated crop plant tomato. The aim of this thesis is to gain a better understanding of the impact of low R:FR on tomato immunity towards the necrotrophic pathogen *B. cinerea* when grown at high densities. By combining phenotypic and gene expression data, we managed to unravel several pathways affected by low R:FR leading to higher susceptibility.

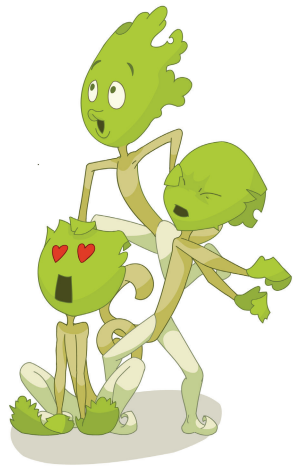
In **chapter 2**, we study the impact of FR-enriched light conditions on tomato growth and morphology and observe a strong stem elongation in tomato plants after a few days of treatment. We also demonstrate that the increase in susceptibility in tomato cv. MoneyMaker towards *B. cinerea* is dictated by the light quality that plants experience prior to the pathogen challenge. This is true under the two light background tested (white LEDs or a combination of red and blue LEDs).

The previous chapter constitutes a solid basis for data presented in **chapter 3**. In this chapter, we use next generation RNA-sequencing to unravel which processes are altered by low R:FR at early timepoints which lead to higher susceptibility several days after infection. We observe that low R:FR alters defense hormone signaling such as jasmonic acid (JA) and/or ethylene but also primary metabolism which might be the cause of increased susceptibility.

In **chapter 4**, we focus on a set of six genes encoding PROTEINASE INHIBITORS (PI) selected from the gene expression analysis. Those genes were found to be upregulated upon infection only in control light conditions but not upon low R:FR treatment. We managed to validate the involvement of jasmonic acid and ethylene in the induction of *PI* genes which was reduced in low R:FR. We observe a decrease in hormone sensitivity in plants experiencing low R:FR which partly explain the increase in susceptibility.

Chapter 5 covers the effect of low R:FR on primary metabolism. We reveal that low R:FR is associated with higher soluble sugar levels in tomato leaves which promotes lesion development. By experimentally modulating glucose levels in tomato leaves, we manage to associated sugar accumulation with increased susceptibility to *B. cinerea*. We also study the effect of local FR illumination of soluble sugar partitioning which appears to match with the disease phenotypes.

Finally, I conclude this thesis with a general discussion presented in **chapter 6**.



Mo

Chapter

2

Far-red light enrichment induces changes in tomato architecture and immunity towards *Botrytis cinerea*

Sarah Coubier¹, Saskia C.M. Van Wees² & Ronald Pierik¹

¹*Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, the Netherlands*

²*Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, the Netherlands*

Abstract

Light is a key parameter for plant growth. Red (R) and blue (B) light are used for photosynthesis and far-red (FR) light is reflected towards neighboring vegetation. At high densities, the light availability decreases whereas FR does not, resulting in a subsequent decrease of the R:FR ratio. Low R:FR light conditions induce strong morphological changes, called shade avoidance, as well as a downregulation of defense mechanisms in *Arabidopsis thaliana* referred to as “FR-induced susceptibility”. Even though this phenomenon has been studied for over a decade, its relevance to crop species has not received much scientific attention yet. We, therefore, investigate FR-induced susceptibility in tomato. To this end, we designed *in vitro* and *in planta* experiments following *Botrytis cinerea* growth under FR-enriched light conditions as well as bioassays assessing the effect of FR on immunity towards *B. cinerea* in tomato. Our data indicate that tomato is a strong shade avoider exhibiting a strong phytochrome-dependent susceptibility to *B. cinerea*. This work ultimately aims to contribute to understanding how light quality manipulations through LED lighting can be used to modify plant susceptibility to pathogens in greenhouses.

Introduction

Plants are in constant need for sufficient light capture to sustain their photoautotrophic growth. At high planting densities, leaf tissues are close together and even overlap, resulting in strongly reduced light availability for leaves and in dramatic changes in light quality due to the absorption of red (R, 600-700 nm) and blue light (B, 400-500 nm) for photosynthesis and the reflection of far-red light (FR, ~750 nm) towards neighboring plants in a canopy. As a result, the red: far-red ratio (R:FR) within the canopy is reduced and detection of this change elicits rapid hypocotyl and petiole elongation as well as increased leaf angles (hyponasty) in *Arabidopsis thaliana* (Arabidopsis), characterized as the shade avoidance syndrome (SAS) (Casal, 2012; Franklin, 2008). Changes in the R:FR are sensed by a specialized family of photoconvertible photoreceptors known as phytochromes where phytochrome B (phyB) plays the major role (Franklin, 2008). A decrease of R:FR leads to the conversion of the active (Pfr) into its inactive (Pr) form. This relieves active phytochrome-mediated suppression of the PIF (phytochrome-interacting factors) transcription factors, which then subsequently activate shade avoidance-associated gene expression under FR-enriched light.

Plants are not only competing with each other but are also frequently subjected to abiotic and biotic stresses. In addition, plant defense mechanisms against pathogens are modulated by FR light. The interplay between light-dependent growth and defense mechanisms in plants known as the “growth-defense tradeoff” has been intensively studied in the last decades (reviewed in Ballaré, 2014; Ballaré and Pierik, 2017). Recent studies have shown that low R:FR downregulates both salicylic acid (SA) and jasmonic acid (JA) defense pathway involved in defense against biotrophic and necrotrophic pathogens respectively (De Wit et al., 2013). Moreover, it has been shown that the downregulation of JA-mediated defense is caused by an increased stability of JAZ10 known as a negative regulator of JA signaling (Cerrudo et al., 2017; Leone et al., 2014). Dampening of JA-mediated defense by FR, referred to as the “FR-induced susceptibility” is explained by a physical interaction between JAZ proteins and DELLA proteins that are negative regulators of gibberellic acid (GA)-mediated growth responses. Upon shade, the inactivation of phyB and the production of GA leads to the degradation of DELLA proteins. This results in the release of JAZ proteins that sequester MYC transcription factors from activating JA-associated defense gene expression. The interaction between JA and GA signaling plays a crucial role in the

balance between growth and defense responses (Hou et al., 2010). Research on light-triggered modulation of susceptibility has mainly been performed in *Arabidopsis*, but has sporadically been translated to crops. Studies in tomato showed that low R:FR drastically reduces tomato defense against chewing and piercing insects (Izaguirre et al., 2006) and modifies the JA-mediated volatile organic compounds (VOCs) composition, a major defense component against herbivores (Cortés et al., 2016). Also, red and purple light have been shown to inhibit *Botrytis cinerea* lesion development on tomato leaves (Xu et al., 2017). Nonetheless, published research on the effect of FR on tomato pathogen resistance mechanisms is absent.

In this chapter, we demonstrate that supplemental FR illumination of tomato plants induces strong morphological changes as well as a dampening of defense responses towards *B. cinerea*. This mechanism seems to be partly regulated by phyB in tomato. Interestingly, the increased susceptibility relies on an extended WL+FR exposure prior to the pathogen challenge and appears to be a robust phenomenon occurring under distinct light backgrounds.

Results

FR-enriched light elicits a strong elongation response in tomato.

In order to investigate the morphological changes induced by FR light enrichment, tomato plants cv. Moneymaker were grown for three weeks in white LED light conditions (WL). The plants were treated for five days in WL supplemented with FR LEDs (WL+FR) or in WL as a control (fig. S2.1) and growth parameters were recorded every day (fig. 2.1). Upon additional FR exposure, the plants exhibit a strong stem and petiole elongation of the leaves n°3 and n°4 from the bottom compared to WL conditions (fig. 2.1B and D). Surprisingly, tomato plants did not show a hyponastic response, a typical shade avoidance response in *Arabidopsis*. Instead, the leaf angle of WL+FR-illuminated plants remained constant while the WL-treated plants exhibited some epinastic leaf movement over time (fig. 2.1C), probably as a result of aging. Although, an increase in specific leaf area (SLA) was observed in *Arabidopsis* upon shade (Evans and Poorter, 2001), tomato plants showed an increased lamina dry mass and area but no difference in SLA (fig. 2.1E-G). Interestingly, even though the leaf area increases slightly, the leaf thickness remained unchanged for leaves already formed before the start of the WL+FR treatment (fig. S2.2).

FR light enrichment duration matters for the modulation of tomato immunity

To study the effects of light quality on plant immunity, tomato plants were pretreated in WL or WL+FR prior to drop-inoculation with *B. cinerea* spores (fig. 2.2A). In order to investigate the effect of the WL+FR treatment duration on tomato immunity, leaflets were infected after being exposed to WL or WL+FR for 0, 1, 3 or 5 days. Interestingly, the lesion area was significantly increased on WL+FR-treated leaflets compared to WL after three and five days after the start of the FR enrichment but not before (fig. 2.2B) indicating that the effect of low R:FR on immunity in tomato is not an immediate but more of a gradual process. Also, the susceptibility of the WL+FR-treated plants was much higher after five days of WL+FR pretreatment compared to other time points (fig. 2.2B) indicating that the effect of FR light on immunity increases with the duration of WL+FR exposure. Similar results were obtained on whole plant systems after five days of WL or WL+FR pretreatment by using a small clip cage allowing a local high humidity for the infection to succeed (fig. S2.3). We also investigated the effect of gradual phytochrome inactivation by progressively increasing the intensity of FR (fig. 2.2C). For this experiment, we took into account PSS values (Photo-Stationary State of the phytochrome) as a proxy for phytochrome inactivation ranging from WL (PSS = 0.8), corresponding to active phytochromes, to FR-enriched conditions used throughout this chapter (PSS = 0.5). Interestingly, the enhanced susceptibility of the plants was closely correlated with the intensity of FR added in the environment (fig. 2.2C) indicating a phytochrome-dependent mechanism acting through a progressive effect on immunity, an effect that facilitates lesion development. Based on the results shown in fig. 2.2B, we tested whether the addition of supplemental FR would modulate tomato immunity towards other pathogens such as another strain of *B. cinerea*, isolated from pepper (Denby et al., 2004), the hemobiotrophic bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 and the oomycete *Phytophthora infestans* (fig. 2.3). Tomato leaflets were inoculated with the different pathogens after five days in either WL or WL+FR conditions. For all pathogens tested, the presence of FR enhanced symptom development in tomato compared to WL conditions (fig. 2.3) showing that FR-induced susceptibility in tomato is a general mechanisms that seems to be pathogen-independent.

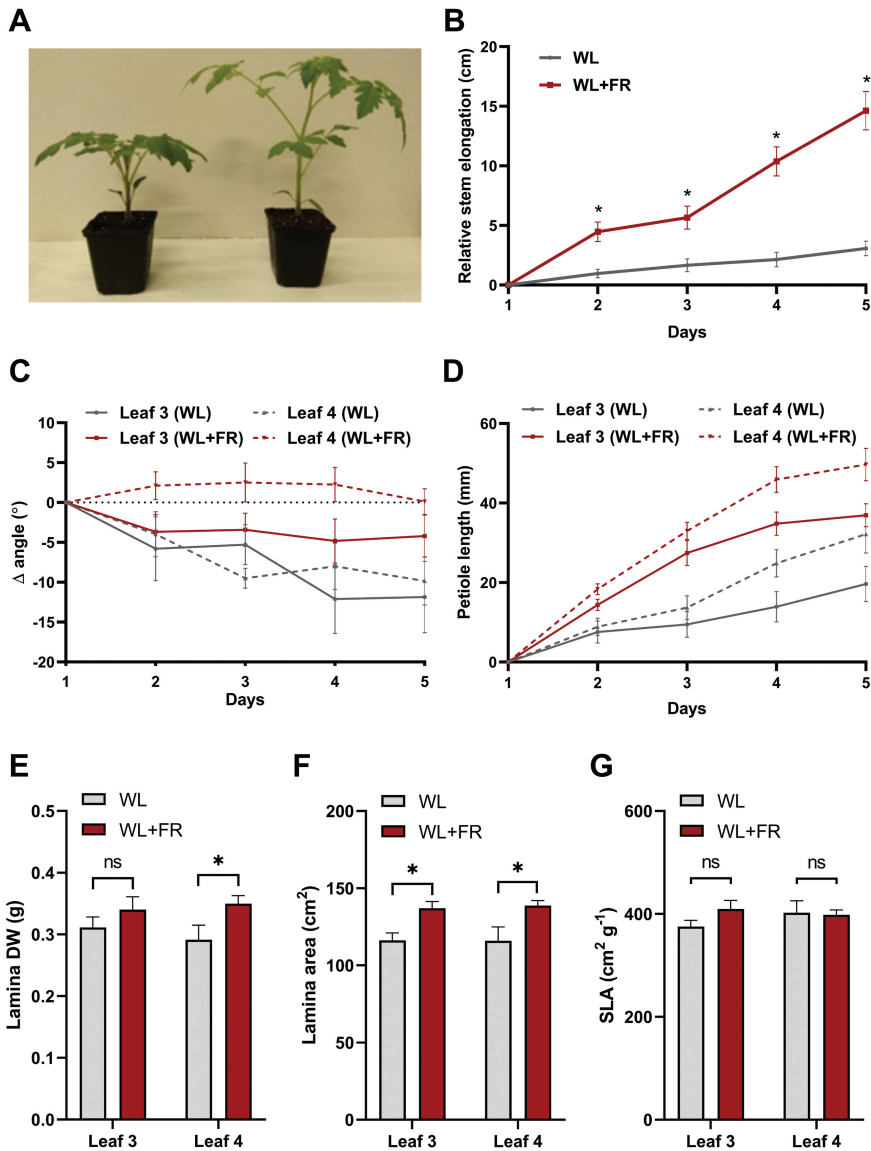


Figure 2.1 : FR-enriched light enhances tomato growth. (A) Pictures and (B) stem elongation measurements of three-week-old tomato cv. Moneymaker plants after five days in WL (A; left picture; R:FR ratio = 5,5; PSS = 0.8) or WL+FR (A; right picture; R:FR = 0,14; PSS = 0.5). (C) Leaf angle as well as (D) petiole elongation measurements on the leaf n°3 and n°4. Measurements were performed at day 1 to day 5 under WL or WL+FR light treatment. (E-G) Lamina dry weight and area measurements for leaf n°3 and n°4. Both parameters were used to determine the specific leaf area of both leaves under the two light treatments. Data represent mean ± SEM, n = 8. Asterisks represents significant differences ; Student's t-test; p-value < 0,05. ns corresponds to a non-significant difference between two groups.

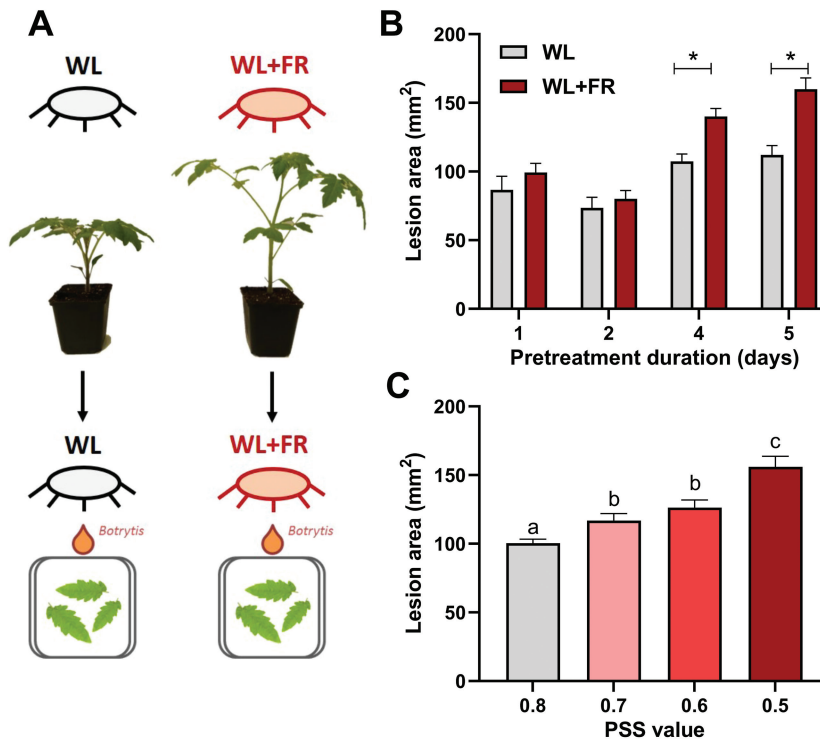


Figure 2.2 : Relative FR fluence rate and duration influence tomato immunity. (A) Scheme representing the experimental setup used for the bioassays. Plants were pretreated in white light (WL) or WL supplemented with far-red LEDs (WL+FR) prior to inoculation with *B. cinerea* spores on detached leaflets. The infection took place in the same light conditions as for the pretreatments. Disease rating on three-week-old tomato leaflets drop-inoculated with *B. cinerea* spores (B) after 0, 1, 3 and 5 days in WL or WL+FR conditions or (C) after 5 days of WL (PSS value = 0,8) and WL+FR treatment with increasing FR intensity (PSS values = 0,7; 0,6 and 0,5). Plants were scored after 3 dpi. Data represent data \pm SEM. Asterisks (Student's t-test, p-value < 0.05) and different letters (ANOVA, Tukey's post-hoc test) indicate significant differences. n = 7 - 8 plants per light treatment.

Both FR-enrichment and genetic phyB disruption enhance susceptibility

To study the involvement of phyB in the FR-induced susceptibility in tomato, we used the *phyB1phyB2* double mutant as tomato plants have two phyB-encoding genes (Pratt et al., 1995). Unlike the *Arabidopsis phyB* mutant, *phyB1phyB2* mutant plants were still quite responsive to WL+FR after five days (fig. 2.4B). This would suggest a role of other phytochromes in the elongation response. Nevertheless, *phyB1phyB2* displayed the classic, very severe constitutive shade avoidance phenotype irrespective

of light quality, consistent with their role in shade avoidance control (fig. 2.4A and 2.4B). In terms of susceptibility, *phyB1phyB2* mutants show a constitutively increased susceptibility to *B. cinerea* (fig. 2.4C). The lesion area measured on *phyB1phyB2* mutant in WL is similar to Moneymaker plants in WL+FR conditions (fig. 2.4C) which shows that the mutation or inactivation of phyB leads to a higher susceptibility in tomato towards *B. cinerea*.

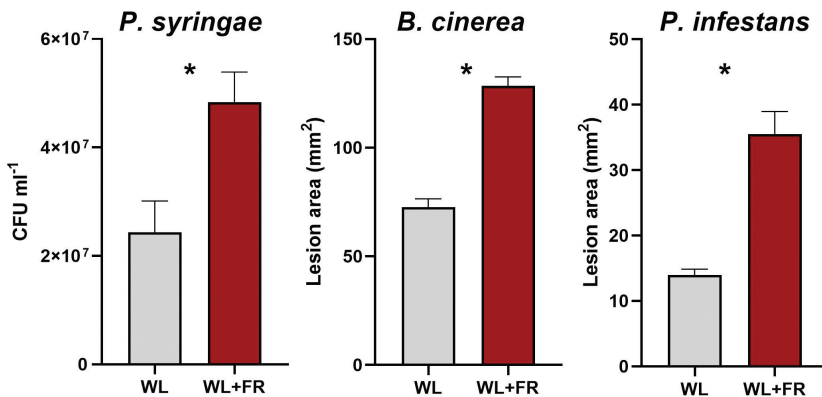


Figure 2.3 : FR light enhances plant susceptibility to various pathogens. Disease rating on tomato leaflets pretreated in WL or WL+FR for five days, infected in the same conditions by a pepper isolate of *Botrytis cinerea*, *Phytophthora infestans* and *Pseudomonas syringae* pv. tomato DC3000. Data indicate mean + SEM. Asterisks represent significant differences (Student's t-test; p-value < 0,05), n = 8 plants.

FR-enriched light triggers higher susceptibility before a pathogen attack

We investigated the effect of a five day light treatment (WL or WL+FR) applied before and/or during interaction with *B. cinerea*. After five days of light pretreatment in WL or WL+FR, leaflets were detached and inoculated with *B. cinerea* spores (fig. 2.5A). Half of the inoculated WL-treated leaflets were placed in WL+FR and the other half in WL (WL-FR and WL-WL respectively). The same procedure was carried out with the inoculated WL+FR-treated leaflets (FR-WL or FR-FR) (fig. 2.5A). The lesion area was measured at 3 dpi (days post inoculation) and was clearly more expanded on the leaflets treated with WL+FR before inoculation (FR-WL and FR-FR conditions) compared to WL-treated plants (fig. 2.5B). Surprisingly, the outcome of the infection was not influenced by the light quality applied after inoculation. These results indicate that the infection outcome is strongly correlated with the light quality that plants experienced before the inoculation takes place. We could associate a larger lesion

size with a higher *B. cinerea* genomic DNA content in WL+FR-treated tissue from 30 hpi onwards showing a positive effect of supplemental FR on *B. cinerea* development *in planta* (fig. 2.5C). A WL+FR light pretreatment mediates the FR-induced susceptibility in tomato resulting in an increase of *B. cinerea* development in infected plant tissue.

FR light promotes *B. cinerea* biomass production *in vitro*.

Since we observed an increase in *B. cinerea* gDNA in WL+FR-treated plants (fig. 2.5C), we also investigated the direct effect of WL+FR light on *B. cinerea* growth *in vitro*. We measured the newly-formed mycelium diameter after depositing and incubating a 5 μ l-droplet of spore solution onto nutrient medium in WL or WL+FR conditions. After three days, we did not observe any significant difference in mycelium diameter between the two light treatments (fig. 2.6B). We then also studied the effect of light quality on biomass production by placing 1 ml of spore suspension on a sterile mesh membrane and measured the weight of the membrane before and after three days in WL and WL+FR light conditions. Strikingly, the biomass production was increased by 30% in FR-enriched conditions compared to WL in our conditions (fig. 2.6B) showing the substantial effect of supplemental FR light on mycelium biomass production but not its 2D expansion *in vitro*.

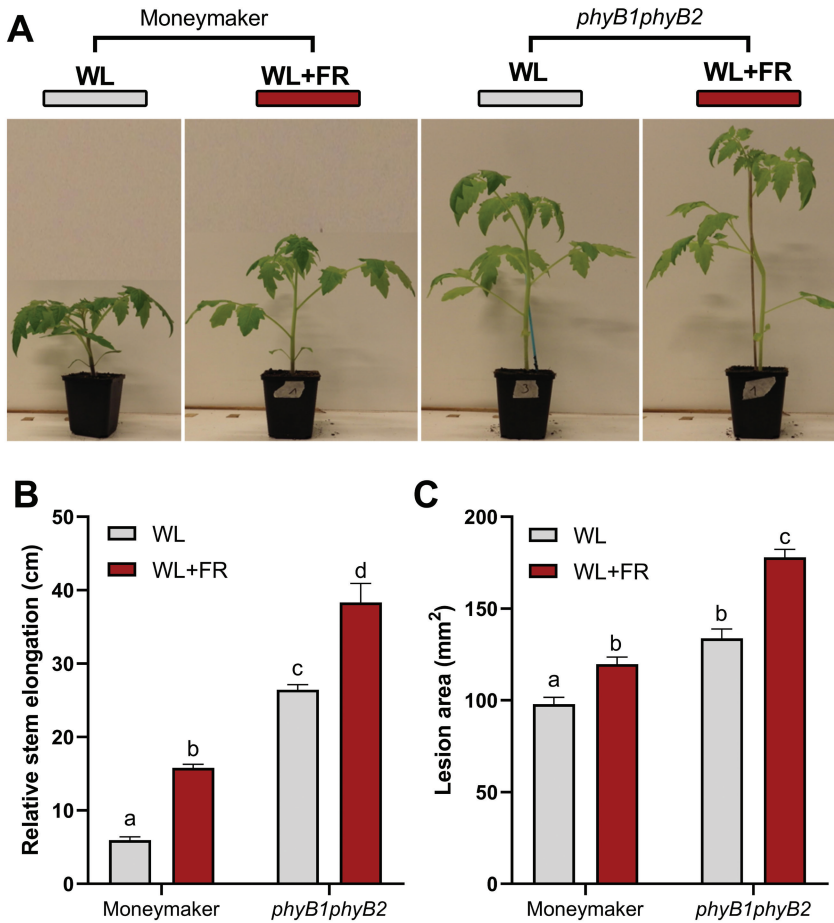


Figure 2.4 : The *phyB1phyB2* double mutant displays enhanced susceptibility in WL conditions (A) Pictures and **(B)** relative stem elongation measurements of Moneymaker and *phyB1phyB2* at day 5 in WL and FR compared to day 1. **(C)** Disease rating on three-week-old tomato leaves drop-inoculated with *B. cinerea* spores after a WL or WL+FR treatment before and during inoculation. Plants were scored after 3 dpi. Data represent mean \pm SEM and different letters indicate significant differences (ANOVA, Tukey's post-hoc test; p-value < 0,05), n = 8.

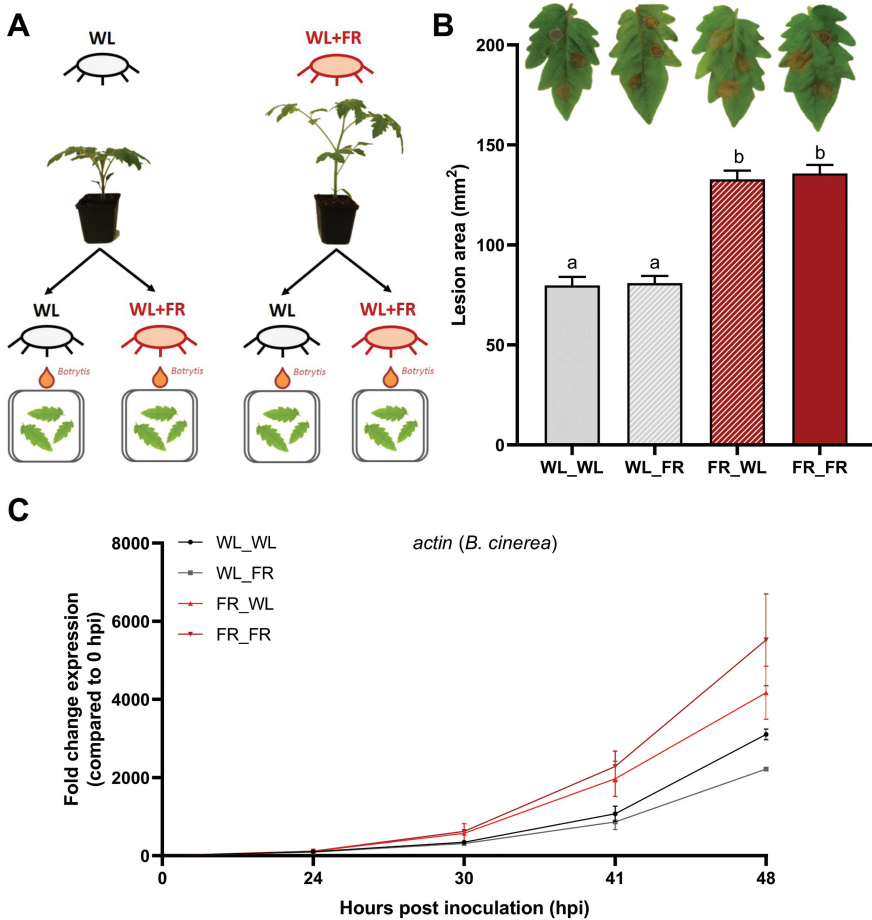


Figure 2.5 : Tomato susceptibility is triggered by FR exposure before *B. cinerea* infection. (A) Scheme of the infection setup and (B) disease rating on three-week-old tomato leaves drop-inoculated with *B. cinerea* spores after a WL or WL+FR pretreatment before and after inoculation. Plants were scored after 3 dpi. (C) *B. cinerea* gDNA was also measured at 24, 30, 41 and 48 hpi (hours post inoculation) in infected plant tissue. Data represent mean \pm SEM, n= 7 – 8 plants. Different letters represent significant differences (ANOVA, Tukey’s post-hoc test; p-value < 0,05).

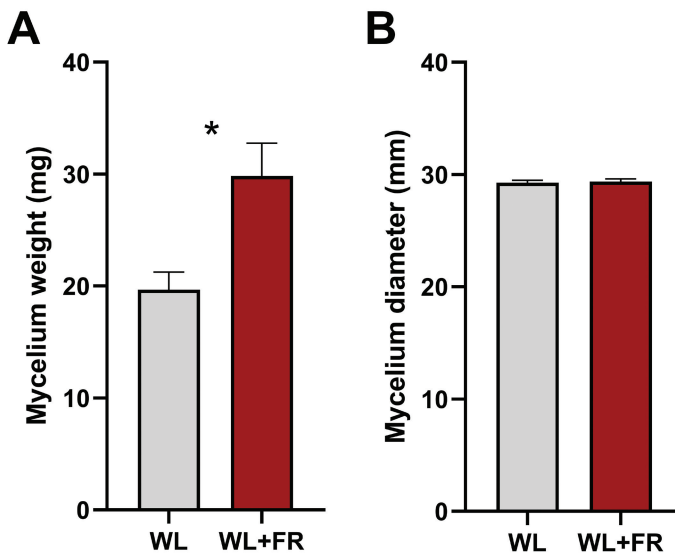


Figure 2.6: FR affects *B. cinerea* growth *in vitro*. (A) Biomass and (B) newly grown mycelium diameter on ½ PDA medium after three days in white light (WL) or white light supplemented with FR light (WL+FR). Data represent mean \pm SEM, n = 10. Asterisk represents statistical difference (Student's t-test, p-value < 0,05).

FR-induced shoot elongation and susceptibility are independent of the background light.

Red (R) and blue (B) light are known to be the major fuel for photosynthesis and are now used in greenhouses to enhance plant growth. In order to test the effect of R and B (RB) LED lighting on the resistance of tomato plants, we compared our results previously obtained under white LED background with RB conditions (spectra are displayed in fig. S2.1). Tomato plants were treated either under control conditions (RB) or under RB supplemented with FR LEDs (RB+FR). Upon addition of FR, the plants exhibited a strong stem elongation that was also observed in WL+FR conditions (fig. 2.7A and fig. 2.1B). However, we observed petiole elongation from the leaf 3 and a trend for the same in leaf 4 in response to RB+FR compared to RB (fig. 2.7B). Interestingly, when we progressively increased the FR intensity from RB (PSS = 0.9) to RB+FR (PSS = 0.6), we observed that the plants showed a maximum susceptibility already at a PSS of 0.8 which did not increase further at 0.7 and 0.6. This is different from the gradual increase in susceptibility seen in the WL background (fig. 2.2C). However, when the plants are exposed to RB or RB+FR before and/or during

the infection process, the infection outcome was influenced by the pretreatment light conditions but also by the light treatment used during the infection (fig. 2.7D). Altogether, these results show that under RB light background, similar to the WL background, the addition of FR light triggers shoot elongation as well as an increase in plant susceptibility indicating that FR is dominant factor in enhancing plant susceptibility independently of the overall light background.

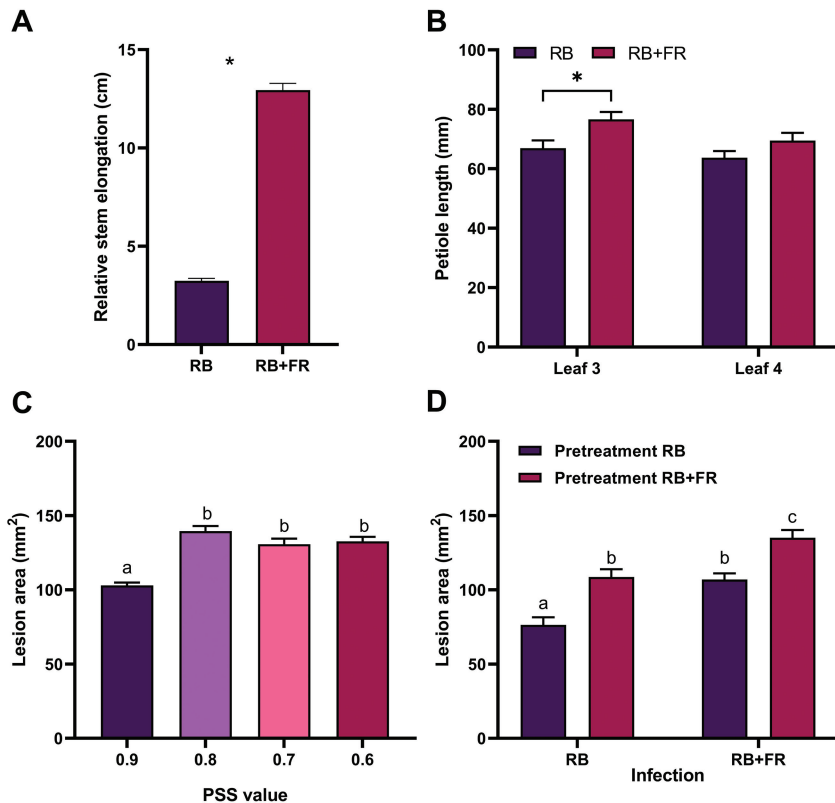


Figure 2.7 : The FR-induced shoot elongation and susceptibility is independent of the background light quality. (A) Stem and (B) petiole elongation measurements after five days under red and blue LEDs (RB) or RB supplemented with far-red LEDs (RB+FR) compared to day 1 (Data represent mean \pm SEM. Asterisks represent significant differences, Student's t-test, p-value < 0,05, n = 7 - 8 plants). Growth measurements were performed prior to inoculation with *B. cinerea* spores on detached leaflets. Disease rating on three-week-old tomato leaves drop-inoculated with *B. cinerea* spores (C) after five days in RB (PSS value = 0,9) and RB+FR treatment with increasing FR intensity (PSS values = 0,8; 0,7 and 0,6) or (D) after a 5-day RB or RB+FR pretreatment before and after inoculation. Plants were scored after 3 dpi. Data represent mean \pm SEM and different letters indicate significant differences (ANOVA, Tukey's post-hoc test; p-value < 0,05), n = 7 - 8 plants.

Discussion

The shade avoidance syndrome allows plants to outgrow neighboring vegetation and secure their light capture, a process that often goes accompanied by inhibition of resistance mechanisms against pathogens and herbivores. In this chapter, we showed that a FR-enriched light environment leads to strong architectural changes such as stem and petiole elongation.

Our results indicate that tomato plants require at least three days of supplemental FR exposure before exhibiting an increased susceptibility to *B. cinerea* (fig. 2.2B). This phenomenon points towards an effect of phytochromes on immunity by gradually, but progressively changing aspects of the plant. Perhaps FR-induced changes in plant architecture facilitate pathogen entry or growth inside plant tissue. Since we observed no changes in the specific leaf area, which is indicative of the number of cell layers in the leaf, after five days of WL+FR exposure compared to WL (fig. 2.1G), we do not expect that the number of colonizable cells would be different enough between WL and WL+FR to explain the different lesion areas. This idea was also confirmed by the fact that leaves already fully expanded before the start of the WL+FR treatment have no changes in thickness indicating that the increased susceptibility is due to other parameters (fig. S2.2). Even though WL- and WL+FR-treated leaves have similar thickness, we cannot exclude a possible effect of supplemental FR on cuticle or cell wall thickness which are the principal barriers that pathogens need to cross at the leaf surface (Ziv et al., 2018). The increased lesion size in WL+FR as compared to WL is indicative of increased fungal growth in plant tissue based on determining *B. cinerea* genomic DNA levels (fig 2.5C). Although WL+FR promotes *B. cinerea* biomass, but not 2D expansion, on plates (fig. 2.6), this is unlikely to contribute directly to the increased lesions sizes since these also develop in the absence of FR during the infection (fig 2.5B).

It is possible that WL+FR would affect tomato defense mechanisms against *B. cinerea* in a similar way as in Arabidopsis by dampening JA-associated defense gene expression in a phyB-dependent manner (Cargnel et al., 2014; Cerrudo et al., 2017; De Wit et al., 2013). Bioassays performed on the *phyB1phyB2* double mutant in tomato partly confirmed this hypothesis. The mutant displayed a constitutively higher susceptibility to *B. cinerea* compared to MoneyMaker and still exhibited an increased susceptibility upon WL+FR exposure compared to WL conditions (fig. 2.4C). This would suggest the additional involvement of other phytochromes in the FR-induced susceptibility in

tomato. Recent studies revealed an important role of phyE in shade avoidance in tomato when phyB1 and phyB2 were absent (Schrager-Lavelle et al., 2016) opening the possibility of phyE playing a role in modulating directly or indirectly immune responses in tomato. The hypothesis of FR dampening JA-associated defense in tomato is also in line with studies showing that FR radiations leads to an increased susceptibility to chewing insects (Cortés et al., 2016; Izaguirre et al., 2006). However, it remains unclear why WL+FR exposure during infection is not effective, whereas pre-exposure is. One explanation could be the detachment of the leaflets prior to the inoculation that could interfere with short versus long distance signaling. Whatever it is that is controlled by phytochrome inactivation apparently has to build up through several days to become effective at the level of disease development. Direct exposure during infection has clearly no effect, and this does help generate hypotheses about the putative mechanisms underpinning these observations. For example, although FR modulation of JA-mediated defense gene expression may be key, we would expect a long-term, rather than an immediate impact of FR enrichment on increased disease development. Thus, we could speculate about a change in the leaf surface properties through time or a progressive build-up of primary or secondary metabolites that could explain our observations rather than for example the immediate abundance of JAZ transcription factors themselves. A change in primary and/or secondary metabolism could explain why we found that supplemental FR light enhances tomato susceptibility not only against *B. cinerea* but also towards hemibiotrophic pathogens such as the bacterial pathogen *P. syringae* and the oomycete *P. infestans* which have very distinct ways to colonize plants and mediate different hormonal pathways upon infection (fig. 2.3).

Interestingly, the results obtained under both WL and RB light background were relatively similar in terms of shoot elongation and disease resistance (fig. 2.2 and fig. 2.7). Altogether, these observations demonstrate that the increased plant susceptibility relies on the presence of FR in the environment which is confirmed by the fact that susceptibility is gradually increased as the intensity of FR increases in the WL background (fig. 2.2C). This shows that the FR-induced susceptibility is a cumulative process and could get worse at increasing intensities. However, in the RB background, we already observed a dramatic increase in susceptibility at PSS = 0.8 compared to the RB control (PSS = 0.9) that cannot be exceeded at higher FR intensities (fig. 2.7C). This can be due to the extremely low R:FR in RB control conditions (R:FR > 99), compared to WL (where R:FR = 5.5), meaning that the plants

were grown in quasi-absence of FR light. The plants suddenly experience FR for the first time as the 5-day pretreatment starts which might drastically influence disease resistance irrespective of the intensity of FR added in the environment. Recently, we achieved similar results in greenhouse settings where tomato susceptibility was increased upon FR enrichment in RB backgrounds showing the robustness of the FR-induced susceptibility response in tomato (Ji et al., 2019).

Conclusion

In conclusion, this chapter describes the effect of supplemental FR radiation on tomato architecture and defense responses against the necrotrophic pathogen *B. cinerea*. Our results show the interplay between growth and defense in a phytochrome-dependent manner. These data show the importance of the light quality given to the plants for growth and immunity, and constitute a solid base to study the effect of FR light on defense mechanisms at the gene expression level at early infection stages. We aim to dissect the FR-induced susceptibility based on transcriptomics studies shown in **chapter 3**.

Acknowledgements

We would like to thank Ankie Ammerlaan for performing the leaf thickness measurements. We thank Prof. Katherine Denby from the University of York (United Kingdom), Dr. Jan van Kan and Prof. Francine Govers from Wageningen University & Research (the Netherlands) for kindly providing *Botrytis cinerea* pepper, 05.10 and *Phytophthora infestans* 88069, respectively. We also kindly thank all members from the “LED it be 50%” program for advice and useful feedback along the project. This work was funded by the Dutch Research Council, TTW Perspectief grant nr 14125 (“LED it Be 50%”) and supported by Signify, WUR Greenhouse Horticulture and LTO Glaskracht.

Material and methods

Plants growth conditions and light treatments

Seeds from tomato cv. Moneymaker and the *phyB1phyB2* double mutants were sown in vermiculite. After 10 days, tomato seedlings were transferred into 9 x 9 cm pots with regular potting soil (Primasta® soil, the Netherlands). Tomato plants were grown

for four weeks after sowing in climate chambers (MD1400; Snijders, the Netherlands) in long day photoperiod (8 h dark / 16 h light) at $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) using the Philips GreenPower LED research modules (Signify B.V.) white (WL, R:FR = 5.5) or red and blue (RB, R:FR > 99) reaching a Phytochrome Stationary State (PSS) value of 0.8 and 0.9, respectively. For additional FR treatments, plants were exposed for five days (started 3 h after dawn on the first day) under WL or RB supplemented with Philips GreenPower LED research modules FR referred to as WL+FR (R:FR = 0.14, PSS value = 0.5) and RB+FR (R:FR = 0.7, PSS value = 0.6), respectively. The first two lateral leaflets of the 3rd leaf were used for the experiments. Light spectra used in this experiment are displayed in fig. S2.1.

2

Growth measurements

Stem and petiole length were measured with a digital caliper. Petiole angles were measured from pictures with the ImageJ software. All measurements were calculated as $t_{\text{day5}} - t_{\text{day1}}$.

Leaf thickness measurements

Fresh leaf pieces were cut and immediately incubated in 1-2 ml of Karnovskii's fixative solution: 2.1 % formaldehyde, 2.5 % glutaraldehyde and 0.1 M phosphate buffer (0,2 M NaH_2PO_4 ; 0,2 M Na_2HPO_4) at pH = 7. The samples were vacuumed 10-15 min for a proper fixation and slowly shaken for 1 h before 3 washes with MilliQ. Samples were dehydrated by adding 1-2 ml of ethanol 30 % and replacing the ethanol every 30-60 min from 30 % to 96 %. Dehydrated leaf samples were embedded following the Technovit® 7100 plastic embedding system and shaped into a trapezoid with until the leaf tissue was visible in the middle. Sections of 10 μm were made using a Leica Om-U3 microtome and put on a microscope slide to dry on a heating plate (80 °C) supplemented with a drop of MilliQ. The sections were stained with 0.5 % toluidine blue for 30-60 sec and washed thoroughly with MilliQ. Images were taken and leaf thickness was measured using a Olympus fluorescent microscope BX50-WI.

Pathogen growth conditions and bioassays

Botrytis cinerea

B. cinerea strain Bc 05.10 and strain pepper (Denby et al., 2004) were maintained on half strength Potato dextrose agar medium (PDA ½) and grown for approximately 2 weeks under natural daylight conditions. The spore suspension was prepared according to Van Wees et al. (2013) and diluted to a final concentration of 1.5×10^5 spores ml^{-1} in half strength Potato dextrose broth (PDB ½) prior to the inoculation. Bioassays were performed on detached tomato leaflets previously pretreated in WL or WL+FR for five days. The adaxial side of the leaflets were drop-inoculated 3-6 times with 5 μl of spore suspension. Pictures were taken 3 days post inoculation (dpi) and lesion areas were measured using the software imageJ.

Phytophthora infestans

P. infestans strain 88069 (Kamoun et al., 1997) was maintained on Rye sucrose agar medium (RSA) for 10 days in darkness at room temperature. Cultured plates were flooded with 10 ml of sterile MilliQ water and incubated at 4°C for 3 h. Zoospores were collected, counted and adjusted to a concentration of 5×10^4 zoospores ml^{-1} prior to the bioassays. The abaxial side of detached tomato leaflets were drop-inoculated several times with 10 μl of zoospores suspension. The lesion areas were measured after 3 dpi with the ImageJ software. Statistical analysis was performed with Student's t.test, p-value <0.05.

Pseudomonas syringae

P. syringae pv. tomato DC3000 (Whalen et al., 1991) was grown in liquid King's medium B (KB) and incubated overnight in an orbital shaker at 220 rpm at 28°C. The next day, the bacterial culture was centrifuged at 7800 rpm for 5 min. The bacterial pellet was washed 3 times with sterile MilliQ water and resuspended in 10 mM MgSO_4 . The inoculum was adjusted to $\text{OD}_{660} = 0.025$ ($\text{OD}_{660} = 1 = 10^9$ cells mL^{-1}) and supplemented with 0.02 % Silwet L-77. The two first lateral leaflets of the 3rd leaf of WL- and WL+FR-pretreated intact tomato plants were dipped in the bacterial inoculum for 3 seconds and placed in their respective light treatment for 3 days at 95% RH. Bacteria were extracted by grinding 2 leaf discs from each leaflets in 400 μl of 10 mM MgSO_4 and diluted from 10^{-2} to 10^{-5} . The dilution series was plated and incubated at 28°C on KB medium supplemented with 25 $\mu\text{g ml}^{-1}$ rifampicin until colony forming units (cfu) appear. Bacterial growth was calculated in cfu per leaf discs.

qPCR on *B. cinerea* gDNA

B. cinerea gDNA was extracted from three infected leaf discs per samples, ground and incubated at 65°C for 45 min in 250 µl of DNA extraction buffer (EDTA 25 mM, Tris-HCl 250 mM, NaCl 250 mM and SDS 1%). The samples were supplemented with 250 µl of PCI and centrifuged for 7 min at full speed. The upper phase was transferred into a new tube and 250 µl of ice-cold isopropanol were added. Precipitated DNA was pelleted by spinning 7 min at full speed prior to 2 washes in EtOH 70%. The air dried pellets were eluted in 100 µl of MilliQ and stored at -20°C. All DNA samples were diluted to 5 ng µl⁻¹ and 15 ng µl⁻¹. The qPCR reaction volume was 5 µl and was performed with SybrGreen Supermix (Bio-Rad) and specific primer for *B. cinerea actin* (Fw : GCCAGTCAATCCTCCAGCTT ; Rv : AATCGACCGTGATACGCTGT and *tomato elongation factor α* (Fw : TTTGCTTTAATTCGTAGATGGAA ; Rv : CGAGGTTGGTAGACCTCTCA) in a Vii7 PCR machine. Amplification data were analyzed using the 2^{-ΔΔCt} method (Livak and Schmittgen, 2001).

In vitro growth assays

B. cinerea mycelium diameter measurements were performed by depositing a 5 µl droplet of a 1.5 x 10⁵ spores ml⁻¹ solution onto PDA ½ plates and incubated for three days in WL or WL+FR conditions. The newly grown mycelium diameter was measured by using a digital caliper. For biomass assays, a sterile mesh membrane was placed onto PDA ½ plates and soaked with 1 ml of 1.5 x 10⁵ spores ml⁻¹ suspension. The plates were sealed and incubated for three days in WL or WL+FR and the membranes were detached from the plates, dried and weighted. The mycelium biomass was calculated as $m_{\text{day3}} - m_{\text{day0}}$.

Supplemental data

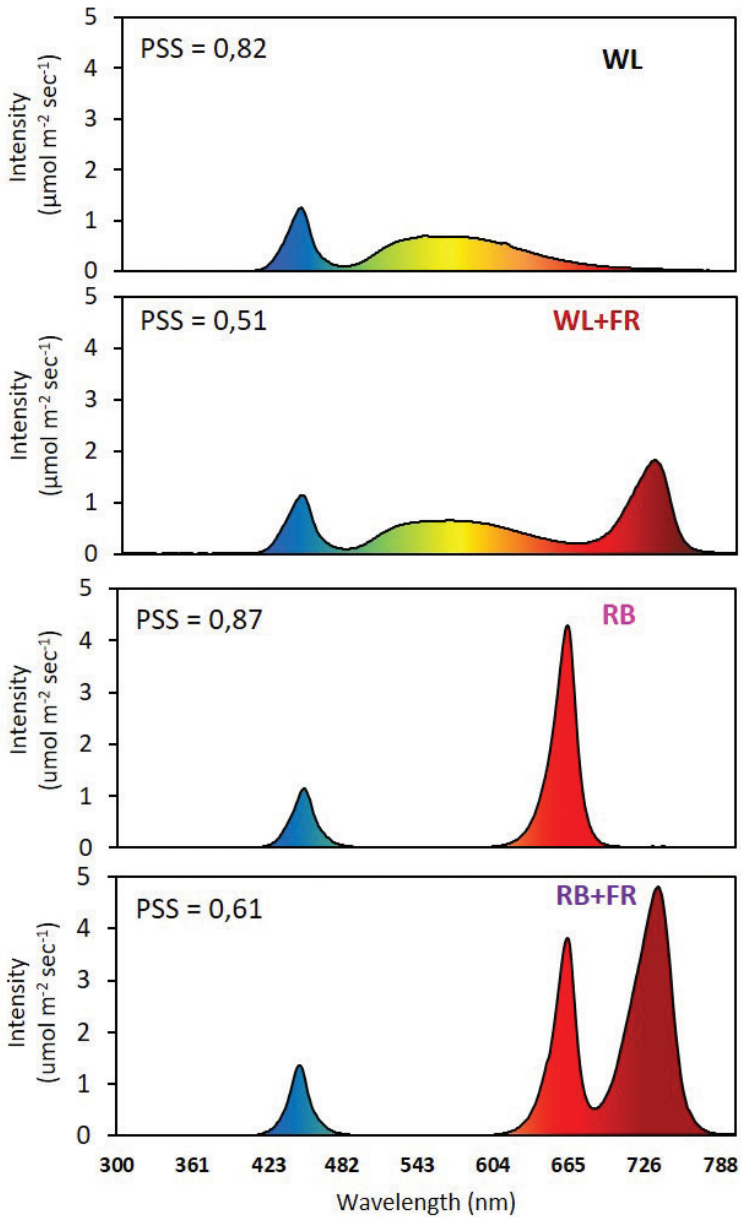


Figure S2.1 : LED spectra and color composition used for the experiments described in this thesis. White LEDs (WL) , White + additional FR LEDs and Red and Blue LEDs (RB) supplemented or not with FR LEDs.

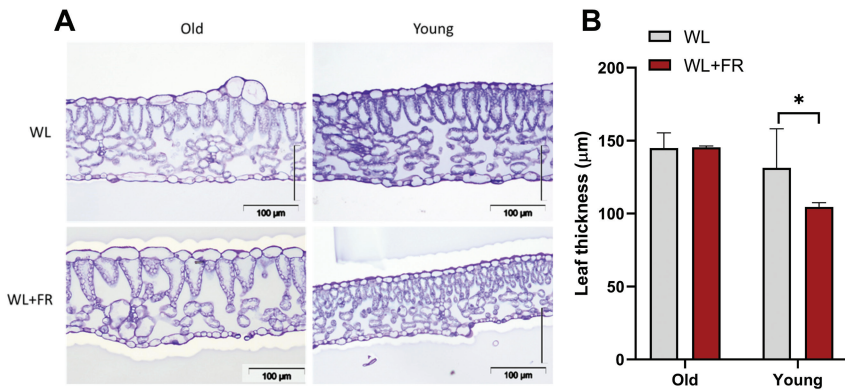


Figure S2.2 : Low R:FR influences newly formed leaf thickness. (A) Cross sections and (B) leaf thickness measurements on 4 week old tomato leaflets after five days in WL and WL+FR light conditions. Measurements were performed on fully expanded and newly-formed leaflets. Asterisk represents significant difference, Student's t-test, p-value < 0,05, n = 4 plants.

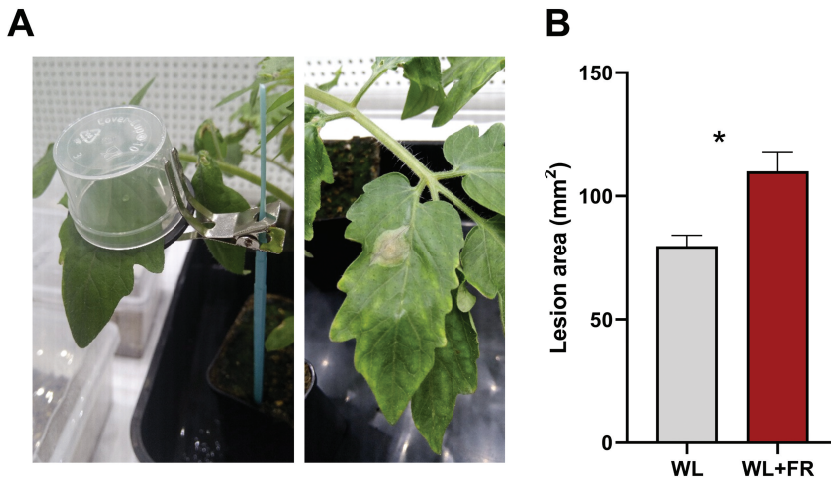
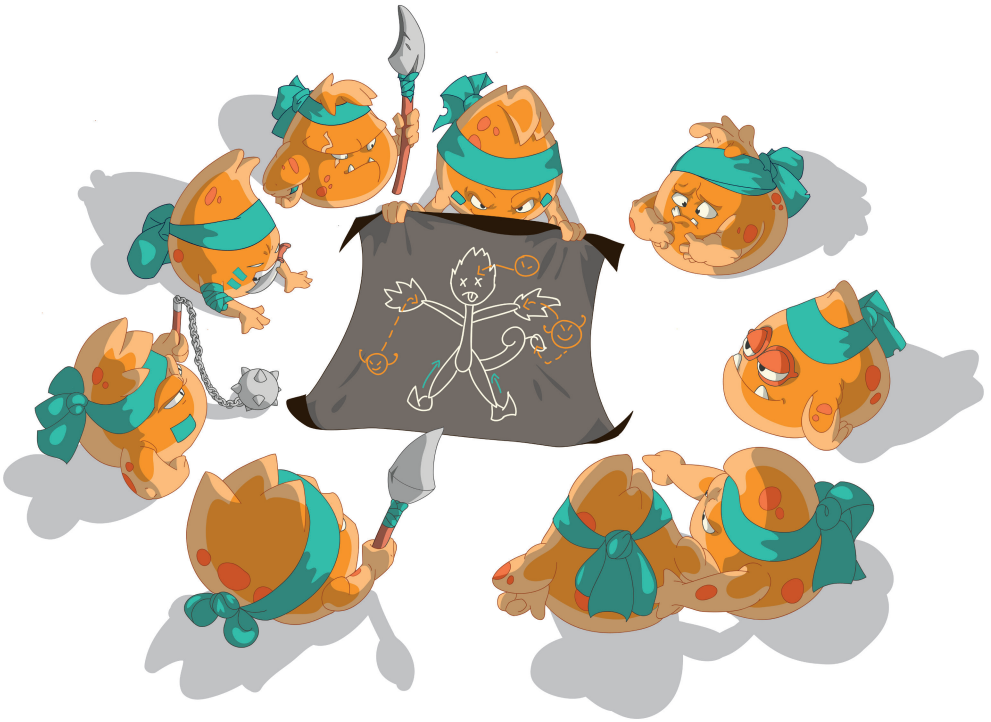


Figure S2.3 : FR light increases susceptibility in whole plant system. (A) Pictures of the high local humidity clip device consisting of a bended hair clip glued onto a polyethylene terephthalate (PET) cup and a rubber ring to avoid wounding. After three days of infection by *B. cinerea* in WL and WL+FR, (B) lesions were measured with the ImageJ software (Asterisk represents significant difference (Student's t-test ; p-value < 0,05).



Mod

Chapter

3

Transcriptome changes of tomato induced by *Botrytis cinerea* infection are modulated by far-red light

Sarah Courbier¹, Basten L.B. Snoek³, Kaisa Kajala¹,
Saskia C.M. Van Wees² & Ronald Pierik¹

¹*Plant Ecophysiology, Institute of Environmental Biology, Utrecht
University, the Netherlands*

²*Plant-Microbe Interactions, Institute of Environmental Biology,
Utrecht University, the Netherlands*

³*Theoretical Biology and Bioinformatics, Institute of Biodynamics
and Biocomplexity, Utrecht University, the Netherlands*

Abstract

Plants absorb red (R) and blue (B) light to fuel photosynthesis and are able to detect high planting density through the reflection of far-red light (FR) from neighboring plants leading to a decrease in the R:FR ratio. In addition to eliciting shade avoidance responses, low R:FR has been shown to increase plant susceptibility to pathogens and herbivores known as “FR-induced susceptibility”. By using RNA sequencing at multiple time points, we aim to decrypt this phenomenon in the high value crop species tomato in response to a combination of low R:FR and infection by the necrotrophic fungus *Botrytis cinerea*, causal agent of serious yield losses in diverse crops. We show that exposure to FR-enriched light prior to the pathogen challenge strongly dampens and/or delays pathogen detection and subsequent defense-related gene induction upon infection with *B. cinerea*. Our data also indicate that FR-enrichment modulates genes associated with jasmonic acid (JA), ethylene (ET) and possibly salicylic acid (SA) upon infection. In addition, FR enrichment alone modulates a large number of genes associated with primary metabolism, suggesting that this process might be FR-dependent. These transcriptome data serve as an important starting point to unravel the mechanisms underlying FR-associated susceptibility of tomato to biotic attackers.

Introduction

Plants under stressful conditions make use of a broad set of defense mechanisms against biotic and abiotic stresses. Plant defense against pathogens is rather well understood as it has been extensively studied for the last decades (Anderson et al., 2010; Jones and Dangl, 2006). In the arms race between the plant and its pathogens both organisms keep evolving their arsenal to counteract each other. Plants have evolved to recognize specific and conserved signatures from attackers such as Microbe-, Herbivore- or Damage-Associated Molecular Patterns (MAMPs, HAMPs and DAMPs) inducing rapid downstream defense signaling cascades referred as “Pattern-Triggered Immunity” (PTI) (Ferrari et al., 2013; Hogenhout and Bos, 2011; Hou et al., 2019; Zipfel, 2014). The suppression of PTI by pathogen effectors or “Effector Triggered Susceptibility” (ETS) is crucial for disease establishment that can be counteracted by plants recognizing those effectors and inducing the “Effector-Triggered Immunity” (ETI ; Cui et al., 2015; Peng et al., 2018). However, the recognition of effectors by plants leads to programmed cell death, which contains biotrophic pathogens to the infection site while in the case of infection by a necrotrophic pathogen, that thrives on dying plant tissue, can result in increased infection rate (Govrin and Levine, 2000). Transcriptome analyses have been performed on *Arabidopsis thaliana* in interaction with the necrotrophic pathogen *Botrytis cinerea* focusing on the chronology of events during plant infection and colonization (AbuQamar et al., 2006; Windram et al., 2012). Other studies focused on the impact of single and combined stresses in *Arabidopsis* and revealed a drastic transcriptome reprogramming upon attack by *B. cinerea* (Coolen et al., 2016).

Interestingly, plant immunity has been shown to be modulated by far-red light (FR) or in response to shade through the phytochrome photoreceptors that detect changes in the relative abundances of red (R) and FR light in the environment (Ballaré, 2014; Ballaré and Pierik, 2017). Changes in the red: far-red ratio (R: FR) are triggered by the absorption of R light by neighboring vegetation and the subsequent reflection of FR within the plant canopy. In *Arabidopsis*, additional FR radiation has been shown to enhance plant susceptibility towards an array of pathogens showing a strong interplay between growth and defense signaling pathways (De Wit et al., 2013). Growing evidence shows that the FR-induced susceptibility in *Arabidopsis* is based on the connection between gibberellin and jasmonic acid (JA) signaling, namely between the negative growth regulator DELLAs and the negative defense regulator

JAZ, respectively (Cerrudo et al., 2012; Leone et al., 2014). In tomato, FR has been shown to enhance growth, fruit set and dry mass partitioning towards the fruits in tomato at the expense of defense responses against *B. cinerea* (Ji et al., 2019). In addition, *phyB1phyB2* double phytochrome mutants in tomato were shown to exhibit increased leaf damage caused by *Mamestra brassicae* caterpillars compared to wild type plants (Cortés et al., 2016). The inactivation of phyB in tomato also resulted in changes of the JA-associated pool of volatile organic compounds (VOCs) allowing plants to be more attractive to the predatory insect *Macroplophus pygmaeus* in turn promoting indirect defense (Cortés et al., 2016). Nevertheless, the exact role of phyB in the regulation of defense responses to pathogens and pests in tomato remains to be elucidated and requires further investigation.

In this chapter, we aim to unravel the effect of supplemental FR on tomato susceptibility towards *B. cinerea*. To our knowledge, it is the first time series experiment describing the chronology of events during tomato colonization by *B. cinerea* after a FR exposure. To this end, we performed an RNA sequencing experiment on white light (WL) controls and WL+FR-pretreated plants infected by *B. cinerea* spores, and followed the transcriptome changes through time. Here, we show that supplemental FR, received before pathogen inoculation, triggers FR-induced susceptibility, which is associated with a delay in pathogen recognition and defense activation via JA and ethylene signaling. Although hormones seem to have a major role in this process, our results also point towards an effect of FR enrichment on downregulating cell biogenesis-related processes which might benefit the growth of *B. cinerea* in plant tissue. We hypothesize that supplemental FR enhances plant susceptibility both in a direct and indirect manner mediated by defense hormones or primary metabolism respectively both leading to higher susceptibility towards *B. cinerea*.

Results

Transcriptomics on WL- and WL+FR-pretreated plants challenged by *Botrytis cinerea*.

In **chapter 2**, we demonstrated that supplemental FR exposure prior to inoculation has a negative effect on tomato susceptibility towards *B. cinerea*. Here, we aim to understand the mechanisms underlying this FR-induced susceptibility in tomato by investigating the associated transcriptome response. Expression data were obtained from leaf tissue pre-exposed to WL or WL+FR prior to being inoculated by *B. cinerea* spores (*B.c.*) or treated with a mock solution (mock) (fig. 3.1). During the infection, all detached leaflets were placed under WL conditions to prevent the growth promoting effect of FR light on *B. cinerea* observed in **chapter 2** (fig. 2.6A), and thus allowing us to study the plant responses specifically. Per treatment*time combination five leaf discs were collected per replicate at 0, 6, 12, 18, 24 and 30 hours post inoculation (hpi). The experiment consisted of 96 samples collected over two light pretreatment conditions (WL and WL+FR), two treatments (*B.c.* and mock), six time points and four replicates per sample (fig. 3.1). Gene expression analysis was performed using a 75-bp single-end NextSeq500 sequencing technique. All 96 libraries that carried a specific barcode were pooled and two sequencing runs resulted in 3.000.000 sequenced reads per library, which were mapped on the tomato reference genome version SL3.0 with ITAG3.20 annotations.

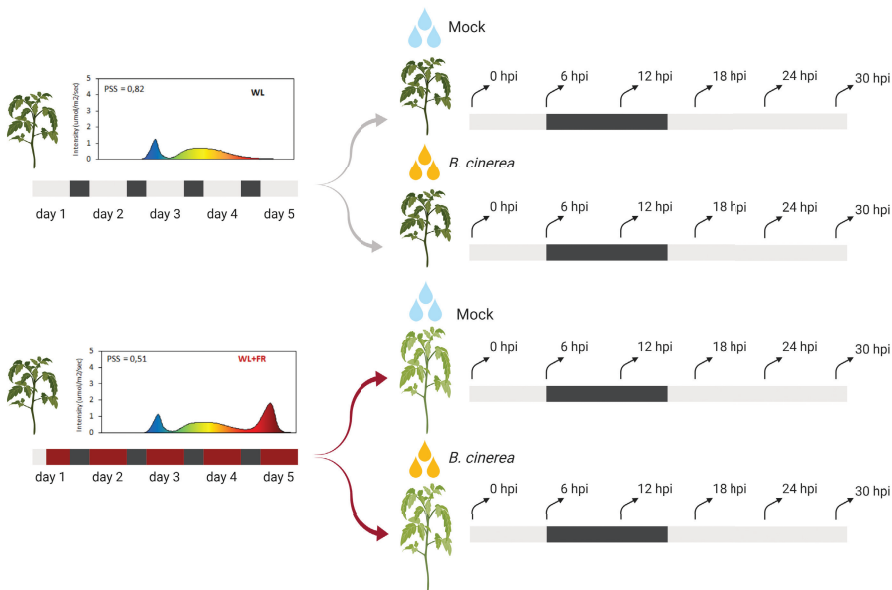


Figure 3.1 : Experimental set up and harvest time points for the time series of *B. cinerea* infection on tomato leaflets of WL- or WL+FR-pretreated plants. Plants were grown for three weeks in WL before five days of treatment in WL (grey) or WL+FR (red) condition before inoculation: Spectra represent the light background. The dark grey area represents an 8-h dark period. After five days of light pretreatment, leaflets were detached and drop-inoculated with *B. cinerea* spores or a mock solution as a control and harvested at 0, 6, 12, 18, 24 and 30 hpi represented by the arrows. The inoculation was performed under WL conditions.

Gene expression dynamics are affected by FR light and *Botrytis cinerea*.

We first determined the number of differentially expressed genes (DEGs) upon changes in light conditions in the mock-treated tissue and the effect of *B. cinerea* infection under the different light conditions at the different time points (fig. 3.2). We observed a strong gene modulation induced by WL+FR pre-exposure at 6 hpi and 12 hpi indicating the early effect of the light quality switch on the plant transcriptome (fig. 3.2A). On the contrary, *B. cinerea*-responsive genes are modulated at later time points, which is likely related to a time lap between the inoculation and the actual infection of the leaf cells. In WL-treated plants, *B. cinerea*-responsive genes start to be modulated from 12 hpi on and are strongly modulated at 24 hpi and 30 hpi (fig. 3.2B). Interestingly, although in WL at 12 hpi there was pronounced gene regulation, barely any DEGs were observed in response to infection in the WL+FR-treated samples at this timepoint, indicating a possible delay in defense gene activation (fig. 3.2C). Also, the

total number of modulated genes is much higher in WL (11784 DEGs) compared to WL+FR-treated samples (7939 DEGs) that could underlie *B. cinerea*-responsive gene expression dampening. In parallel, by performing principal coordinate analysis (PCoA), we observed similar patterns as in fig. 3.2 where the samples segregated according to the light treatment at 6 hpi and the infection at the later timepoint (24 hpi and 30 hpi) (fig. S3.1). Altogether, these observations predict drastic FR-mediated transcriptome changes of *B. cinerea*-responsive genes.

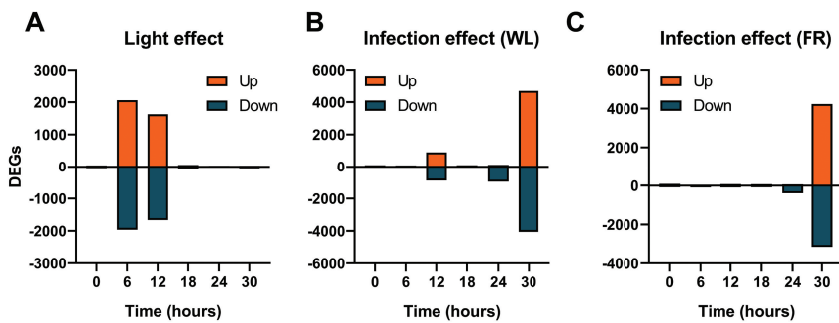


Figure 3.2: Number of differentially expressed genes (DEGs) through time upon mock treatment or *Botrytis cinerea* inoculation. Graphs showing the number of upregulated (orange) and downregulated (dark blue) DEGs by comparing mock-treated samples in WL+FR compared to WL (A), *B. cinerea*-inoculated samples compared to mock samples after a WL (B) or WL+FR pretreatment (C). Selected p-value < 0,001.

Light impacts gene expression at early timepoints

In order to investigate the direct effect of light quality on gene expression over time, we selected DEGs affected by the WL+FR pretreatment in the absence of the pathogen. Most of the FR-mediated gene modulation takes place in the first 12 h (fig. 3.2A and fig. S3.2). Surprisingly, at 0 hpi, only a hand full of genes were modulated (37 up and 41 downregulated genes), which may imply that light quality does not influence the tomato transcriptome after an extended period of time. By performing a GO terms enrichment on WL+FR-responsive genes for each timepoint, we observed the upregulation of GO categories related with oxidoreduction processes, regulation of transcription, proteasome activity and even glycolytic process indicating an effect of WL+FR pre-exposure on gene expression and metabolism through time (fig. 3.3A). To investigate further the effect of supplemental FR light on oxidoreduction-related processes occurring at 12 hpi that could affect oxidative stress responses, we performed a luminol-based hydrogen peroxide (H₂O₂) quantification in WL- and WL+FR-pretreated plants.

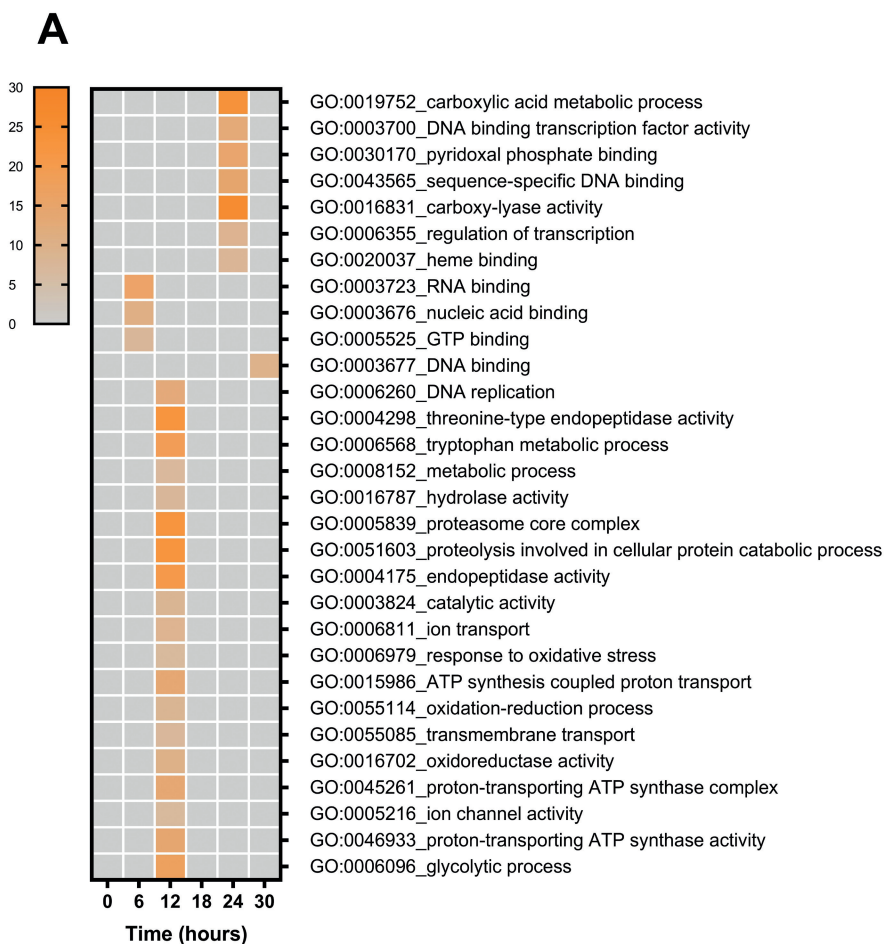


Figure 3.3: Gene ontology analysis on DEGs modulated by WL+FR pretreatment under non-infected conditions. Heatmap of the Gene ontology categories (GO) based on $-\log_{10} p$ -value where shades of orange represent upregulated and blue the downregulated categories. GO IDs and descriptions are indicated in the text.

We observed that WL+FR-pretreated plants had a severe decrease in their basal H_2O_2 production compared to WL (fig. 3.4B). No significant difference was observed between control and *B. cinerea*-inoculated samples (fig. 3.4B). However, a strong and rapid increase in H_2O_2 production occurred upon elicitation with the bacterial MAMP flagellin (flg22), which was higher in WL- versus FR-pretreated samples, indicating that WL-treated plants are better prepared to respond to a pathogen attack by activating enhanced ROS signaling (fig. 3.4A).

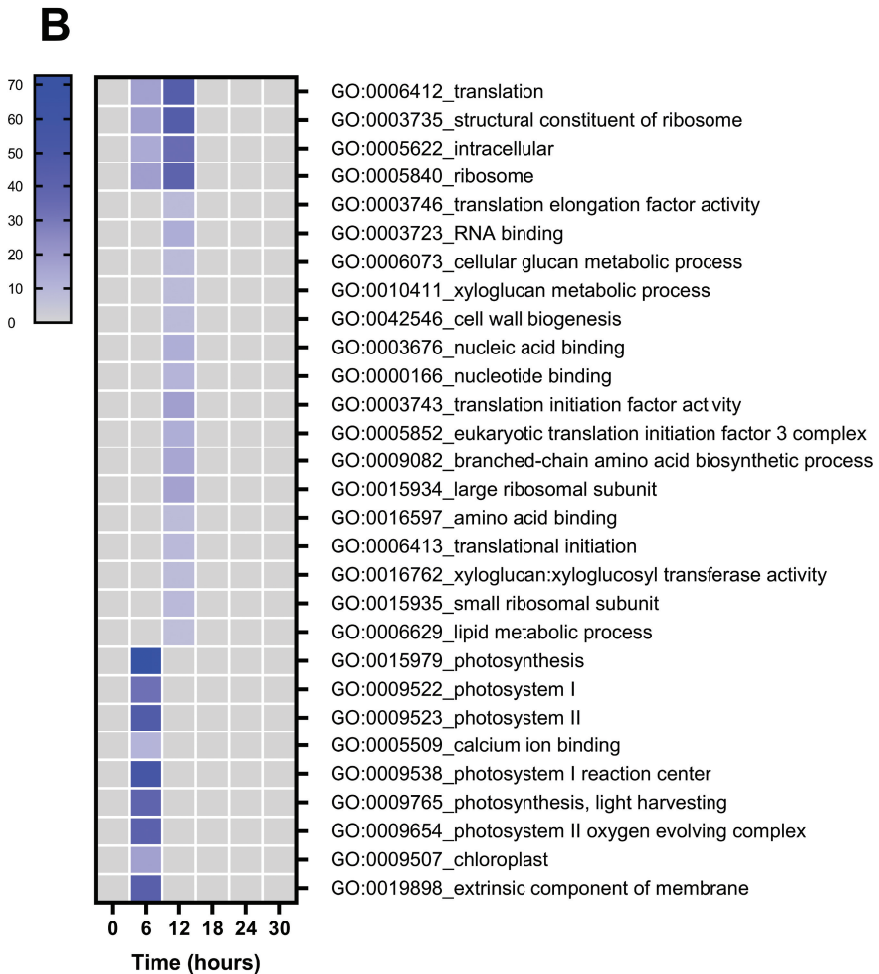


Figure 3.3 : Continued.

At 6 hpi, genes associated with photosynthesis-related processes are strongly downregulated by WL+FR pre-exposure as are genes associated with cell metabolism such as glucan- and cell biogenesis-related processes at 12 hpi (fig. 3.3B). In order to test the effect of additional FR on cell wall and membrane integrity, we performed an electrolyte leakage experiment on WL- and WL+FR-pretreated plants and observed a slight increase in the percentage of electrolyte loss in the WL+FR-pretreated plants compared to WL (fig. 3.5). This possibly implies that FR negatively affects the membrane integrity, which could in principle benefit pathogen entry. Genes related

to translation are also strongly downregulated (fig 3.3), which correlates with a delay in defense gene expression upon a *B. cinerea* infection in FR-pretreated plants (fig 3.1). Altogether, these results indicate that the negative effect of FR on susceptibility could involve metabolic changes and dampening of gene expression responses.

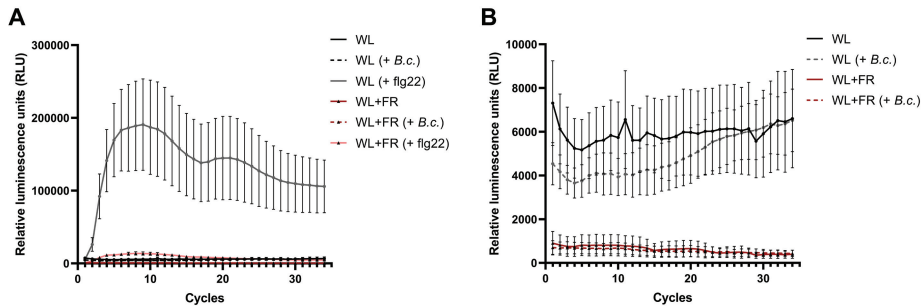


Figure 3.4 : ROS quantification by luminescence. (A) Leaf discs originating from three-week-old tomato plants treated for five days in WL or WL+FR light conditions were drop-inoculated with *Botrytis cinerea* spores (+ *B.c.*) or exposed to the bacterial elicitor flagellin (+ flg22). Leaf discs which received none of the elicitors were taken as controls. (B) Same results as (A) excluding the flagellin-treated samples. Each measurement (cycle) lasted approximately 100 sec. Error bars represent mean \pm SEM, n = 8.

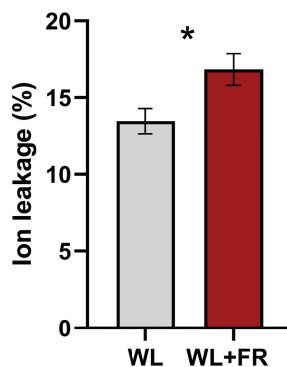


Figure 3.5 : Electrolyte leakage quantification on WL and WL+FR treated leaf discs. Percentage of electrolytes loss from tomato leaf discs after five days in WL or WL+FR light conditions. Data represent mean \pm SEM. Asterisk represents significant difference, Student's t-test, p-value < 0.05, n = 8.

FR light delays pathogen recognition and dampens defense activation

To follow the differences in chronology of gene induction during infection of tomato by *B. cinerea* between the two tested light conditions, we selected significant *B. cinerea*-responsive DEGs in both light pretreatments at any of the time points (fig. S3.3). These gene expression profiles show that overall gene modulation starts at 12 hpi in WL but not in WL+FR-pretreated samples, while a strong modulation of gene expression occurs at 24 hpi and 30 hpi in both light treatments. These observations confirm the analysis of the cumulative number of up- and downregulated DEGs shown in fig. 3.2B and C. However, we observed that some of the genes, which are modulated by *B. cinerea* under the two light conditions (fig. S3.3, fig 3.2B and C) differed in their dynamics and/or amplitude at 24 hpi and 30 hpi. This shows that *B. cinerea*-responsive gene modulation is dampened or delayed in WL+FR-pretreated samples compared to WL. This could indicate that the detection of pathogen attack is delayed and/or signal processing is different between the light treatments, thereby affecting defense activation.

Next, we searched for overrepresentation of GO terms representing *B. cinerea*-responsive genes in the dataset (fig. 3.6). As for the upregulated processes, approximately half of the GO categories found enriched in the WL-treated samples were simply missing in the WL+FR-pretreated samples. Most of the other categories that were shared between the light treatments were delayed by FR light (fig. 3.6A). At 12 hpi, we observed a striking inhibition of protein catabolic processes (GO:0006511 and GO:0051603) via the proteasome (GO:0005839 and GO:0019773) or endopeptidase activity (GO:0004175, GO:0004190 and GO:0004298) by FR in the GO analysis (fig 3.6A). This may indicate plant response against cell wall degrading enzymes (CWDEs) secreted by the fungus upon infection. At later timepoints, we found GO terms associated with chitin binding (GO:0008061) and chitin degradation processes (GO:0006032 and GO:0004568) in WL but not in WL+FR. This could suggest induction of a plant response directed at degradation of the fungal cell wall in WL, but not WL+FR. Consistently, genes associated with responses to biotic stimulus or defense response upregulated at 24 hpi in WL, are absent or delayed in WL+FR. Those observations are consistent with the notion that WL-treated plants recognize the pathogen and actively restrict its progression. The delay or absence of those categories in WL+FR-pretreated samples suggests a delay in pathogen recognition and/or downstream signaling and associated defense responses.

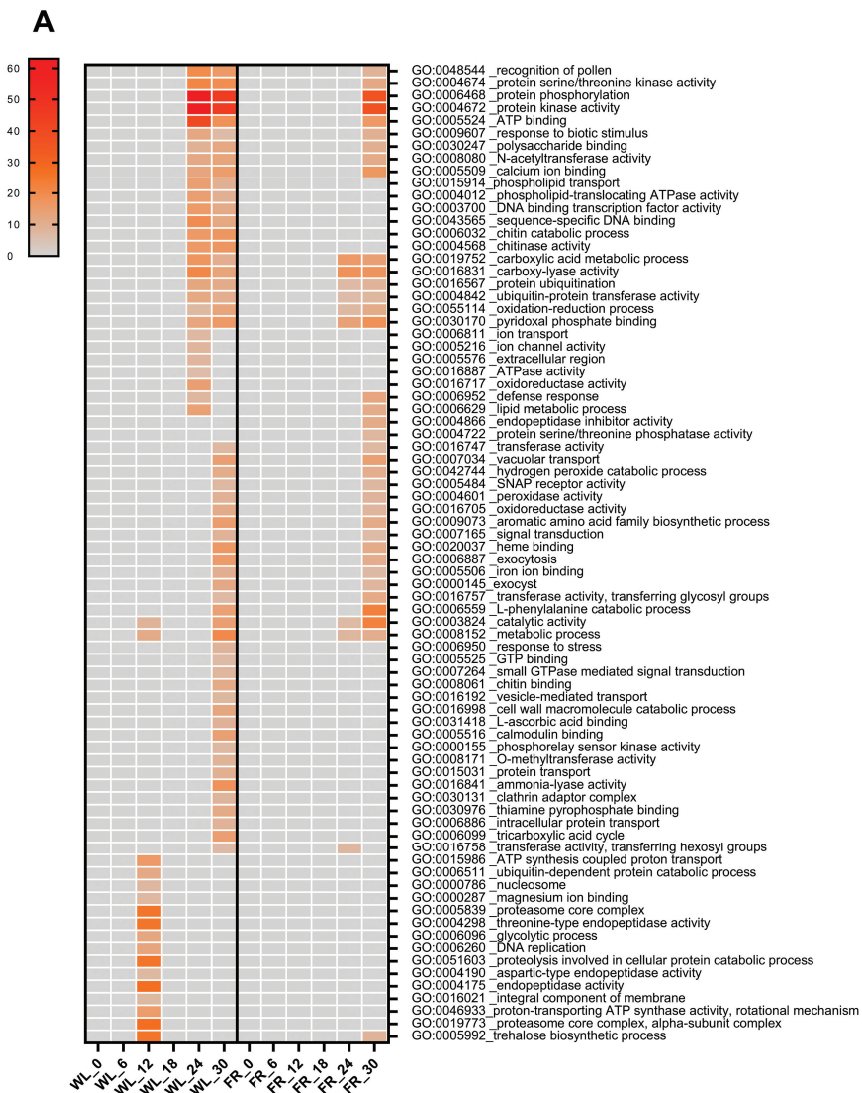


Figure 3.6 : Gene ontology analysis on *Botrytis cinerea*-responsive genes. Heatmap of the Gene ontology categories (GO) based on $(-)\log_{10}$ (p-value) of DEGs in WL and WL+FR light exposed plants where orange (A) represent upregulated and blue (B) downregulated categories. GO identifiers and descriptions are indicated in the figures.

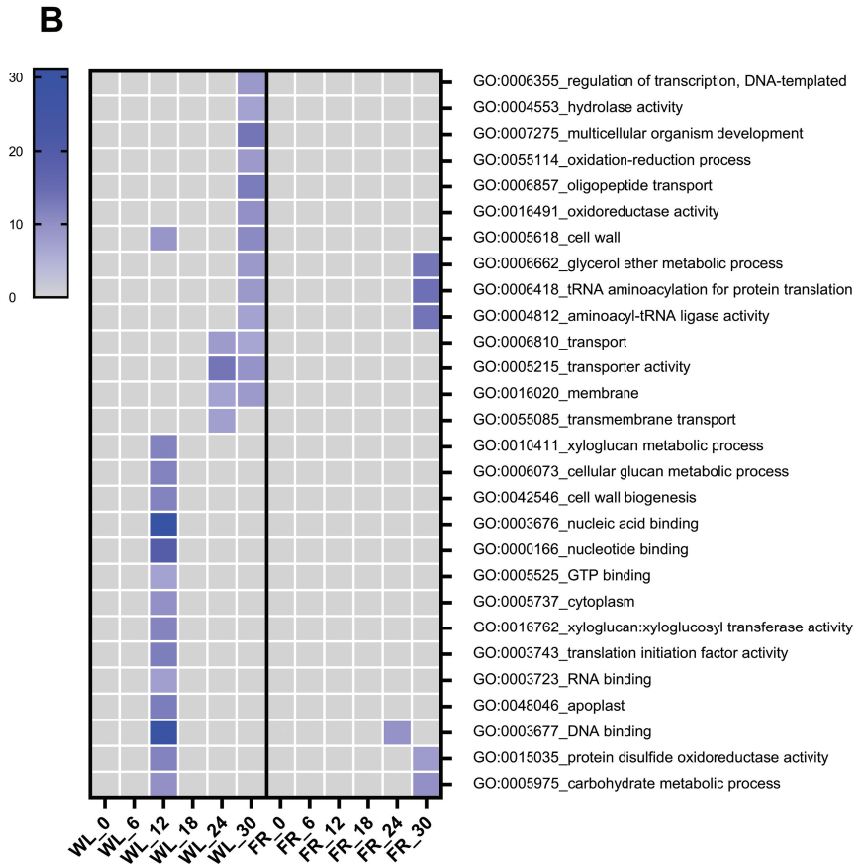


Figure 3.6 : Continued.

At 24 and 30 hpi in FR-treated samples, we also observed a delay in the upregulation of genes associated with protein phosphorylation (GO:0006468) and protein kinase activity (GO:0004674 and GO:0004672), possibly involved in the signaling cascade downstream of pattern-triggered pathogen detection. Also, DNA binding transcription factor activity (GO:0003700) is upregulated in WL-treated samples at 24 hpi onwards while absent in WL+FR-pretreated samples. In this GO category, we found genes encoding WRKY transcription factors of which some might be involved in salicylic acid (SA) and JA signaling (Pandey and Somssich, 2009) and ERF transcription factors regulated by ethylene (ET) mainly (Müller and Munné-Bosch, 2006). The involvement of ET was also confirmed by an upregulation of genes encoding the ACO and ACS ethylene biosynthesis enzymes at 24 and 30 hpi in WL and at 30 hpi in FR.

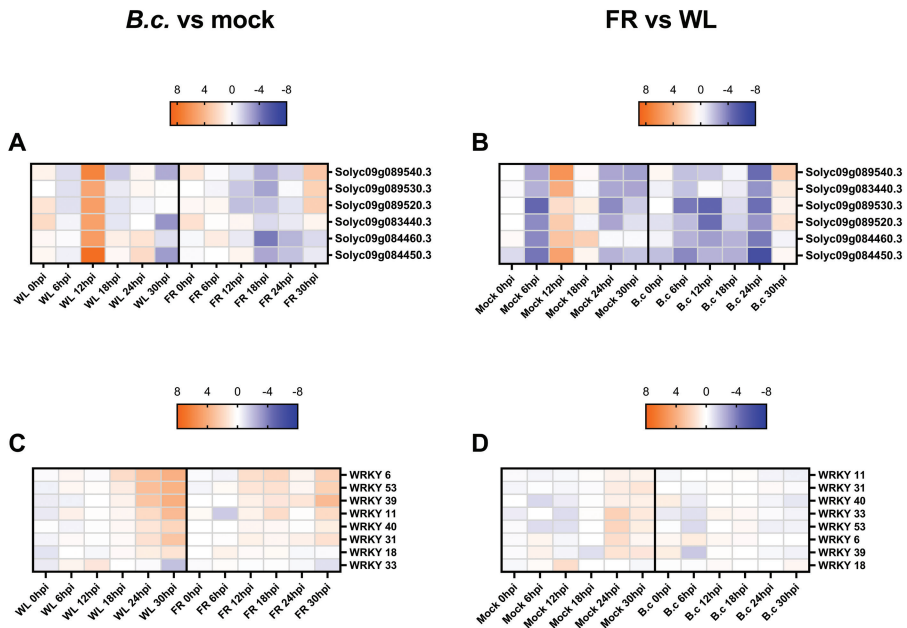


Figure 3.7 : Dynamics of expression of *PROTEINASE INHIBITOR (PI)* and *WRKY* genes from the 3-way ANOVA light*infection*time. Heatmaps corresponding to the \log_2 FC of six *PI* genes and eight *WRKY* transcription factor genes in response to *Botrytis cinerea* infection after a 5-day WL or WL+FR pretreatment at 0, 6, 12, 18, 24 and 30 hpi (hours post inoculation) (**A and C**) or in response to FR under mock conditions (**B and D**). Orange and blue colors represent up and downregulation, respectively.

Upon infection by *B. cinerea*, only five out of 28 downregulated GO categories were shared between WL and WL+FR-treated samples (fig. 3.6B). Interestingly, most GO categories downregulated by *B. cinerea* in WL conditions correspond to primary metabolism processes. GO categories associated with xyloglucan and cellular glucan metabolism as well as xyloglucan:xyloglucosyl transferase activity and cell wall biogenesis (GO:0010411, GO:0006073, GO:0016762 and GO:0042546, respectively) were strongly downregulated at 12 hpi in WL conditions only (fig. 3.6B). Taken together, these results indicate that *B. cinerea* infection triggers massive transcriptome reprogramming in WL which is significantly dampened, delayed, or fully suppressed in WL+FR-pretreated samples. This likely contributes to the observed less effective defense to *B. cinerea*. The delayed upregulation of biological processes associated with protein degradation response to stress and the downregulation of processes associated with primary metabolism could possibly explain the increased susceptibility observed in WL+FR conditions via a delayed pathogen detection,

defense response activation and differential carbohydrates partitioning. Our results also point towards the involvement of JA, ET and possibly SA hormone pathways through WRKY and ERF transcription factors in tomato defense against *B. cinerea*.

FR-enhanced plant susceptibility coincides with downregulation of hormonal signaling

In order to investigate the interplay between light and defense signaling and the core regulation of the FR-induced susceptibility in tomato, we performed a 3-way ANOVA on light*infection*time to select genes showing differential expression patterns which are dependent on the light conditions, infection and time. A total of 131 genes were significant. By looking at the expression profile of those genes, we observed strong differences between WL and WL+FR-treated samples upon infection with *B. cinerea* especially at 12 hpi where a group of genes stands out as being temporarily highly induced in WL-treated plants specifically (fig. S3.4). Next, we performed a GO terms enrichment and identified 11 significantly over-represented categories shown in table 3.1. The category that stands out most clearly was associated with response to wounding (GO:0009611) and was composed of 6 genes encoding PROTEINASE INHIBITORS (PI) (table 3.1). *PI* are JA-dependent and highly induced upon *B. cinerea* infection in tomato (El Oirdi et al., 2011) opening the possibility of an association with JA in the FR-induced susceptibility in response to *B. cinerea*. We also found an enrichment in genes associated with sequence-specific DNA binding (GO:0043565) mostly encoding WRKY transcription factors (table 3.1) which have been shown to be regulated by SA- and JA/ET-mediated defense pathways (Pandey and Somssich, 2009; Zheng et al., 2006).

We looked at the expression patterns of *PI* and WRKYs upon *B. cinerea* infection compared to mock conditions (fig. 3.7A and C) and comparing WL and FR-treated samples in the absence of the pathogen (fig. 3.7B and D). Interestingly, *PI* expression was upregulated at 12 hpi upon *B. cinerea* infection only in WL-treated samples. In the WL+FR-pretreated samples, such an induction was not visible indicating a possible involvement of those genes in tomato resistance via JA signaling (fig. 3.7A). Interestingly, we observe that these *PI* genes were downregulated at 6 hpi, thus preceding their upregulation at 12 hpi in response to WL+FR alone. This seems to indicate that FR exposure creates an arrhythmic regulation of *PI* (fig. 3.7B). Interestingly, those genes were all downregulated upon *B. cinerea* infection in WL+FR

compared to WL fitting the differential gene induction seen in fig. 3.7B. As for the WRKY transcription factor expression patterns, the strongest modulation of those genes happened upon *B. cinerea* infection (fig. 3.7C and 3.7D) showing a clear delay in WL+FR-pretreated samples. Altogether, these analyses indicate that differential regulation of hormone-dependent defense genes is associated with FR-induced susceptibility in tomato.

Table 3.1 : Gene ontology categories based on DEGs selected from the light*infection*time interaction.

GO ID	Category name	Adj.p.value	# genes
GO:0009611	Response to wounding	3.7E-06	6
GO:0008171	O-methyltransferase activity	2.8E-05	6
GO:0008152	Metabolic process	5.5E-05	52
GO:0006629	Lipid metabolic process	5.6E-05	17
GO:0004867	Serine-type endopeptidase inhibitor activity	6.2E-05	6
GO:0004866	Endopeptidase inhibitor activity	4.5E-04	4
GO:0043565	Sequence-specific DNA binding	2.9E-03	21
GO:0004190	Aspartic-type endopeptidase activity	4.4E-03	9
GO:0016747	Transferase activity, transferring acyl groups other than amino-acyl groups	5.9E-03	9
GO:0030170	Pyridoxal phosphate binding	6.9E-03	10
GO:0006869	Lipid transport	8.9E-03	4
GO:0016627	Oxidoreductase activity, acting on the CH-CH group of donors	8.9E-03	4

Discussion

Previous work has shown that tomato plants experiencing FR light as a pretreatment before inoculation exhibit increased susceptibility towards *B. cinerea* (**chapter 2**). Here, by using transcriptomics on WL and WL+FR-pretreated tomato plants infected or not with *B. cinerea*, we studied the chronology of events upon infection and unraveled multiple potential processes that could underlie the FR-induced susceptibility in tomato. Collectively, our findings demonstrate that supplemental FR by itself affects general metabolic processes such as cell biogenesis and photosynthesis-related processes that subsequently, upon challenge with *B. cinerea*, delays immune signaling at different levels.

FR control of cell wall biogenesis and ROS-mediated plant defense.

The cell wall is one of the first layers that pathogens encounter upon infection. The thickness and permeability of the cell wall and cell membrane might even be key to control pathogen penetration success (Underwood, 2012). In our conditions, we observed a downregulation of cell wall biogenesis upon WL+FR exposure as well as upon *B. cinerea* infection (fig. 3.3B and 3.6B) showing active cell wall remodeling that could potentially facilitate the pathogen penetration in plant tissue. This hypothesis was supported by WL+FR-pretreated plants showing higher electrolyte leakage than WL-treated plants, pointing to having more permeable membranes (fig. 3.5). Upon a pathogen attack, the production of reactive oxygen species (ROS) by plants is essential for the establishment of resistance (Chinchilla et al., 2007). In our dataset, we observed a clear upregulation of genes associated with oxido-reduction processes in WL+FR-pretreated samples compared to WL upon *B. cinerea* infection (fig. 3.3A). In addition, physiological data demonstrated that WL+FR-pretreated plants had a lower basal level of hydrogen peroxide (H_2O_2) and a much weaker H_2O_2 production burst upon elicitation with the bacterial MAMP flagellin (fig. 3.5). Even though *B. cinerea* spores did not significantly change H_2O_2 levels compared to the control, we observed a slight increase in H_2O_2 production towards the end of the measurement. This possibly indicates that the effect of *B. cinerea* on H_2O_2 production takes more time and might occur at a later timepoint which was not included in the experiment. Interestingly, although genes associated with oxido-reduction processes are upregulated by WL+FR, the H_2O_2 production is reduced, which is contradictory. However, this strong upregulation could lead to a redox unbalance where plants cannot produce

ROS as efficiently as in WL. We also cannot exclude that WL+FR-pretreated plants over-scavenge ROS even in unstressed conditions resulting in reduced basal ROS production and a weaker ROS-mediated defense responses.

Primary metabolism adjustments

Pathogens target carbohydrate supplies from their host in order to colonize them successfully. Upon infection, we observed a strong downregulation of glucan and xyloglucan metabolism in WL conditions at 12 hpi while none of these processes appear in WL+FR conditions (fig. 3.6B). This possibly corresponds to the fact that WL-treated plants limit the pathogen access to carbohydrates by shutting down the biosynthetic processes at least at the site of infection. Despite this, a WL+FR pretreatment led to a stronger inhibition of photosynthesis-related processes at 6 hpi in the absence of the pathogen compared to WL conditions (fig. 3.3B). Recent literature showed that *Arabidopsis phyB* mutant plants accumulate more soluble sugars compared to wild type plants (Yang et al., 2016) showing the effect of phytochrome B inactivation on sugar metabolism. If this is true for tomato plants experiencing WL+FR, it would be consistent with the observation that genes associated with photosynthesis processes get inhibited at the end of the day as the carbohydrate supplies produced during the day are sufficient to sustain the dark period. It has been shown recently that the relative fructose content (RFC) in tomato stems, corresponding to the proportion of fructose in the total soluble sugar pool (glucose, fructose and sucrose) was increased by limited water or nitrogen conditions. In parallel, an increase in RFC was shown to promote tomato resistance towards *B. cinerea* via the induction of the JA-mediated defense pathway highlighting the importance of sugars in tomato immunity (Lecompte et al., 2017). It is still unclear how sugar signaling is modulated by WL+FR in tomato however as *B. cinerea* feeds on glucan resources from the host to complete its life cycle, a FR-dependent modulation of the sugar pool in tomato would be likely to affect pathogen fitness and growth capacity in plant tissue.

FR delays pathogen detection and downstream activation of defense signaling

Plants are able to recognize pathogen-associated conserved motifs known as MAMPs at the plasma membrane as a very early signal preceding downstream defense gene activation (Jones and Dangl, 2006). Chitin is the main component of fungus

cell wall and constitutes a very efficient MAMP that can trigger immediate defense gene activation upon a pathogen challenge (Zipfel, 2014). In our dataset, WL-treated plants that are infected by *B. cinerea* show an upregulation of chitin binding- and chitin degradation-associated processes while WL+FR-pretreated plants do not (fig. 3.6A). Also, GO categories associated with “protein serine/threonine kinase activity”, “protein phosphorylation” and “protein kinase activity” that are enriched in upregulated DEGs at 24 hpi and 30 hpi in *B. cinerea*-challenged WL-treated plants are present only at 30 hpi in WL+FR (fig. 3.6A). Most of the DEGs associated with those 3 categories encode receptor like kinase (RLKs), which are often associated with MAMP detection at the plasma membrane (Zipfel, 2014). The increased disease susceptibility triggered by WL+FR light could be explained by a delay in pathogen detection via RLKs at the cell surface, subsequently delaying the induction of chitinases that target degradation of the fungal cell wall. In turn, this would indirectly promote pathogen colonization in WL+FR-pretreated leaf tissue compared to WL. A delayed pathogen detection at the cell surface correlates with a reduction of subsequent defense signaling, which is supported by a delayed representation of the GO categories associated with “defense response” and “response to biotic stimulus” in the WL+FR-pretreated plants challenged with *B. cinerea* (fig. 3.6A). Altogether, these observations indicate a clear FR effect in response to infection, possibly because of a delay in pathogen recognition at the membrane that could result in a delay in chitin degradation and defense gene activation through the RLK-mediated defense signaling cascade.

FR affects JA and ET-mediated defense gene activation

The 3-way ANOVA light*infection*time analysis revealed the core gene expression patterns associated with FR-induced susceptibility. Out of the 131 genes selected, we found six *PI* genes highly induced at 12 hpi in WL and not in WL+FR-pretreated samples (fig. 3.7A and fig. S3.4). *PI* genes have been reported to be strongly induced upon *B. cinerea* infection and promote plant resistance in a JA-dependent manner in tomato (El Oirdi et al., 2011). In addition to plant defense responses against *B. cinerea*, *PI* are also involved in plant defense response against herbivores partly by inactivating digestive proteases thereby inducing plant resistance (Hamza et al., 2018; Ryan, 1990; Tanpure et al., 2017). *PI* genes are also induced upon wounding and depend on a coregulation by JA and ET signalling (O'Donnell et al., 1996). Interestingly, we also observed modulation of *PI* genes by WL+FR in non-infected conditions (fig. 3.7B).

This response might reflect a wounding response initiated by cutting the leaflets from the petiole at 0 hpi. Strikingly, *PI* genes were downregulated at 6 hpi and then upregulated at 12 hpi by WL+FR, which indicates a delay in the induction of those genes upon wounding. We propose that the negative effect of FR enrichment on tomato resistance towards *B. cinerea* could be associated with a delay in JA- and ET-dependent induction of *PI*. However, we cannot exclude a possible effect of FR on enhancing the JA/SA antagonism through induction of SA-dependent *WRKY* transcription factor expression (fig. 3.7C). Interestingly, additional FR has been shown to dampen both JA- and SA-mediated defense pathways in Arabidopsis, which could also be the case in tomato (De Wit et al., 2013).

Conclusion

In this chapter, we show that WL+FR strongly modulates genes associated with plant susceptibility, upon infection with *B. cinerea*, which suggests pronounced metabolic and hormonal alterations. *PI* are likely to have an important role in the FR-induced susceptibility in tomato as they are only present in WL-treated samples and involved in plant immunity through JA and ET signalling. By targeting key players involved in the FR-induced susceptibility in tomato in future studies, it might be possible to generate relatively pathogen-resistant tomato cultivars that could still be grown at high planting density. This chapter constitutes a foundation for studying hormonal regulation upon WL+FR pre-exposure on disease resistance following in **chapter 4**.

Acknowledgements

We thank the Utrecht Sequencing Facility team (USEQ) and the Utrecht Bioinformatics Center (UBEC) for performing the sequencing as well as the demultiplexing and mapping of the RNA sequencing output. We also thank Dr. Tom Raaymakers for his help with the ROS quantifications and Dr. Scott Hayes for helping with the electrolyte leakage assays. We also thank the members from the “LED it be 50%” consortium for help and feedback on the project. This work was funded by the Dutch Research Council, TTW Perspectief grant nr 14125 (“LED it Be 50%”) and supported by Signify, WUR Greenhouse Horticulture and LTO Glaskracht.

Material and methods

Bioassays and harvest

For time series experiments, we use the bioassay protocol described in **chapter 2**. Three-week-old tomato cv. Moneymaker were pretreated for five days in WL or WL+FR respectively. The first two lateral leaflets of the third leaf from the bottom were detached and drop-inoculated 10 h after dawn with a *B. cinerea* spore solution or a mock solution only containing $\frac{1}{2}$ PDB medium. Each leaflet was placed in an individual round Petri dish (9 cm) and the infection took place in WL conditions and five leaf discs (0.5 cm diameter) were collected per replicate at 0, 6, 12, 18, 24 and 30 hpi. In the end, 96 samples were collected corresponding to two pretreatment light conditions (WL and WL+FR), two infection conditions (*B.c.* and mock), following six time points with four replicates per samples. Samples were snap-frozen in liquid nitrogen and kept at -80 °C until RNA isolation.

RNA isolation and fragmentation

We prepared non-strand-specific RNA-seq libraries according to Townsley et al. (2015) with minor modifications. Leaf discs were finely ground with a tissue lyser (MM 400, Retsch) and resuspended in 400 μ l of lysis/binding buffer (LBB; 100 mM Tris-HCl pH = 8, 1 M LiCl, 10 mM EDTA, 1% SDS, 5 mM DTT, 1.5% full strength Antifoam A). LBB was supplemented with 5 μ l ml⁻¹ β -mercaptoethanol right before use. The samples were bead beaten again for 2 x 30 seconds and let at room temperature for 10 min before spinning 10 min at 13000 rpm. Total mRNA was isolated from 200 μ l of lysate. Then, 1 μ l of 12.5 μ M biotin-20nt-dT oligo primer (5'-Biotin-ACAGGACATTCGTCGCTT CCTTTTTTTTTTTTTTTTTTTTTT-3') was added to the lysate and incubated for 10 min at room temperature before addition of 20 μ l of washed streptavidin coated beads (Biolabs). The samples were placed on a Magwell® 96 well magnetic separator and the supernatant was discarded. The pellets were washed in 200 μ l of Washing buffer A (WBA) containing 10 mM Tris-HCl, 150 mM LiCl, 1 mM EDTA and 0.1% SDS then with 200 μ l of washing buffer B (WBB) corresponding to WBA without SDS. The bead pellets were washed one last time in low salt buffer (LSB) containing 20 mM Tris-HCl, 150 mM NaCl and 1 mM EDTA. The process was repeated another time to improve RNA purity. RNA fragmentation and cDNA priming was performed by mixing 1.5 μ l of 5 X Thermo scientific RT buffer, 0.5 μ l random primers (Invitrogen®) and 8 μ l of RNA.

The mixed reagents were placed in a thermocycler (25°C – 1 second, 94°C – 1.5 min, 4°C – 5min, 4°C – hold).

cDNA synthesis

For cDNA synthesis, 5 µl of fragmented RNAs was added to the following reagents mixture: 1.5 µl of 5X Thermo scientific RT buffer, 0.1 M DDT, 1 µl H₂O, 0.5 µl 25 mM dNTPs and 0.5 µl RevertAid Reverse Transcriptase enzyme (Thermo scientific). The total reaction volume was 15 µl and was incubated in a thermocycler for reverse transcription of the first cDNA strand (25°C – 10 min, 42°C – 50 min, 50°C – 10 min 70°C – 10 min and 4°C - hold). The second strand synthesis was performed as follows with 1.5 µl H₂O, 0.4 µl 25 mM dNTPs, 1 µl Poll (Thermo scientific), 0.1 µl RNaseH (Biolabs), 0.4 µl End repair module (T4 Pol + PNK mix), 0.2 µl Taq polymerase and 1.4 µl End repair buffer. The total reaction volume was 10 µl and was placed in a thermocycler following the program : 16°C – 20 min, 20°C – 20 min, 72°C – 20 min, 4°C – hold.

Adapter ligation

Non-strand-specific universal primer adapters were prepared by mixing 8 µl of 100 µM PE1-lig primer (5'-CACTCTTTCCTACACGACGCTCTTCCGATCT-3') and 8 µl of 100 µM 5'phosphorylated ILL-lig primer (5'-P-GATCGGAAGAGCACAGTCTGAACTCCAGTCAC-3') in 784 µl of H₂O. The total volume was equally divided over a 8-well strip and followed the PCR program : 94°C – 1 min ; (94°C – 10 seconds) x 60 -1°C per cycle ; 20°C – 1 min ; 4°C – hold). cDNAs were cleaned up by a 5 min incubation at room temperature with 30 µl of XP Ampure beads (Beckman coulter), washed twice with 200 µl of 80% ethanol. The bead pellets were put to dry at room temperature on magnetic separator. Non-strand-specific adapters were ligated onto cDNAs by adding 3 µl of annealed 1uM universal primers on dry cDNA pellets. Once eluted, the cDNA/universal primers mix was supplemented with 7 µl the following reaction mix : 1.75 µl H₂O ; 5 µl 2X Rapid T4 ligase buffer ; 0.25 µl DNA ligase (Biolabs) and let to sit for 15 min. 10 µl of 50 mM EDTA and 25 µl of ABR resuspension buffer (15% PEG 8000 ; 2.5 M NaCl) were added to the mix and let to incubate at room temperature for 5 min before placing it on the magnetic tray. The bead pellets were washed twice with 200 µl of 80% ethanol without resuspension. The samples were eluted in 22 µl of 10 mM Tris-HCl and enriched by PCR.

Library indexing and PCR enrichment

For library indexing, 5 µl of each cDNA sample coupled with the universal adapters was supplemented with 0.5 µl of the appropriate indexed enrichment oligo and 4.5 µl of the following mix : 2 µl 5X Phusion HF Buffer, 1.3 µl H₂O, 0.5 µl PE1 primer (5'-AATGATACGGCACCACCGAGATCTACTCTTTCCCTACACGACGCTCTCCGATCT-3'), 0.5 µl 8 µM each S1 and S2 primers (S1: 5'-AATGATACGGCGACCACCGA-3' and S2: 5'-CAAGCAGAAGACGGCATACGA-3' respectively), 0.1 µl of 25 mM dNTPs and 0.1 µl Phusion polymerase. The mix followed the thermocycler program : 98°C - 30 sec, (98°C - 10 sec, 65°C - 30 sec, 72°C - 30 sec) x 11 cycles ; 72°C - 5 min and hold at 10°C. The non-strand specific libraries were cleaned one last time to select for 350 pb molecules. The libraries were washed with 1.1 volumes of Ampure XP beads per samples and incubated at room temperature for 5 min before washing twice with 80% ethanol on magnetic rack. The final product was eluted in 10 µl 10 mM Tris-HCl.

Sequencing and reference sequence alignment

All cDNA libraries were sequenced using 1 x 75-bp high output Illumina NEXTseq500. All 96 libraries were quantified and pooled in equal concentration as one sample (final concentration of 8 nM in 40 µl) and two sequencing runs were necessary to achieve 3.000.000 sequenced reads per library. Both sequencing outputs were mapped on the Tomato reference genome version SL3.0 with ITAG3.20 annotations (https://solgenomics.net/organism/Solanum_lycopersicum/genome) and then merged into one. The quality of the libraries was controlled with FastQC v0.10.1 (available online at : <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Reads were mapped using STAR v2.4.2a (Dobin et al., 2013) and counted using HTSeq-Count 0.6.1p1 (Anders et al., 2015).

Differential gene expression

Reads were normalized by their size factor via DESeq2 normalization using the R package DESeq v1.18.0 (Anders and Huber, 2010). Principal coordinate analyses were done in R 3.6.0 (R Core team, 2013) using the time and infection components as PCO1 and PCO2, respectively. Differentially expressed genes were determined per timepoint using an ANOVA, with the model: gene expression ~ infection * light treatment + replication. Where gene expression is the per gene, log₂ ratio of each

observation of that gene with the mean expression over all samples of that gene. Infection is the mock or *B.c.* treatment and light is the control of pre-treatment with FR light. To calculate the differences between the four different treatments Tukey HSD was used. All calculations were done in R 3.6.0 (R Core team, 2013). The p-values and effects were recorded for further investigation. A p-value < 0.001 was used to select the differentially expressed genes for GO-term enrichment.

GO-enrichment

GO enrichment was done in R 3.6.0 (R Core team, 2013), using the hypergeometric test on the differentially expressed genes per time point. The GO terms identifiers were obtained from solgenomics.net based on the tomato reference genome version SL3.0 with ITAG3.20 annotations.

Clustering

Clustering behind the heatmaps for GO-enrichments and DEGs were performed using heatmapper.ca with default settings (<http://heatmapper.ca/>).

ROS measurements

Three-week-old tomato plants were exposed to either WL or FR for four days. On day 5, leaf discs (\emptyset 0.4 cm) originating from the 3rd leaf of each plants were kept floating in deionized water for approximately 8 h in the same light conditions as for the pretreatment (either WL or FR) without shaking. Each leaf disc was placed in each well of a white flat-bottomed 96-well plate (Greiner LUMITRAC™ 200) onto 180 μ l of deionized water mixed with 20 μ l of 10 X reaction mix (200 μ M Luminol L-012 and 10 μ g ml⁻¹ horseradish peroxidase) with a final concentration of 20 μ M Luminol and 1 μ g of peroxidase. The luminescence was quantified by using a GloMax luminometer (Promega). The background noise was measured for 15 min prior to adding 1 μ M flg22 (final concentration) to the reaction mix or a 5 μ l droplet of 1.5×10^5 spores ml⁻¹ on the leaf discs. The luminescence was measured for approximately 1 h following 34 cycles of 100 seconds (Albert and Fürst, 2017).

Ion leakage

After experiencing either WL or FR for five days, 4 week-old tomato leaf discs were punched from the 3rd leaf of each plants. The leaf discs were rinsed in deionized water to remove attached electrolytes leftover from the cutting. The conductivity was measured after incubating the leaf discs for 3 h in 10 ml of 400 mM Mannitol (value 1) under ambient light and temperature and again after 20 min at 95 °C (value 0 = 100%). The percentage of electrolyte loss was calculated relative to the total amount of electrolytes in leaf tissue (value 1-value 0).

Supplemental data

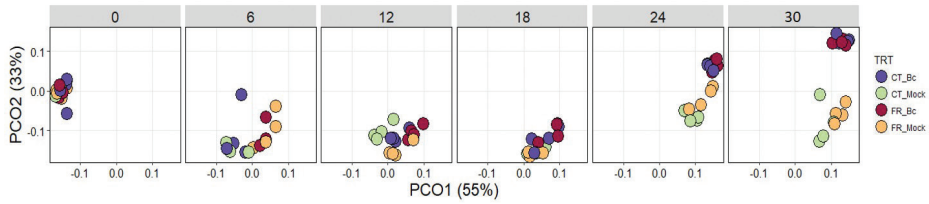


Figure S3.1 : Responses to light and infection are not simultaneous. Visualization of the variation in gene expression by principal coordinate analysis (PCoA). PCO1 and PCO2 represent time and infection, respectively. The analysis was separated per timepoint (0, 6, 12, 18, 24 and 30 hpi) and each color represents a treatment (WL+B.c. spores in purple, FR+B.c. spores in red and mock samples originating from WL-treated plants in green and WL+FR treated plants in orange) and each dot corresponds to a replicate.

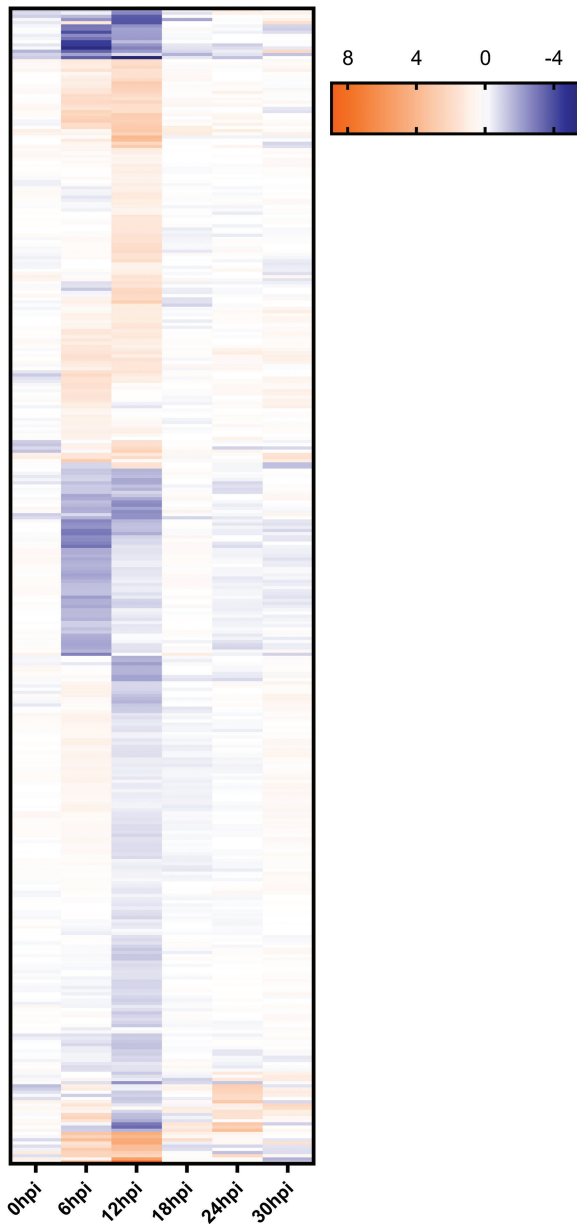


Figure S3.2 : Expression dynamics of FR-responsive genes. (A) Heatmap corresponding to the \log_2 FC for the DEGs (total 364 unique genes) after a 5-day WL and WL+FR pretreatment at 0, 6, 12, 18, 24 and 30 hpi. Blue and orange colors represent the significantly down- and upregulated genes in response to FR respectively.

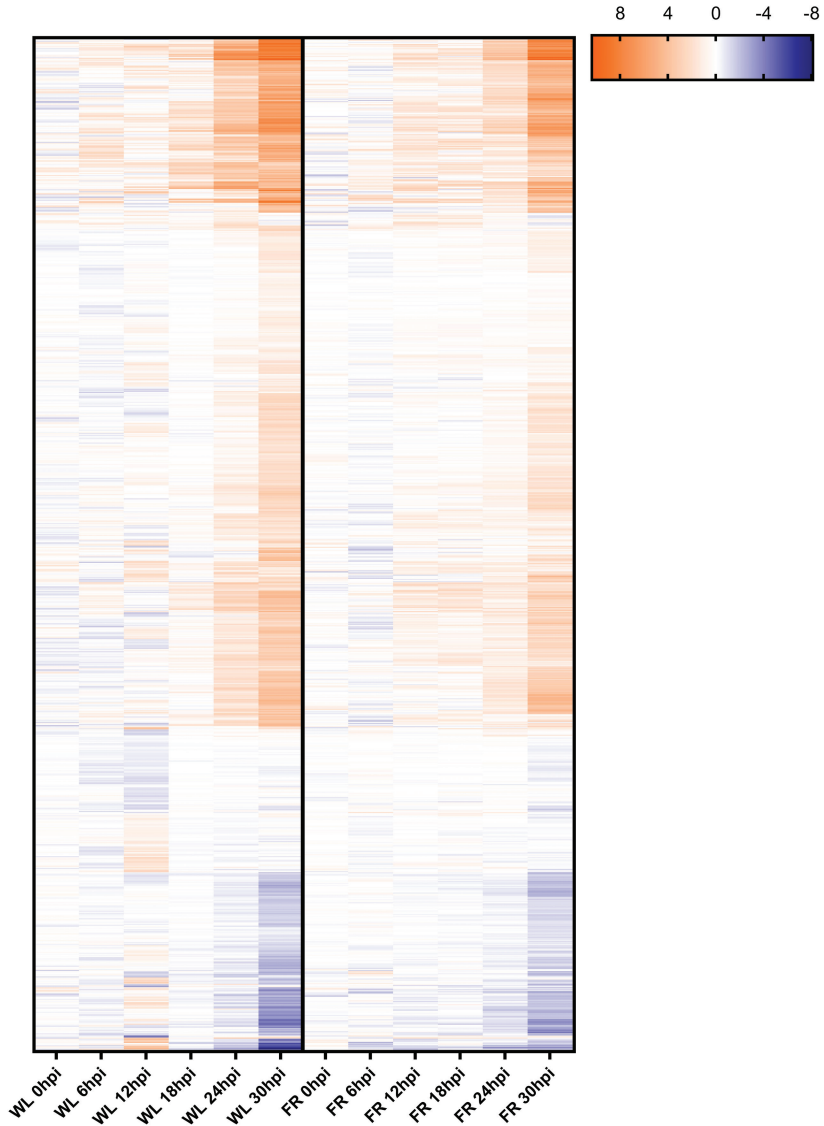


Figure S3.3 : Expression dynamics of DEGs upon *Botrytis cinerea* infection. (A) Heatmap corresponding to the log₂ FC for the DEGs (total 944 unique genes) after a 5-day WL and WL+FR pretreatment and inoculation in WL at 0, 6, 12, 18, 24 and 30 hpi. Blue and orange colors represent the significantly down- and upregulated genes in response to *B. cinerea* infection respectively.

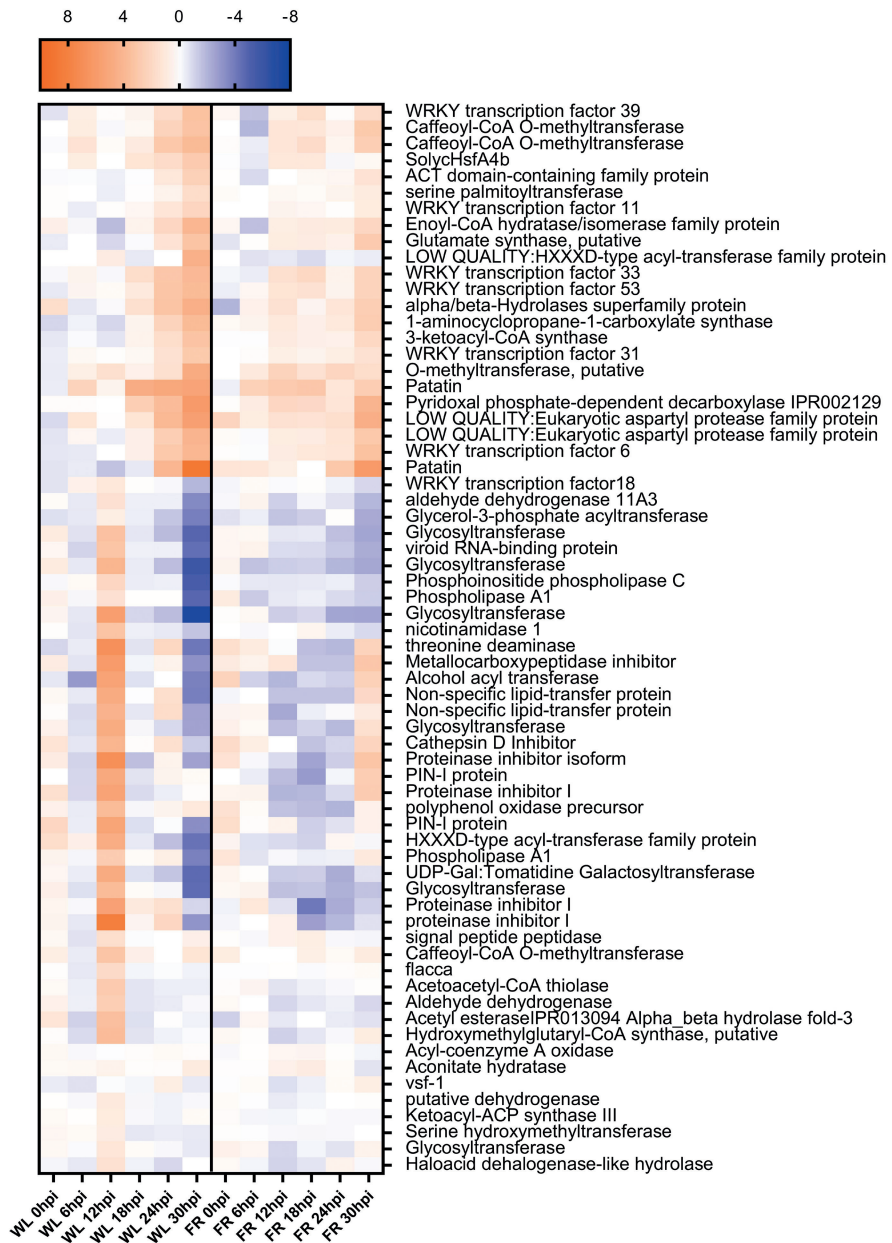


Figure S3.4 : Dynamics of expression of 3 way ANOVA light*infection*time gene selection. Heatmap corresponding to the \log_2FC for the 131 unique selected genes in response to *B. cinerea* infection after a 5-day pretreatment in WL or FR and inoculation in WL at 0, 6, 12, 18, 24 and 30 hpi. Blue and orange colors represent the significantly down- and upregulated genes in response to *B. cinerea* infection respectively.

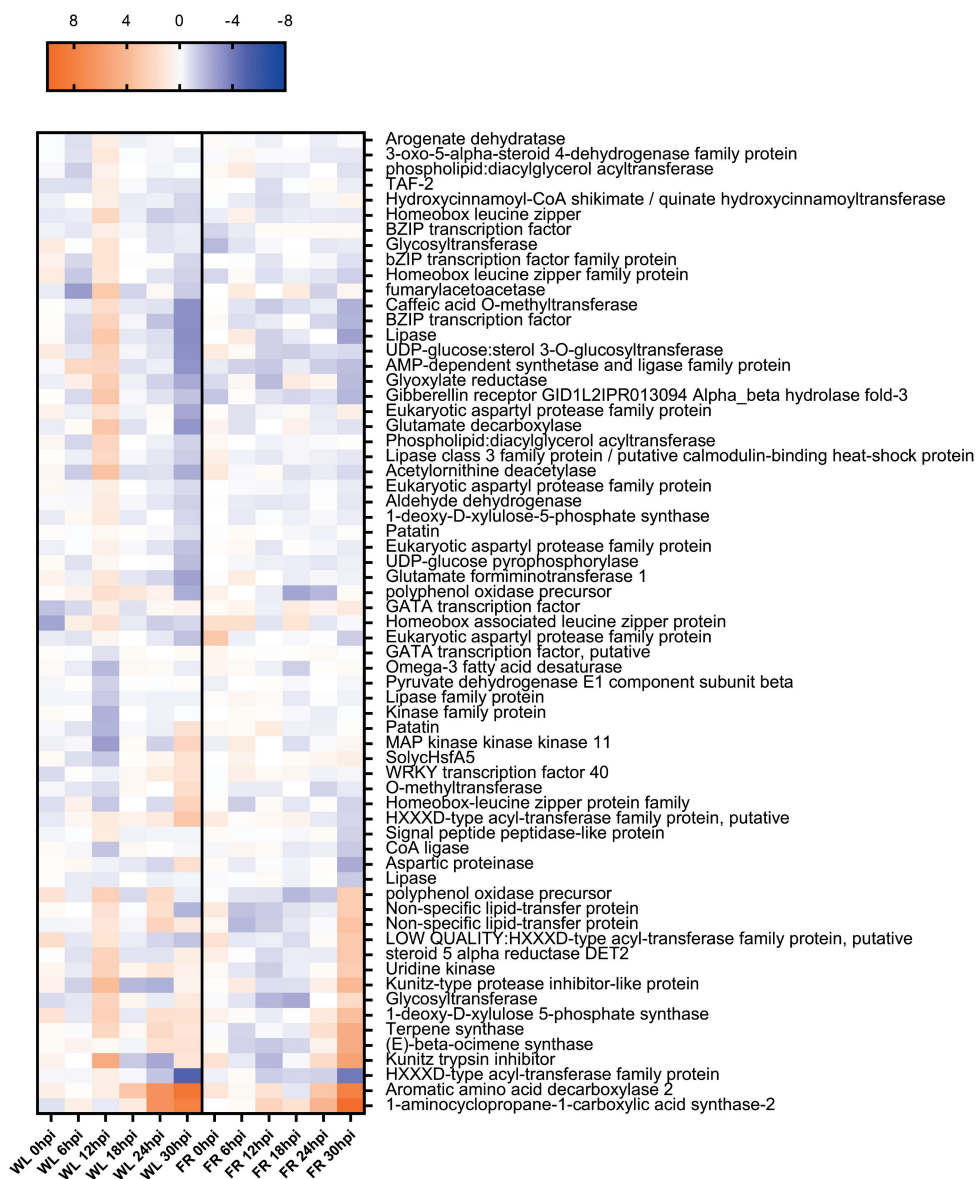
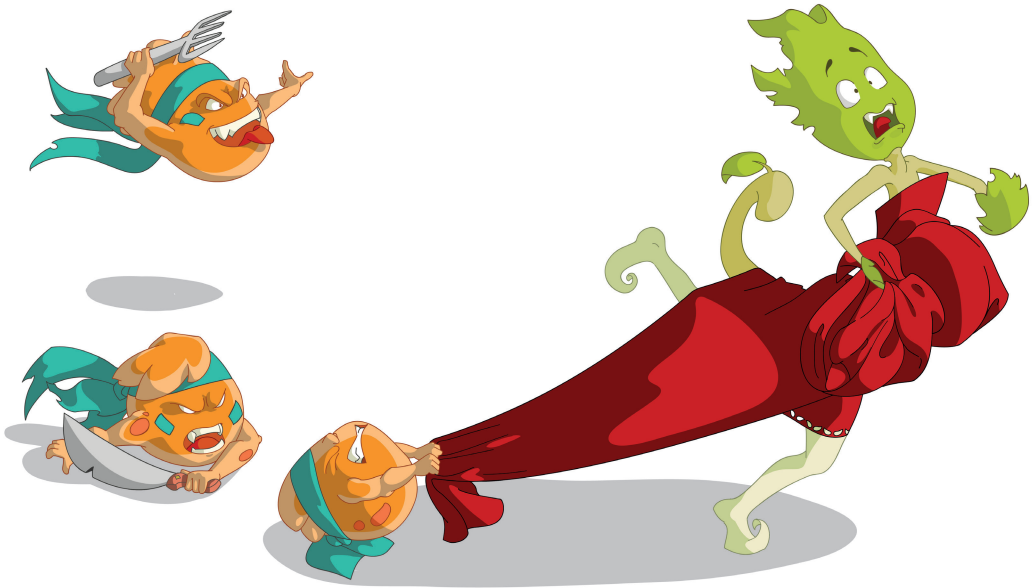


Figure S3.4: Continued



Mo

Chapter

4

Far-red light modulates tomato defenses induced by *Botrytis cinerea* by influencing synergism between jasmonic acid and ethylene pathways

Sarah Courbier¹, Wouter Kohlen², Saskia C.M. Van
Wees³ & Ronald Pierik¹

¹*Plant Ecophysiology, Institute of Environmental Biology, Utrecht
University, the Netherlands*

²*Laboratory of Molecular Biology, Wageningen University, 6708 PB
Wageningen, the Netherlands*

³*Plant-Microbe Interactions, Institute of Environmental Biology,
Utrecht University, the Netherlands*

Abstract

At high planting densities, the ratio between red and far-red light (R:FR) decreases dramatically in the canopy because of absorption of red light (R) and reflection and transmission of far-red light (FR). Plants exposed to low R:FR exhibit the typical shade avoidance response as well as weakened defense responses against various pathogens including *Botrytis cinerea*. As *B. cinerea* is one of the most destructive fungal pathogens worldwide, it is paramount to understand how supplemental FR impacts plant defense responses. Here, we investigate the FR-mediated modulation of the expression of six *PROTEINASE INHIBITOR (PI)* genes identified in **chapter 3** as being induced by *B. cinerea* in WL-treated plants but not in WL+FR-pretreated plants. We show that the FR-induced susceptibility in tomato is associated with a reduced basal expression and weaker induction of *PI* genes upon exogenous methyl-jasmonate (MeJA) treatment. In addition, the synergistic effect by ethylene on jasmonic acid (JA)-activated *PI* gene expression only occurred in WL-treated plants. Exogenous JA and ethylene treatments promoted plant resistance to *B. cinerea* while JA and ethylene inhibitors had opposite effects, with WL+FR-exposed plants remaining more susceptible than WL-treated plants. Our results demonstrate that supplemental FR may induce plant susceptibility partly through a change in JA and ethylene signaling via differential *PI* gene expression. With these data, we highlight *PI* genes as good markers to further investigate the molecular players involved in the FR-induced susceptibility towards *B. cinerea* in tomato.

Introduction

Light is a crucial environmental cue for plant growth. Plant leaves absorb red (R) and blue (B) light while far-red (FR) light is reflected or transmitted by green tissue towards its surroundings. When plant density increases, the reduction in available R light and the high reflection of FR in the environment leads to a reduced ratio between R and FR light (R:FR). Changes in R:FR are sensed by specialized photoreceptors called phytochromes (Franklin, 2008). Upon perception of low R:FR, phytochrome B (phyB) is inactivated which leads to the release of Phytochrome Interacting Factors (PIFs), transcription factors able to initiate downstream gene expression. In parallel, inactive phyB promotes gibberellin (GA) biosynthesis in turn repressing the sequestration of PIFs and the negative growth regulators DELLAs leading to strong PIF-mediated growth responses. This phenomenon known as “shade avoidance” triggers petiole and hypocotyl elongation as well as strong hyponastic responses in Arabidopsis.

Jasmonic acid (JA) is a crucial hormone in the regulation of plant immune responses. The interplay between GA and JA has been shown to have a role in compromised plant resistance in low R:FR conditions. This is explained by the ability of DELLA proteins to physically interact not only with PIFs but also with JAZ proteins, which are repressors of JA-mediated gene expression (Hou et al., 2010; Li et al., 2016; Yang et al., 2012). Upon necrotrophic pathogen attack under normal light conditions, active phytochromes repress PIFs while low levels of bioactive GA levels stabilize DELLAs allowing the formation of the DELLA/JAZ complex, which enables strong JA-mediated defense (Ballaré, 2014; Pieterse et al., 2014). Upon plant infection with a necrotrophic pathogen in low R:FR, both GA and JA levels increase resulting in the release of PIFs through DELLA degradation, and in parallel, JAZ proteins are free to sequester transcription factors preventing them from initiating defense gene transcription. This leads to growth promotion at the expense of defense responses (Pieterse et al., 2014). Although the growth-defense tradeoff was originally thought to result from passive sink-source interactions, it is now known to involve fine-tuned molecular control mechanisms in the plant (Ballaré and Austin, 2019), that interact with the pathways described above.

In **chapter 3**, we observed a strong induction of a set of six genes encoding type 1 PROTEINASE INHIBITORS (PI) in WL but not in FR-pretreated samples upon infection by *Botrytis cinerea*. PI have been identified long ago by Green and Ryan (1972) as

proteins accumulating in tomato leaf tissue after wounding and are thought to interfere with feeding capacity of insects during herbivory. The accumulation of PI proteins has been shown to be JA-mediated since exogenous application of methyl jasmonate (MeJA) on tomato leaves increases PI abundance in plant tissue as well in neighboring untouched plants (Farmer and Ryan, 1990). Upon wounding or herbivory, an 18 amino-acid (aa) peptide called systemin is cleaved off from its precursor known as prosystemin (200 aa). The release of systemin is sensed by specialized receptors initiating JA biosynthesis mediating downstream induction of *PI* expression promoting plant defense (Pearce et al., 1991). More recently, the systemin-mediated induction of *PI* has been characterized during *B. cinerea* infection in tomato plants. A systemin antisense tomato line displayed enhanced susceptibility to *B. cinerea*, which was associated with low levels of prosystemin and weak *PI* gene expression in infected plant tissue (El Oirdi et al., 2011). Wound-induced *PI* gene expression was shown to require both JA and the gaseous hormone ethylene since the inhibition of the ethylene pathway was able to abolish the JA-mediated *PI* induction in tomato (O'Donnell et al., 1996). A pretreatment with ethylene enhanced tomato resistance towards *B. cinerea* while the ethylene receptor blocker 1-methylcyclopropene (1-MCP) gave opposite results (Díaz et al., 2002). Other studies demonstrated that reduced expression of *TOMATO PROTEIN KINASE 1 (TPK1b)* using RNA interference was associated with increased tomato susceptibility to *B. cinerea* via reduced *PI* gene induction (AbuQamar et al., 2008). Paradoxically, even though ethylene enhances tomato resistance low R:FR has been shown to promote ethylene emissions in for example tobacco plants (Pierik et al., 2004). So far, the regulation of JA and ethylene-mediated defense responses in tomato towards *B. cinerea* by low R:FR is unknown. This mechanism remains to be investigated to further improve plant resilience in the field or greenhouses. The transcriptome analysis presented in **chapter 3** highlighted a set of six *PI* genes that are induced only in WL-treated samples. In this chapter, we investigate the role of JA and ethylene in *PI* gene expression and tomato resistance against *B. cinerea*. By performing exogenous hormone treatments, we show that *PI* genes are regulated differently by JA and ethylene upon FR exposure compared to white light conditions. The FR-induced susceptibility in tomato is associated with a dampening of *PI* gene induction and expression affecting defense responses resulting in more susceptible plants.

Results

FR light inhibits *PI* gene expression upon wounding and *B. cinerea* infection.

Based on the 3-way ANOVA gene selection from the transcriptome experiment (131 genes) presented in **chapter 3**, we identified six *PI* genes. RPKM-normalized expression profiles from these RNA-seq data were plotted for the six genes in all conditions tested: WL- or WL+FR-pretreated plant material inoculated or not with *B. cinerea* spores (WL_mock, WL_B.c., WL+FR_mock and WL+FR_B.c.) through time (0, 6, 12, 18, 24 and 30 hours post inoculation) (fig. 4.1). All *PI* genes were upregulated in response to *B. cinerea* in WL conditions (WL_B.c) after 12 hpi (fig. 4.1). On the contrary, this induction was strongly dampened in WL+FR_B.c conditions showing the effect of the supplemental FR pretreatment on *PI* genes induction upon *B. cinerea* infection (fig. 4.1 and fig. 3.7A).

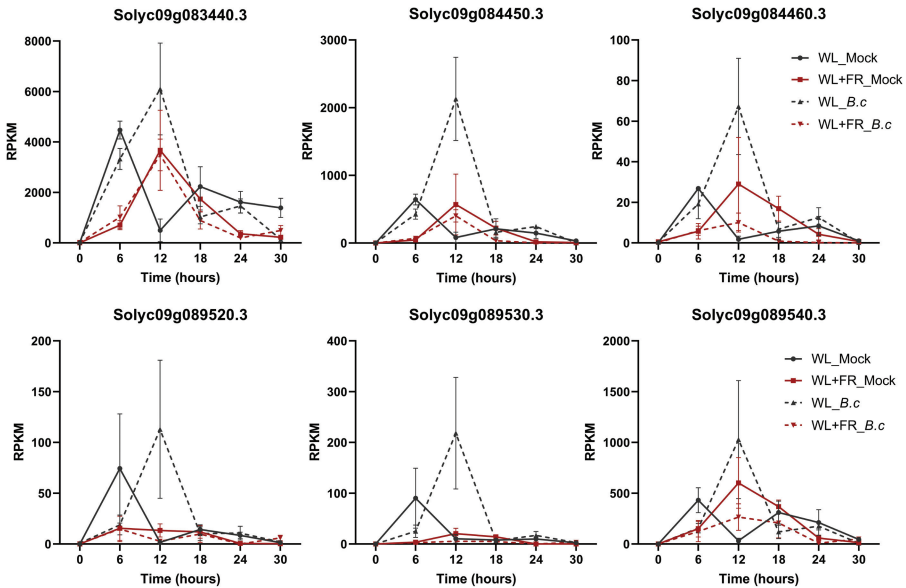


Figure 4.1 : Expression of *PI* genes is upregulated upon *Botrytis cinerea* infection. Expression patterns (RPKM) of six *PI* genes selected from the RNA-seq experiment described in **chapter 3**. Each plot corresponds to the expression patterns of an individual gene through time in the four conditions as determined by RNA-seq. White light (WL) or WL + additional far-red LEDs (WL+FR) pre-treated plants inoculated with *B. cinerea* spores (dashed lines with WL_B.c. in grey and WL+FR_B.c. in red) or a mock solution (plain lines; WL_mock ; grey or WL+FR_Mock ; red). Data represent mean of four biological replicates \pm SEM.

Interestingly, we also observed a peak of expression of all genes at 6 hpi in WL-treated samples in the absence of *B. cinerea* (WL_mock) possibly reflecting the wounding responses triggered by detaching the leaflets from the plants prior to starting the infection assays. Similar to the delayed *PI* induction in WL+FR_B.c, the peak of expression of *PI* in WL+FR_mock conditions was also visible and delayed, for most of the genes, at 12 hpi instead of 6 hpi in WL_mock (fig. 4.1A-C and 4.1F). For some other genes such as *Solyc09g089520.3* and *Solyc09g089530.3*, the peak in expression did not appear at all (fig. 4.1D and E). Altogether, these results show that *PI* genes are responsive to *B. cinerea* and wounding and their level of expression is dampened and/or delayed by supplemental FR light.

FR inhibits the JA-mediated *PI* induction in tomato.

To test whether the effect of additional FR on *PI* induction upon *B. cinerea* infection could be mediated by an effect of FR on JA-mediated signaling, we investigated the effect of FR enrichment on *PI* gene induction by exogenous MeJA treatment. Following a 5-day pretreatment in WL or WL+FR light conditions, the 3rd leaf of intact tomato plants was sprayed with a 100 μ M MeJA or a mock solution (fig. 4.2). *PI* gene expression was measured at 15 min and 4 h after the start of the treatment. After 15 min of MeJA treatment, we already observed a strong induction of *PI* genes under WL condition and this upregulation was significantly reduced in WL+FR conditions for five out of the six *PI* genes. After 4 h, there was still a significant reduction of MeJA-induced expression levels of two of the *PI* genes but for the other four *PI* genes the induction level by MeJA was comparable between WL- and WL+FR-treated samples (fig. 4.2). This suggests a delay rather than an overall inhibition in the induction of *PI* genes by MeJA under FR enrichment.

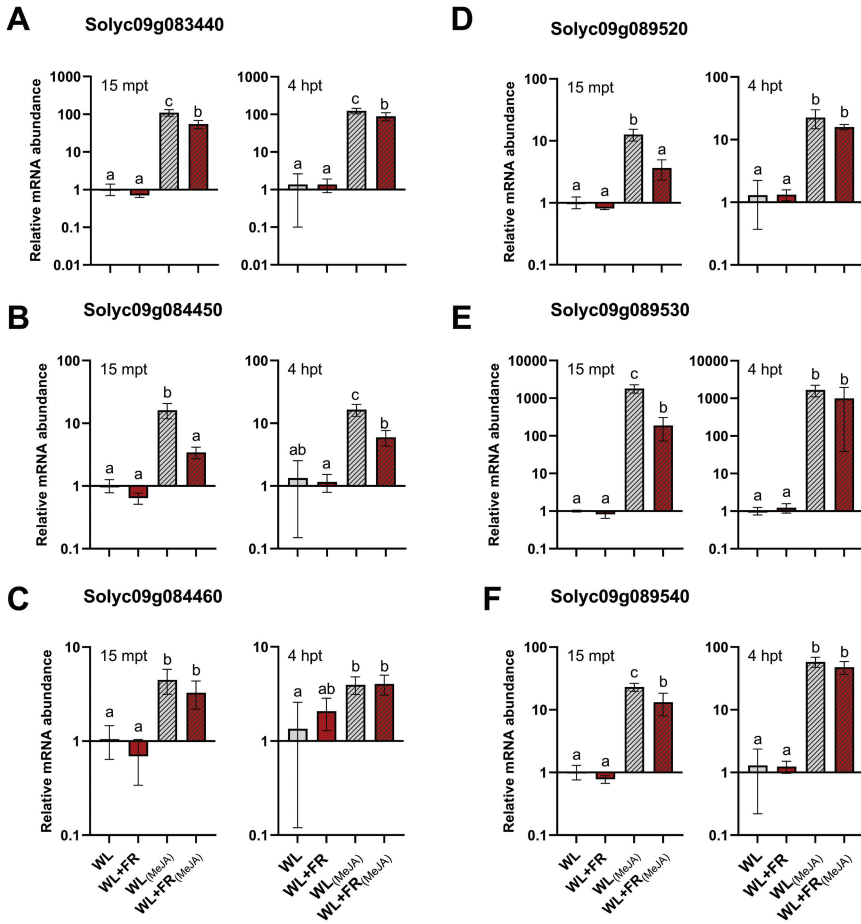


Figure 4.2 : PI induction by exogenous MeJA is delayed by supplemental FR. Gene expression analysis for six *PI* genes after a WL (grey) or WL+FR (red) pretreatment for five days followed by an exogenous meJA (100 μ M) treatment (dashed bars) or a mock solution (plain bars) on intact plants. Leaf material was harvested at 15 min (15 mpt) and 4 h (4 hpt) after the start of the meJA treatment (JA). Values are relative to WL untreated samples for each gene. Data represent mean \pm SD. Different letters represent significant differences (ANOVA with Tukey's post-hoc test, p -value $<$ 0.05), $n = 6 - 8$ plants per treatment.

As our experiments are usually carried out on detached leaflets, we next investigated whether detachment of the leaflets would influence the effect of FR on the JA-mediated *PI* induction. Therefore, leaflets were detached and dipped in a MeJA or mock solution. We collected leaf samples at the start of the experiment and after 4 h (fig. S4.1). Five out of the six genes gave detectable expression values in our conditions. The basal level of expression of all five genes was significantly reduced in FR conditions in untouched plants (timepoint 0) showing that the FR enrichment represses *PI* gene expression already in non-elicited conditions (fig. S4.1). After 4 h, all genes were induced upon MeJA addition irrespective of the light treatment given beforehand, although repressing trends were found for two genes (fig. S4.1B and S4.1D). Overall, supplemental FR delays MeJA-mediated *PI* induction in whole plants (fig. 4.2) and dampens the basal expression of *PI* genes in detached leaflets (fig. S4.1) both showing the negative impact of FR on JA signaling.

FR increases tomato susceptibility via disruptions in jasmonic acid signaling.

Next, we studied the effect of exogenous MeJA treatment on tomato immunity towards *B. cinerea* to verify if this can counteract the FR-mediated susceptibility. After a 5-day pretreatment either in WL or WL+FR, tomato leaflets were detached and dipped in a 50 μ M MeJA, 100 μ M MeJA or a mock solution as a control. As expected, an exogenous MeJA treatment could enhance resistance in WL-exposed plants, confirming a promoting effect of JA on tomato resistance. MeJA could also partly rescue the resistance of WL+FR-pretreated plants. In addition, this promoting effect of MeJA on resistance was stronger at higher concentration (fig. 4.3A). Then, we tested the effect of the JA biosynthesis inhibitor Jarin-1 using the same procedure as used for the MeJA treatments. The addition of Jarin-1 (50 μ M) increased plant susceptibility to *B. cinerea* in WL-treated leaflets. However, it did not significantly affect the resistance of WL+FR-pretreated tissue as the lesion size upon Jarin-1 treatment was similar to the mock conditions (fig. 4.3B). A comparable effect was observed in the JA biosynthesis mutant *def1* (*defenseless 1*) whose susceptibility was increased in WL but not in WL+FR (fig. 4.3C). Interestingly, even though the resistance was modulated by MeJA, jarin-1 and *def1*, FR-treated leaflets remained more susceptible than in WL. Altogether, these results show that the resistance between WL- and WL+FR-treated leaflets cannot become equal through modulation of JA levels in plant tissue. Importantly, we observed that FR reduces the effects of modulation of JA levels on tomato resistance possibly via reduced JA responsiveness.

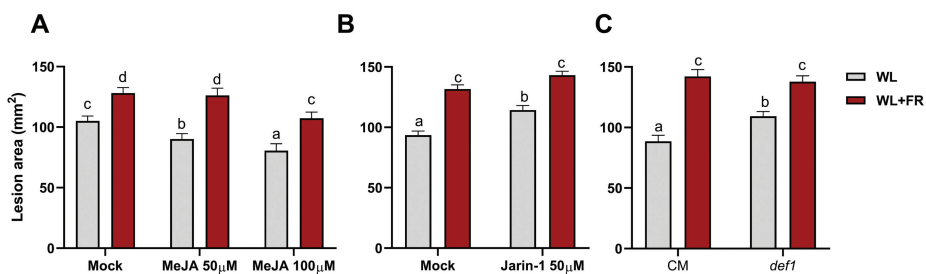


Figure 4.3 : The FR-induced susceptibility is partly JA-dependent. Disease rating on tomato leaflets after an exogenous (A) meJA (50 µM or 100 µM) or (B) Jarin-1 (50 µM) treatment. (C) Disease rating on JA biosynthesis mutant *defenseless 1 (def1)* tomato leaflets and its respective background Castlemart (CM). Data represent mean ± SEM and different letters indicate significant differences (ANOVA with Tukey's post-hoc test, p -value < 0.05), $n = 3 - 8$ plants per treatment.

OPDA and JA levels are only slightly modulated upon FR illumination and *B. cinerea* infection.

In order to investigate whether supplemental FR light and *B. cinerea* interactively modulate JA levels in plants, we quantified JA, JA-Ile and the jasmonic acid precursor OPDA levels in plant tissue in either WL- or WL+FR-pretreated leaflets upon mock or infection by *B. cinerea* at 0 hpi and 12 hpi (fig. 4.4). At 0 hpi, we observed strong variation in the hormone quantifications especially for JA and JA-Ile measurements, which may reflect the wounding response associated with the cutting of the leaflets prior to the inoculation (fig. 4.4B and 4.4C). At 12 hpi, JA and JA-Ile levels were not modulated much by either WL+FR or *B. cinerea* (fig. 4.4B and 4.4C). However, we did observe a slight decrease in OPDA levels in WL+FR-pretreated samples compared to WL-treated plant tissue and a further decrease upon *B. cinerea* infection, although not significant (fig. 4.4A).

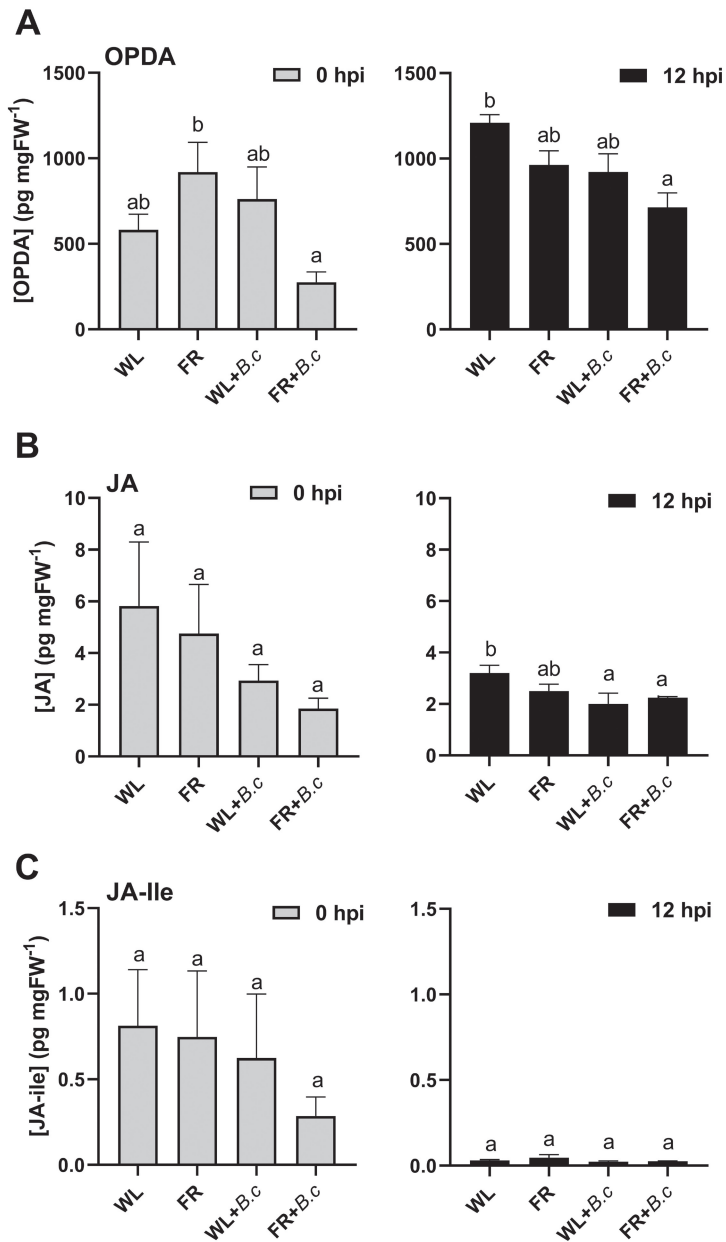


Figure 4.4 : OPDA and JA levels are slightly modulated upon supplemental FR exposure and *B. cinerea* inoculation. (A) OPDA, **(B)** JA and **(C)** JA-Ile quantification by liquid chromatography-tandem mass spectrometry on tomato leaf discs previously exposed to WL or WL+FR for five days and 0 and 12 hours post inoculation with *B. cinerea* spores. Different letters represent significant differences (ANOVA, Tukey's post-hoc test). Data represent mean \pm SEM, n = 6.

Ethylene is involved in the regulation of the FR-induced susceptibility.

To investigate the effect of FR enrichment on ethylene emissions, tomato plants were pretreated in WL or WL+FR for five days and lateral leaflets from the 3rd leaf were detached and carefully placed in separate plastic syringes. After 30 min of incubation, short enough to prevent *de novo* ethylene emissions in response to wounding (Boller and Kende, 1980), the ethylene levels were quantified and found to be elevated in WL+FR-pretreated samples compared to WL (fig. 4.5A). In Arabidopsis, defense responses against necrotrophic pathogens are regulated by the coaction between JA and ethylene signaling (Penninckx et al., 1998). In order to investigate the effect of ethylene on FR-induced susceptibility, we performed bioassays on detached tomato leaflets pretreated for five days under WL and WL+FR and treated with air, the ethylene precursor 1-Aminocyclopropanecarboxylic acid (ACC), ethylene or the ethylene perception inhibitor 1-MCP for 1 h prior to inoculation with *B. cinerea* spores (fig. 4.5B). Addition of ACC to the plates could rescue plant resistance in both WL- and WL+FR-pretreated plant tissue while ethylene alone had more variable effects on resistance in WL+FR from one experiment to another with the overall pattern being that it hardly affects resistance in WL+FR (fig. 4.5B). ACC is converted into ethylene by the leaflets and slowly accumulates in the sealed Petri dishes giving a long-lasting ethylene treatment compared to ethylene treatment alone which is dissipated within minutes after opening the desiccators. Tomato resistance was compromised by a 1-MCP treatment in line with the protective effect of ethylene in tomato resistance against *B. cinerea* previously observed by Díaz et al. (2002) (fig. 4.5B). However, since ethylene is beneficial for plant defense, it is paradoxical that supplemental FR light would simultaneously promote ethylene emission while enhancing susceptibility unless either ethylene sensitivity or the balance between JA and ethylene is affected thus modulating downstream defense activation.

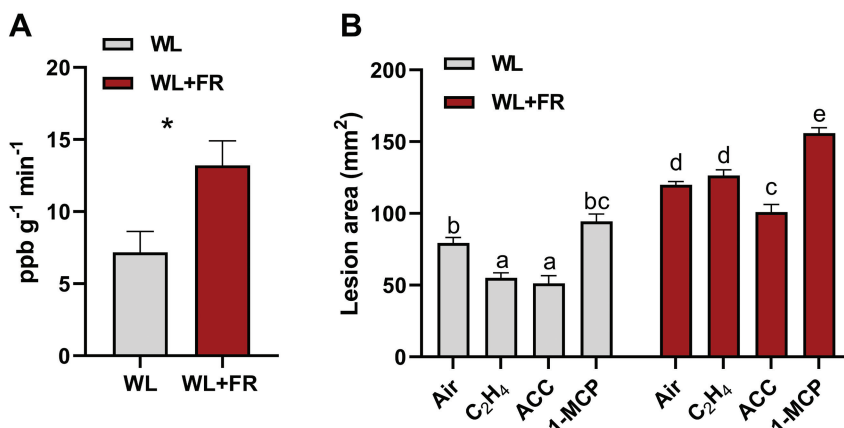


Figure 4.5 : Ethylene enhances tomato resistance. (A) Ethylene emission after five days of WL and WL+FR light treatments. Data represent mean \pm SEM. Asterisk represent significant difference (Student's t-test, p -value < 0.05), $n = 6$. **(B)** Disease rating on tomato leaflets after five days of WL and WL+FR light treatments followed by exposure to either ethylene (C_2H_4), the ethylene precursor ACC or the ethylene receptor blocker 1-MCP for 1 h prior to inoculation (in WL). Data represent mean \pm SEM. Different letters represent significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), $n = 7 - 8$ plants per light treatment.

Low R:FR perception inhibits synergistic action by JA and ethylene on *PI* induction

Plant defense responses against *B. cinerea* require the coaction of JA and ethylene and those hormones have been shown to be able to activate *PI* gene expression. To investigate the effect of FR light on *PI* gene induction upon exogenous JA, ACC and 1-MCP treatments, we pretreated three-weeks-old tomato plants in WL or WL+FR for four days. On day 4, leaflets were detached, placed in Petri dishes and incubated for 24 h in the same light conditions as for the pretreatment to reduce possible effects of cutting the leaves on JA-mediated responses. The leaflets were treated with either ACC, 1-MCP or air (control) prior treatment with a 100 μ M MeJA solution or a mock solution. After 2 h, we observed a slight induction of most *PI* genes by MeJA in WL-pretreated samples (fig. 4.6A-C and G-I) and this induction was less pronounced in WL+FR-pretreated samples (fig. 4.6D-F and J-L). Even though the JA-mediated induction of *PI* is not significant for every gene tested after 2 h of treatment, the trends observed correlate with observations in fig. 4.2 which was measured after 15 min and 4 h of treatment. ACC alone had minor effects on the expression of *PI* genes in

both WL- and WL+FR-pretreated samples (fig. 4.6). However, in WL-treated samples, a combination treatment of ACC with MeJA resulted in a significantly greater induction of four *PI* genes compared to the individual hormone treatments (fig. 4.6A-C and G-I), showing the synergistic effect of both hormones on *PI* induction. Interestingly, we did not observe such synergistic effects in WL+FR-pretreated samples (fig. 4.6D-F and J-L). This suggests that the coaction of JA and ethylene is affected by FR. However, blocking of ethylene receptors with 1-MCP did by itself not affect *PI* gene expression. Altogether, these results show that, in WL conditions, *PI* genes can be strongly induced by a simultaneous JA and ACC treatment and that this synergistic effect between JA and ethylene is reduced in FR-enriched light conditions.

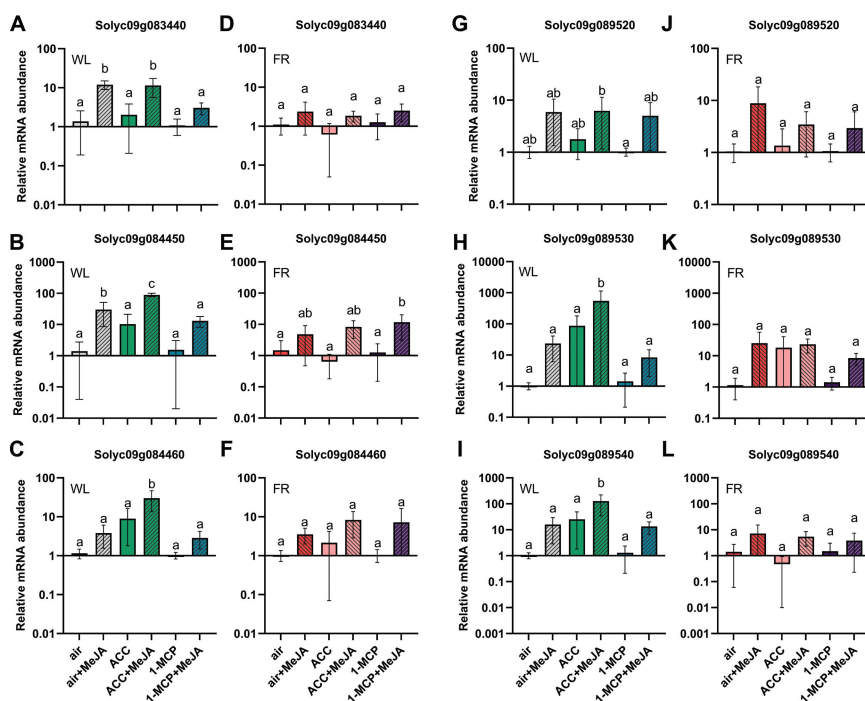


Figure 4.6 : *PI* genes are upregulated via the coaction of JA and ethylene. Relative gene expression analysis for six *PI* genes after a WL (A-C and G-I) or FR (D-F and J-L) pretreatment followed by an air, ACC or 1-MCP treatment. After 1 h, the leaflets were either dipped in a 100 μ M MeJA or a mock solution (dashed and plain bars, respectively) and incubated for 2 h in WL conditions. Data represent mean \pm SD. Different letters represent significant differences (ANOVA Tukey's post-hoc test, p-value < 0.05), n = 3.

Discussion

The induction of *PI* genes by wounding, *B. cinerea* or JA is altered by supplemental FR.

Plants experiencing high planting density often prioritize growth over defense responses (Ballaré and Austin, 2019). From the RNA sequencing results presented in **chapter 3**, we observed that tomato *PI* genes are induced upon infection by the necrotrophic pathogen *B. cinerea*, and this was inhibited by supplemental FR light. Indeed, we observed a strong upregulation of a set of six *PI* genes in WL-treated samples and not in WL+FR-pretreated samples at 12 hpi in **chapter 3** (fig. 3.7A). Here, we observed that MeJA treatment induced the *PI* genes within 15 min and that the level of expression was reduced in WL+FR-pretreated plants compared to WL. Interestingly, *PI* gene expression reached similar levels between WL and WL+FR-exposed plants after 4 h of MeJA treatment showing either a reduced JA sensitivity or a delay in gene induction by FR enrichment (fig. 4.2, fig. S4.1). As supplemental FR seems to reduce JA responsiveness, it is possible that the plants do not respond to JA-inducing *B. cinerea* infection at the same speed, therefore allowing the pathogen to develop faster in supplemental FR conditions. Also, the expression profiles of the six *PI* genes based on the RNA sequencing results revealed a strong induction at 6 hpi in uninfected plants (WL_Mock ; fig. 4.1). Since *PI* have been identified as wound-responsive in the first place (Green and Ryan, 1972), the peak in *PI* expression could be the result of cutting the leaflets prior to starting the experiment. In addition, we observed relatively high variation in the JA and JA-Ile quantifications (fig. 4.4B and 4.4C) correlating with a JA-induced wounding response due to cutting the leaflets. Interestingly, the wounding-mediated *PI* induction is repressed in FR_Mock samples correlating with a dampening of JA-induced responses (fig. 4.1). Upon *B. cinerea* infection, the induction of *PI* becomes even stronger at 12 hpi in WL and stays low in supplemental FR conditions (fig. 4.1). Altogether, our data show that *PI* genes are strongly upregulated by MeJA, wounding and *B. cinerea* infection and FR downregulates JA responsiveness.

Dampening of JA responsiveness by FR reduces *PI* expression and enhances *B. cinerea* susceptibility.

Even though *PI* induction in response to an exogenous MeJA treatment was delayed by supplemental FR light (fig. 4.2), we also observed that *PI* genes were already repressed in untreated plants experiencing WL+FR compared to WL (fig. S4.1). These observations match with the slight decrease in OPDA levels and reduced JA sensitivity in WL+FR conditions compared to WL (fig. S4.1, 4.4A and 4.6). The increase in plant susceptibility towards *B. cinerea* observed in WL+FR might be associated with decreased basal OPDA levels prior to stress encounter. The levels of OPDA are decreased further upon infection which would typically be associated with the production of bioactive JA-Ile. However, the levels of JA-Ile remained low at 12 hpi (fig. 4.4C). We cannot exclude that OPDA itself could play a role in the activation of other defense processes such as callose deposition (Scalschi et al., 2015). It is also possible that the OPDA-mediated JA biosynthesis happened after 12 hpi. The possibility that *B. cinerea* would directly downregulate JA biosynthesis via salicylic acid (SA) antagonism (Caarls et al., 2015; El Oirdi et al., 2011) is unlikely in our conditions since the levels of SA stayed unchanged at 12 hpi (fig. S4.1). Indeed, increased susceptibility to *B. cinerea* in FR-exposed Arabidopsis was also not mediated by the putative SA-mediated repression of JA (Cerrudo et al., 2012; De Wit et al., 2013). Tomato resistance against *B. cinerea* was rescued by exogenous MeJA treatment while it was compromised by Jarin-1 or in the *def1* mutant (fig. 4.3). However, we could only observe an increased resistance in WL+FR samples at a concentration of 100 μ M MeJA which might be explained by low JA sensitivity in WL+FR (Cerrudo et al., 2012; De Wit et al., 2013; Izaguirre et al., 2013; Moreno et al., 2009). Even with supplemental MeJA, the WL+FR-exposed plants never reached lesion areas similarly small as those in WL, pointing at additional routes being also affected by FR light, one of which could be related to sugar status as investigated in **Chapter 5**.

Ethylene emissions and response are modulated by FR.

We observed that supplemental FR enhances ethylene emissions by plant leaves, while exogenous ACC or ethylene treatment promote plant resistance to the fungus (Díaz et al., 2002; fig. 4.5). It would be possible that the increased levels of ethylene in WL+FR-exposed plants leads to a desensitization to ethylene via a negative feedback loop that is well known for this hormone (Chen et al., 2007; Kendrick and Chang, 2008;

Rai et al., 2015). This would explain why susceptibility to *B. cinerea* stays rather high in WL+FR-pretreated plants after 1 h of exposure to high ethylene levels that cause a clear increase of resistance to *B. cinerea* in WL-treated plants (fig. 4.5B). The ethylene gas exposures were very brief (1 h), relative to the infection period (3 d), because the desiccator had to be opened again for the assays, and the ethylene gas would escape. Therefore, ACC treatments were used as an alternative. ACC is gradually converted into ethylene and since the plates are sealed during the infection, ethylene accumulates and the treatment lasts longer (fig. 4.5B). Even though ethylene sensitivity might be reduced by supplemental FR, the fact that plants exhibit an increase in ethylene emissions could be beneficial for disease resistance. In addition, a 1-MCP treatment significantly increased susceptibility in WL+FR conditions but not in WL suggesting that the increased basal ethylene emissions in WL+FR as compared to WL (fig. 4.5B) still somewhat promote resistance in these plants.

Synergism between ethylene and JA on *PI* induction is affected by supplemental FR.

Synergy between the JA and ethylene pathways is of great importance to optimally regulate plant defenses against necrotrophic fungi (Glazebrook, 2005; Lorenzo et al., 2003; Penninckx et al., 1998; Pré et al., 2008). In Arabidopsis, the JA and ethylene pathways are indispensable for plant resistance against *B. cinerea* and the JA-ethylene markers *ERF1* and *PDF1.2* have been shown to be downregulated upon infection in low R:FR compared to WL in Arabidopsis (Cerrudo et al., 2012; Zhang et al., 2014). In our study, *PI* genes were not very responsive to ACC alone, but were significantly induced by a combination of ACC and MeJA (fig. 4.6A-C and G-I). This suggests that ethylene acts as a catalyzer of the JA-mediated response rather than a direct regulator of defense. Interestingly, the synergy between JA and ethylene to induce *PI* does not occur in WL+FR conditions (fig. 4.6D-F and J-L). It is possible that the JA and ethylene sensitivity in supplemental FR is reduced to the point where the ethylene-mediated *PI* induction via JA signaling is prevented (fig. 4.2 and fig. 4.5). Other hypotheses could be that the coaction between JA and ethylene exists in WL+FR but goes through a *PI*-independent process or that we miss the necessary time resolution in the current data set. As supplemental FR light reduces plant sensitivity to JA and probably ethylene, it is paramount to estimate the involvement of both hormones separately and how they affect downstream defense activation.

Conclusion

To summarize, in this chapter we investigated the roles of JA and ethylene in *PI* gene expression and resistance against *B. cinerea* under WL versus WL+FR. JA and ethylene are both needed for the full induction of *PI* genes but supplemental FR light decreases plant responsiveness to these hormones leading to decreased *PI* gene induction, which is associated with enhanced susceptibility to *B. cinerea*. To investigate whether *PI* contribute themselves to FR-induced susceptibility, tomato lines would have to be created carrying antisense *PI* sequences or use CRISPR-Cas9 technology to create mutant versions of *PI* genes to validate their roles.

Acknowledgements

We would like to thank Dr. Sjon Hartman for help with the ethylene treatments and measurements. We kindly thank Dr. Maria J. Pozo from the Department of Soil Microbiology and Symbiotic Systems in Granada (Spain) for providing tomato cv. Castlemart and *def1* mutants seeds. We also thank the members from the “LED it be 50%” consortium for help and feedback on the project. This work was funded by the Dutch Research Council, TTW Perspectief grant nr 14125 (“LED it Be 50%”) and supported by Signify, WUR Greenhouse Horticulture and LTO Glaskracht.

Material and methods

Plants growth conditions and light treatments

Tomato plants cv. Moneymaker, Castlemart and the JA biosynthesis mutant *defenseless1 (def1)* in the Castlemart background were grown in long day conditions under WL LEDs as described in **chapter 2**. Three-week-old tomato plants were exposed for five days under White LEDs (WL research LED modules Philips ; R:FR = 5.5 ; Phytochrome stationary state (PSS) value = 0.8) or WL supplemented with FR LEDs (WL+FR ; R:FR = 0.14, PSS value = 0.5). The first two lateral leaflets of the 3rd leaf were used for the experiments. Light spectra used in this experiment are displayed in fig. S2.1.

Fungus growth conditions and bioassays

Botrytis cinerea (Bc 05.10) was maintained on half strength Potato dextrose agar medium and grown for two weeks under natural daylight conditions. Spores were harvested and used for bioassays as described in **chapter 2**.

Chemical treatments

Five days after the start of the WL or WL+FR pretreatment, tomato leaflets from the 3rd leaf were detached and immediately dipped for 10 sec in a 50 μ M or 100 μ M meJA (Sigma-Aldrich) or mock solution (0.1 % EtOH) supplemented with 0.1 % Tween 20. When performed on whole plants, the 3rd leaf was sprayed with 100 μ M MeJA or a mock solution (0.1 % Tween 20). Hormone treatments started at 10:00 am in either WL or FR. For gene expression experiments, leaf material was sampled for every condition at 0, 15 min and 4 h after the start of the MeJA treatment. For bioassays, tomato leaflets were inoculated in WL conditions with *B. cinerea* spores 4 h after MeJA application. The same procedures and experiments were performed by using the JA biosynthesis inhibitor Jarin-1 (50 μ M).

For ethylene-related experiments, tomato leaflets from the 3rd leaf were detached and placed in square petri dishes containing two discs of Whatman® filter paper soaked with tap water. Petri dishes were placed in separate transparent glass desiccators. Control plates were placed in a desiccator of which the lid was slightly opened to allow for air circulation. Ethylene treatments were performed by either replacing tap water by 6 ml of a 10 μ M ACC (ethylene precursor) solution or by injecting gaseous ethylene (10 ppm) in the desiccator. The last desiccator was injected with the ethylene receptor blocker 1-MCP (10 ppm) with plastic syringes. All treatments lasted for 1 h prior to inoculation with *B. cinerea* spores as previously described.

Simultaneous JA and ethylene treatments

Simultaneous hormonal treatments consisted in treating isolated leaflets with either air, ACC or 1-MCP for 1 h in glass desiccators as described above prior to dipping the leaflets in a 100 μ M MeJA or mock solution. Plates were incubated in WL for 2 h prior to sampling for gene expression analysis.

RNA isolation and gene expression

Treated leaf discs were sampled for RNA isolation (three or four biological replicate per treatment) and ground with silica beads for 1 min in a tissue lyser and supplemented with 300 μ l of cell lysis buffer (2% SDS, 68 mM sodium citrate, 132 mM citric acid and 1 mM EDTA) and incubated for 5 min at room temperature prior to adding 100 μ l of protein/DNA precipitation buffer (4 M NaCl, 16 mM sodium citrate and 32 mM citric acid) followed by 10 min of incubation on ice. Samples were centrifuged for 15 min at 13000 rpm, the supernatant was transferred in a new tube (300 μ l) and supplemented with an equal volume of ice cold isopropanol. All tubes were centrifuged for 5 min at 13000 rpm, pellet was washed with 300 μ l of 70% EtOH. RNA pellet were air dried for 5 min before elution in 30 μ l RNase-free water. cDNA synthesis was carried out using RevertAid H minus Reverse transcriptase (Thermo Scientific). Gene expression analysis were performed by quantitative RT-PCR in a Viiia7PCR device with 5 μ l reaction mix containing SyberGreen Supermix (Bio-Rad). Tomato actin was used as a reference gene and fold change in expression were calculated based on the $2^{-\Delta(\Delta Ct)}$ method (Livak and Schmittgen, 2001). Primer sequences used are displayed in table S4.1.

Ethylene quantification by gas chromatography

Tomato leaflets were detached from WL- or WL+FR-treated plants, carefully wound and inserted in a 1-ml syringe. After 30 min, the gas contained in the syringe was injected in a gas chromatography device (Syntech Spectras GC955) to determine ethylene levels.

Extraction of plant growth regulators

For the extraction of plant growth regulators, tomato cv. Moneymaker leaf discs (~20 mg corresponding to 4 - 5 leaf discs) were snap-frozen and used per sample. Tissue was ground to a fine powder at -80°C using 3-mm stainless steel beads at 50 Hz for 1 min in a TissueLyser LT (Qiagen, Germantown, USA).

Ground samples were extracted with 1 mL of 10% methanol (MeOH) containing stable isotope-labeled internal standards (IS, Supplemental Table S4.2). Internal standards were used at an end concentration of concentration of 100 nM per compound per sample. Samples were vortexed, ultrasonicated for 30 seconds and extracted at 4°C overnight on an shaker. Subsequently, samples were centrifuged at 12000 rpm for

10 min in a tabletop centrifuge set at 4°C. Supernatants were transferred to amber 4-mL glass vials. Pellets were re-extracted with 1 mL of 10% MeOH for 1 h at 4°C. After centrifugation as above, both supernatants were pooled before loading on a 30 mg Oasis HLB Cartridge (Waters, Milford, USA). The cartridge was equilibrated with 1 mL of MeOH and Equilibrated with acetonitrile/water (0.1% formic acid) (10/90, v/v) prior to sample loading. Subsequently, the cartridge was washed with 1 mL of 10% MeOH and eluted with 1 mL of 80% MeOH. The 80% MeOH was evaporated in a speed vacuum system (SPD121P, ThermoSavant, Hastings, UK) at RT and the residue stored at -20°C until further analysis.

Detection and quantification of plant growth regulators by liquid chromatography-tandem mass spectrometry

Samples were resuspended in 100 µL of acetonitrile/water (0.1% formic acid) (10:90, v/v) and filtered through a 0.45 mm Minisart SRP4 filter (Sartorius, Goettingen, Germany). Analyses of plant growth regulators from *S. lycopersicum* leaf material was performed by comparing retention times and mass transitions with those of unlabeled standards (Supplemental Table S4.2) using a Waters XevoTQs mass spectrometer equipped with an electrospray ionization source coupled to an Acquity UPLC system (Waters, Milford, USA). Chromatographic separations were conducted on an Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 mm; Waters, USA) by applying an acetonitrile/water (0.1% formic acid) gradient. The column was operated at 40°C with a flow rate of 0.25 mL min⁻¹. The column was equilibrated for 30 min using either solvent composition at the start of a run.

The acetonitrile/water (0.1% formic acid) gradient started from 20% (v/v) acetonitrile, increasing to 70% (v/v) acetonitrile in 17 min. To wash the column, the water/acetonitrile gradient was increased to 100% (v/v) acetonitrile in a 1.0 min gradient, which was maintained for 1.0 min before going back to 20% acetonitrile using a 1.0 min gradient, prior to the next run.

The sample injection volume was 3 µL. The mass spectrometer was operated in positive and negative electrospray ionization mode when required. Cone and desolvation gas flows were set to 150 and 1000 l h⁻¹, respectively. The capillary voltage was set at 3.5 kV, the source temperature at 150°C, and the desolvation temperature at 550°C. The cone voltage was optimized for each standard compound using the IntelliStart MS Console (Waters, Milford, USA). Argon was used for fragmentation by collision-induced dissociation. Multiple reaction monitoring (MRM) was used for

quantification. Parent–daughter transitions for the different (stable isotope labeled) compounds were set using the IntelliStart MS Console. MRM transitions selected for compound identification and quantification are shown in Supplemental Table S4.2. The cone voltage was set to 40 eV.

To determine sample concentrations, a 10-point calibration curve was constructed for each compound ranging from 1 μ M to 190 pM and each dilution also contained a known amount of an appropriate deuterium-labelled internal standard.

Supplemental data

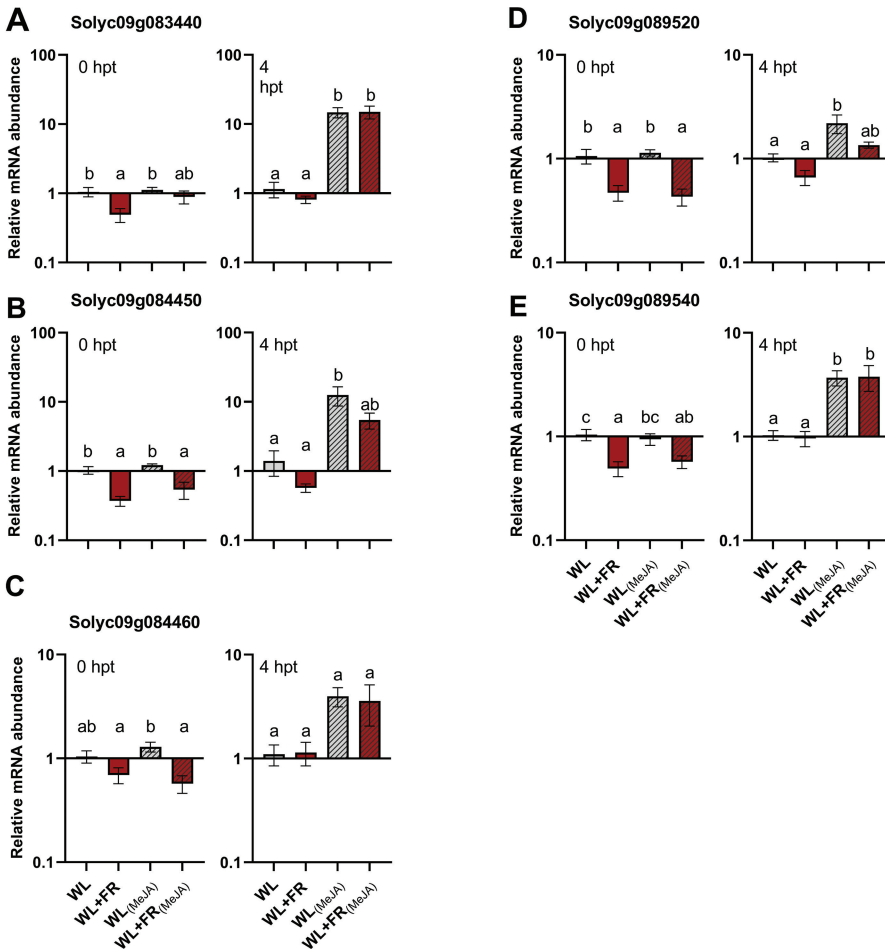


Figure S4.1 : PI genes are upregulated upon by exogenous MeJA. Gene expression analysis for five PI genes after a WL (grey) or FR (red) pretreatment followed by an exogenous MeJA (50 μ M) treatment (dashed bars) or a mock solution (plain bars). Leaf material was harvested at the start and 4 h after the start of the treatment. Data represent mean \pm SEM. Different letters represent significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), n = 6 - 8 plants per treatment.

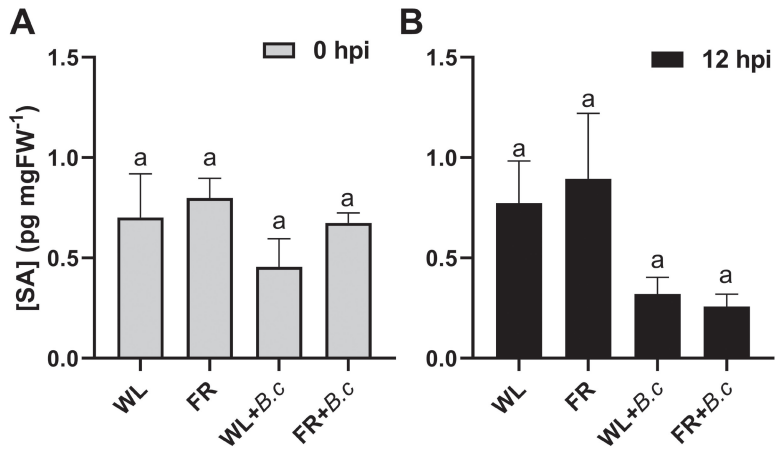


Figure S4.2 : Salicylic acid (SA) levels are not modulated upon either supplemental FR exposure or *B. cinerea* infection. SA quantification by liquid chromatography on tomato leaf discs exposed to WL or FR for 5 days and (A) 0 and (B) 12 hours post inoculation (hpi) with *B. cinerea* spores. Data represent mean + SEM. Letters represent significant differences (ANOVA, Tukey's post-hoc test, $p < 0.05$), $n = 6$.

Table S4.1. Primers used for *PI* gene expression analysis by qPCR in this chapter.

Oligo Name	Orientation	Seq (5' - 3')
Solyc09g083440	Forward	AATTGGTGGACCAGAAGTCA
	Reverse	CCGAACATCAGTTATGAATGG
Solyc09g084450	Forward	GAATTTGAATCCGCATCTTG
	Reverse	TCGATCACATCGAAAATCCT
Solyc09g084460	Forward	TTTGAATCCGAATTTTGGTG
	Reverse	TCGAAAATCCATTGTGACTG
Solyc09g089520	Forward	TTCAAACACTGATGGCAAGA
	Reverse	TTGAGTTACTATTGGATTTTCCTTC
Solyc09g089530	Forward	GCGCAAAGAAATGGACTAGA
	Reverse	CAGTTGTGATTGGAGAACCA
Solyc09g089540	Forward	TTCAATCACTGATGGCAAGA
	Reverse	GCCTAGGCATACCAGGAAAT
Actin	Forward	GGAAC TTGAAACCGCTAGGAGCA
	Reverse	GAGTTGTATGTAGTCTCATGGATACC

Table S4.2. Multiple reactions monitoring (MRM) transitions table for all plant growth regulators and corresponding internal standards used in this study.

Number	Compound	Retention Time	Mass*	MRM transition	Cone V.	Coll. Energy
1	SA	3.64	-137.1	93.1 [‡]	40	15
			+139.1	121.0	18	12
2	[^{2H4}]SA	3.60	-141.1	97.1 [‡]	40	15
			+143.1	124.9	20	14
3	JA	5.78	-209.06	59.1 [‡]	40	12
			+211.1	133.1	16	16
4	[^{2H5}]JA	5.76	-214.25	61.9 [‡]	40	12
			+216.2	135.29	14	12
5	OPDA	12.43	+293.25	95.25	30	22
				133.25	30	20
				275.25 [‡]	30	10
6	[^{2H5}]OPDA	12.41	+298.25	95.25	30	22
				133.25	30	20
				275.25 [‡]	30	10
7	JA-Ile	8.38	+324.45	86.25 [‡]	35	20
				151.3	35	15
				278.45	35	10
8	[^{2H2}]JA-Ile	8.35	+326.4	151.3 [‡]	35	15
				280.45	35	10

*Mass in positive (+) or negative (-) ion mode. ‡transition used for quantification



Chapter

5

Host sweet host: Far-red light increases susceptibility by changing the sugar pool in tomato

Sarah Courbier¹, Sanne Grevink¹, Pierre-Olivier Bonhomme¹, Saskia C.M. Van Wees² & Ronald Pierik¹

¹Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, the Netherlands

²Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, the Netherlands

Abstract

As autotrophic organisms, plants possess the ability to sustain growth by harnessing light energy into sugars through photosynthesis. At high planting densities, red (R) and blue light availability is strongly decreased and far-red (FR) light reflected towards neighboring vegetation leading to a decrease in the R:FR ratio. A low R:FR ratio results in the inactivation of phytochrome (PHY) photoreceptor family triggering the “shade avoidance syndrome” (SAS). The *Arabidopsis* phytochrome triple mutant *phyBDE* exhibits a constitutive SAS phenotype and matches this to elevated soluble sugar and starch levels compared to wild type plants. Also supplemental FR light has been shown to enhance plant susceptibility to pathogens referred to as “FR-induced susceptibility”. Although pathogens principally feed on sugar supplies provided by the plant, the link between plant immunity and increased sugar levels in plants upon phytochrome inactivation has never been studied before. In order to investigate this, we studied the effect of endogenous addition of glucose in plant tissue on tomato immunity towards the necrotrophic fungus *Botrytis cinerea*. Our data indicate that FR enrichment changes the sugar pool in tomato leaves and that additional sugars benefit *B. cinerea* growth *in vitro* and *in planta*. In addition, we observed that FR illumination on single tomato leaflets could affect sugar concentration and immunity in distal leaves. With these data, we wish to stress the importance of primary metabolism in the tradeoff between growth and defense signaling in tomato that could control plant susceptibility.

Introduction

Plant growth depends on carbohydrate supplies derived from photosynthesis. Soluble sugars are produced via the conversion of atmospheric CO₂ into fructose-6-phosphate (Fru6P) in the Calvin cycle occurring in the chloroplasts in the presence of water and light (Stitt and Zeeman, 2012). Fru6P is converted into Glucose-1-phosphate (Glc1P) through several enzymatic steps. In addition to enzymatic activity, ATP plays a major role in carbohydrate production in chloroplasts. ATP is produced by ATP synthase via the photophosphorylation pathway requiring the activation of photosystem II in the membrane of thylakoids in the chloroplasts (Arnon et al., 1954). Light excites electrons from the reaction center of photosystem II carried towards the cytochrome b₆-f complex by plastoquinone allowing the internalization of protons from the stroma into the lumen (Tikhonov, 2014). The change in proton gradient between both sides of the thylakoid membrane is balanced by the pumping of protons from the lumen towards the stroma through ATP synthase which allows the phosphorylation of ADP and inorganic phosphate (Pi) into ATP (Arnon et al., 1954). ATP is used to convert Glc1P into ADP-glucose (ADPGlc) that can be stored as glucose polymers forming starch granules in the chloroplast (Stitt et al., 2010). In parallel, Glc1P can also be transformed into UDP-glucose via the reduction of ATP into ADP and Pi to be exported as sucrose from source to sink organs through phloem transport (Kölling et al., 2015). At night, starch is reconverted into simple soluble sugars allowing the plant to continue growing in the absence of light (Stitt and Zeeman, 2012).

Carbohydrates generated by plants are the primary nutrition target of plant pathogens therefore plants must heavily regulate sugar fluxes to restrict pathogens from accessing those resources. Studies reported a strong downregulation of photosynthetic processes in *Arabidopsis* upon infection by both necrotrophic and biotrophic pathogens (Berger et al., 2007; Windram et al., 2012) leading to the transition from source to sink organ in infected tissue. However, plant pathogens have developed the capacity to hijack the sugar transport machinery of their host to redirect carbohydrates towards the infection site (Chen et al., 2010; Doidy et al., 2012; Lapin and Van den Ackerveken, 2013). Pathogenic bacteria and fungi were shown to enhance the expression of different sets of SWEET sugar transporters in *Arabidopsis* upon infection showing a pathogen-specific sugar transport manipulation (Chen et al., 2010). In other plant systems such as grapevine, *B. cinerea* was shown to upregulate *SWEET4* and further analysis on *Arabidopsis* showed

that *sweet4* mutants had increased resistance to the fungus (Chong et al., 2014). However, high sugar concentrations are often associated with stronger plant defense potential. Many studies have shown the involvement of sugars in the induction of secondary metabolites and/or defense gene induction such as MAPK, PR genes or phenylpropanoid metabolic pathway (reviewed in Moghaddam and Van Den Ende, 2012). Nevertheless, the involvement of sugars in plant defense still needs further research as it differs for most pathosystems where pathogens and hosts keep on evolving novel strategies infection – defense mechanisms.

Light quality also plays a major role in regulating carbohydrate production in plants. Red (R) and blue light are used by the plant to fuel photosynthesis and far-red (FR) light is reflected by or transmitted towards neighboring vegetation. At high planting density, FR radiation becomes omnipresent and leads to low R:FR light conditions in the canopy. Changes in light quality are sensed by specialized photoreceptors called phytochromes that are found in two photoconvertible form which are inactivated in the presence of low R:FR (Franklin, 2008). This inactivation results in the release of Phytochrome Interacting Factors (PIFs) that in turn trigger the activation of growth-related genes expression leading to morphological changes known as the shade avoidance response (Ballaré and Pierik, 2017; Casal, 2013). Recently, phytochrome signaling has been described to influence carbohydrate levels in plants. In Arabidopsis, plants lacking phytochromes showed an increase in soluble sugars and starch during daytime compared to wild-type plants indicating the involvement of phytochrome in the control of primary metabolism (Yang et al., 2016). Also, low R:FR was described to lower plant defense towards *B. cinerea* through a decrease in metabolite production and lower defense gene activation (Cargnel et al., 2014). Interestingly, low R:FR treated plants or phytochrome mutants combine enhanced sugar supplies and lower defense potential compared to normal growth conditions or wild-type plants.

It is paramount to understand the relationship between light and primary metabolism in case of a pathogen attack. Interestingly, the impact of sugars has so far only been studied during infection while low R:FR elevates soluble sugar levels prior to a potential infection by a pathogen. As tomato plants are grown at high planting densities in greenhouses, we investigate whether low R:FR impacts soluble sugar levels in crop plant species like tomato and whether it could be associated to higher susceptibility to *B. cinerea*. In this chapter, we observed that low R:FR causes an increase in glucose and fructose content in leaf tissue and that such fluctuations

promoted *B. cinerea* growth *in vitro* as well as tomato susceptibility. Here, we show that in addition of a delayed and dampened JA-related defense activation (**chapter 3 and 4**), elevated sugar levels also play a role in the FR-induced susceptibility in tomato.

Results

FR light increases soluble sugar content in tomato leaves.

Based on findings in *Arabidopsis phy* mutants from Yang et al. (2016), we hypothesize that tomato plants experiencing WL+FR would accumulate more soluble sugars in leaf tissue compared to WL that could greatly influence *B. cinerea* lesion development. To test this hypothesis, we pretreated tomato plants for five days under WL as a control and WL supplemented with FR LEDs (WL+FR) to simulate neighboring vegetation. Leaf discs from the third formed leaf normally used for bioassays were harvested (fig. 5.1A) and the soluble sugar content was quantified to estimate the sugar levels present in the leaves at the time our bioassays started. The total soluble sugar levels were indeed elevated in WL+FR-pretreated plants where we expect phytochromes to be inactivated compared to WL controls (fig. 5.1B). We observed a doubling in the concentration of glucose and fructose which is in line with the observations made in *Arabidopsis* (fig. 5.1C). However, the levels of sucrose did not show a significant increase, making glucose and fructose potential candidates for influencing disease resistance in tomato towards *B. cinerea*.

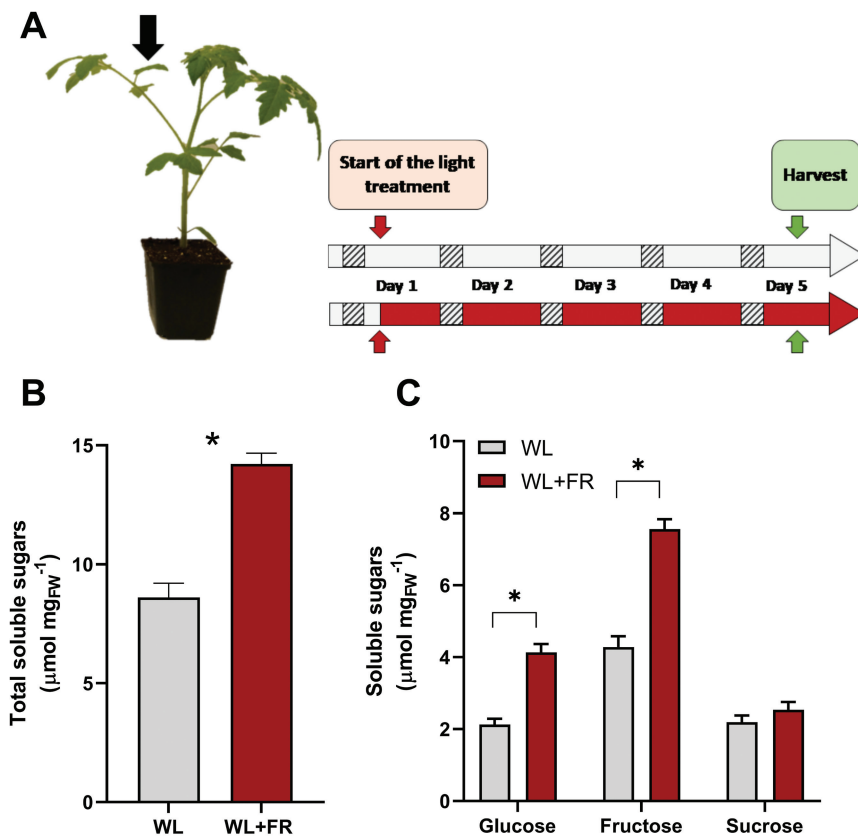


Figure 5.1 : Soluble sugar content is elevated in tomato leaves upon shade avoidance. (A) Three-week-old tomato plants were exposed to WL or WL+FR conditions for five days (grey dashed squares represent dark periods). Soluble sugars were quantified from the first lateral leaflets of the third leaf (indicated by the black arrow). (B) total soluble sugars and (C) glucose, fructose and sucrose content at midday on day five after the WL (grey bars) and FR (red bars) treatment. Data represent mean \pm SEM and asterisks represent significant differences between WL and WL+FR (Student's t-test, p-value < 0.05), n = 8 plants per treatment.

***B. cinerea* benefits from elevated sugars concentration.**

In order to determine whether the elevated glucose and fructose levels in WL+FR-pretreated plants could affect *B. cinerea* growth *in vitro*, we first tested the effect of ground leaf tissue from either WL- or WL+FR-pretreated plants mixed through agar plates (fig. 5.2A). Compared to control agar plates, the mycelium grew faster on agar plates supplemented with plant material, consistent with our hypothesis that elevated

sugars in these leaves would promote growth. We also observed a significantly higher mycelial growth on plates containing WL+FR-treated compared to WL treated plant tissue correlating with our previous findings linking elevated leaf sugar content to higher mycelial growth. We also designed an *in vitro* assay where *B. cinerea* spores were deposited onto PDA ½ medium supplemented with increasing concentrations of glucose or fructose (fig. 5.2B). Even though PDA ½ alone allows the mycelium to grow rapidly, we still observed a significant increase in mycelium diameter upon sugar supplementation indicating that *B. cinerea* indeed benefits from higher glucose and fructose concentration *in vitro*. In addition, we looked whether sugars added to the spore solution could increase disease development in WL. The addition of glucose or fructose in the spore suspension right before inoculation led to a considerable increase in lesion area compared to a regular spore solution only containing PDB ½ (fig. 5.2C). These results show that elevated glucose or fructose concentrations boost *B. cinerea* spore development *in vitro* and *in planta*. Consistently, supplementing plates with plant material promotes *B. cinerea* growth, and more so with WL+FR-treated leaves that contain elevated sugars.

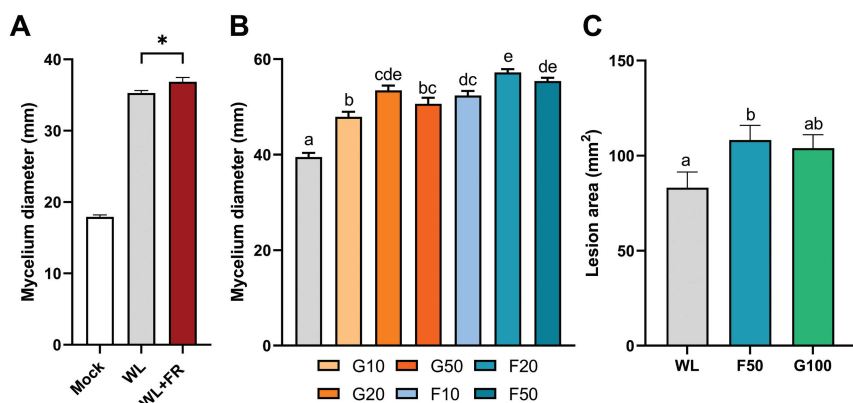


Figure 5.2 : *Botrytis cinerea* growth is enhanced by elevated soluble sugar concentrations, *in vitro* and *in planta*. Mycelium diameter growth measurement after 3 days on (A) agar-based media supplemented with either water (mock), WL-treated or WL+FR-treated ground leaf material and (B) on PDA-based media supplemented with increasing concentrations (10, 20 or 50 g l⁻¹) of glucose (G10, G20 and G50) or fructose (F10, F20 and F50), n = 10. (C) Disease rating on tomato leaflets inoculated with *B. cinerea* spores supplemented with fructose (50 g l⁻¹, F50) or glucose (100 g l⁻¹, G100), n = 8 plants. Data represent mean ± SEM. Asterisk (Student's t-test, p-value < 0.05) and different letters (ANOVA, Tukey's post-hoc test, p-value < 0.05) represents significant differences.

The FR-induced susceptibility is partly sugar-dependent.

To investigate the effect of elevated sugar content on lesion development on tomato leaves, we designed a series of experiments allowing for manipulation of internal glucose content on separated tomato leaflets prior to sugar content analysis or bioassays. Whole tomato plants were pretreated for four days either in WL or WL+FR and two lateral leaflets per plant (from the 3rd leaf) were detached. The leaflets were then placed in a tilted plate with their petiole reaching inside a mock or 0.5 M glucose solution. After 24 h of treatment, we detected an increase in the total sugar content upon glucose supplementation and as expected, which was caused entirely by an increase of glucose, and not fructose or sucrose (fig. 5.3A and C). The same procedure was carried out prior to inoculating the leaflets with *B. cinerea* spores (fig. 5.3B). Interestingly, plant susceptibility was increased upon glucose supplementation in both WL- and WL+FR-pretreated leaflets compared to mock conditions which clearly shows that elevated glucose levels in tomato leaflets is correlated to higher lesion development. Also, lesion area measured on WL + 0.5 M glucose leaflets was very similar to WL+FR-pretreated leaflets indicating that glucose elevations in WL+FR could indeed be partially responsible for the increase in susceptibility.

To test whether sugar elevation in WL+FR could indeed be causal to the susceptibility phenotype, we performed a similar experiment as with sugar additions, except that the leaflets were treated either with a mock solution or 100 μ M DCMU prior to sugar analysis or inoculation with *B. cinerea*. DCMU is a herbicide that disrupts the light reactions of photosynthesis (Velthuys, 1981). After 24 h of DCMU treatment, the total soluble sugar content was indeed reduced, reaching similar levels between WL- and WL+FR-pretreated leaflets (fig. 5.4A) and glucose, fructose and sucrose levels were all reduced to reach similar levels between WL- and WL+FR-treated leaflets (fig. 5.4C). When challenged with *B. cinerea* spores, the lesions were significantly smaller after DCMU treatment compared to mock conditions, confirming the importance of plant sugar accumulation for *B. cinerea* growth. However we could still see an effect of supplemental FR on susceptibility in both treatments compared to WL conditions (fig. 5.4B), indicating that other pathways such as JA (**chapter 3** and **chapter 4**) are also involved in determining pathogen development. DCMU, like any drug, may have additional direct or indirect side effects and we, therefore, tried to find another way to reduce sugar levels in plant tissue.

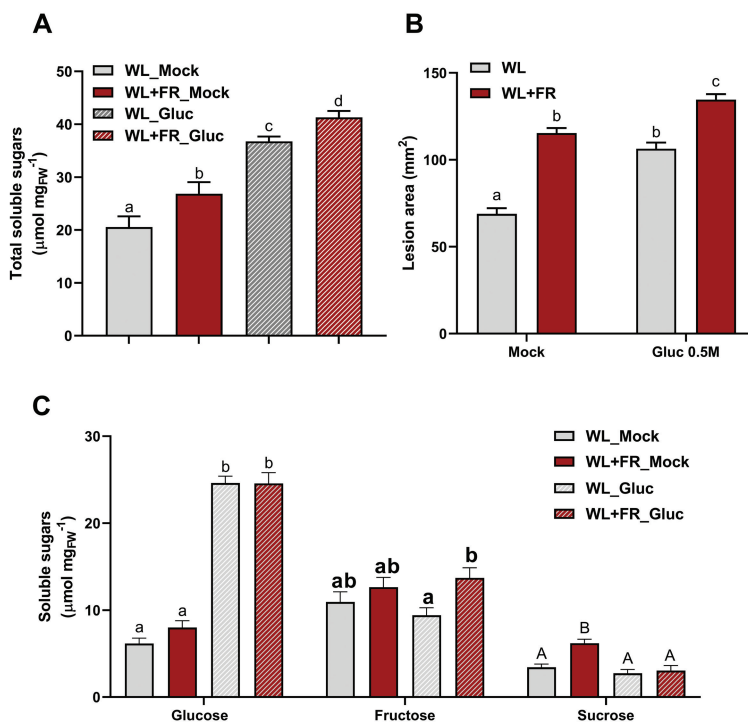


Figure 5.3 : Glucose supplementation *in planta* enhances lesion development by *Botrytis cinerea*.

Quantifications of (A) total soluble sugars and (C) glucose, fructose and sucrose content on three-week-old plants after four days in WL or WL+FR followed by 24 h of either mock (plain) or 0.5 M glucose solution supplementation (dashed) on detached leaflets. The same experimental procedure was performed prior to bioassay with *B. cinerea* spores. (B) Disease rating on tomato leaflets treated as for (A) and (C) at three days post inoculation with *B. cinerea* spores. Data represent mean \pm SEM and different letters indicate significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), $n = 7 - 8$ plants per treatment per experiment.

Plants produce soluble sugars through photosynthesis during the day and consume those during the night (Stitt and Zeeman, 2012). Therefore we expected sugar levels to be reduced to similar levels between WL- and WL+FR-pretreated plants after extending the night period for 3 h. Starting an infection at that time point might thus cancel out a putative sugar effect between WL and WL+FR. However, at the end of night soluble sugar levels turned out to be still significantly increased in WL+FR-pretreated samples compared to WL meaning that plants experiencing additional FR accumulate sugars during the day and do not fully use them during night time. Accordingly, WL+FR-pretreated plants still showed increased susceptibility compared to WL (fig. S5.1).

Altogether, these data clearly show the role of soluble sugar levels in the modulation of tomato susceptibility towards *B. cinerea*. However, the level of susceptibility induced by WL+FR cannot be fully complemented by adding sugars on WL-treated plants which indicates that another pathway is regulating defense responses under supplemental FR conditions such as hormonal regulation through JA-dependent mechanisms presented in **chapter 4**.

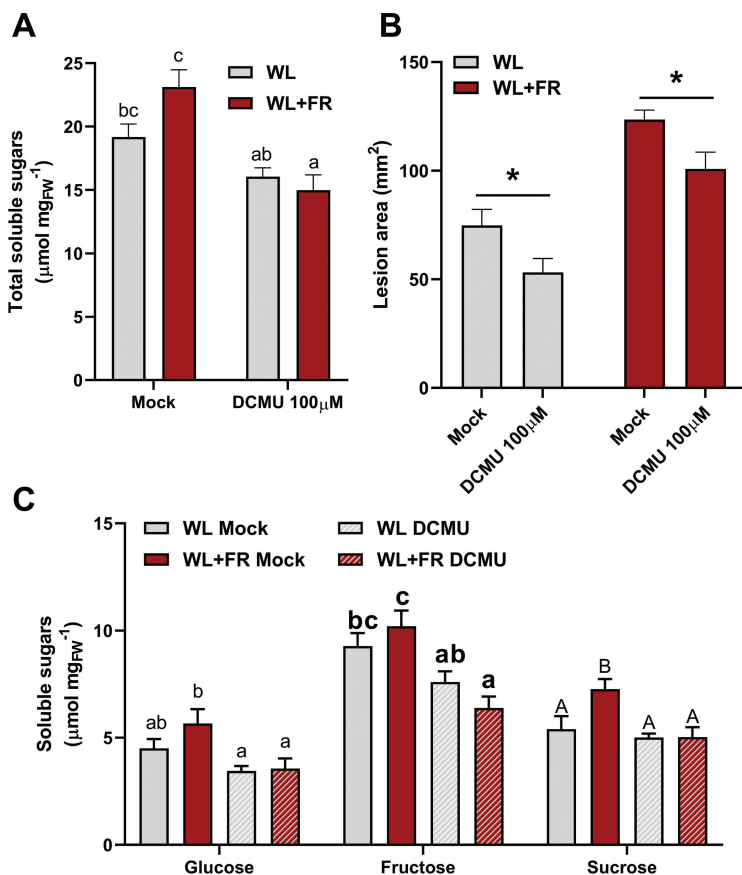


Figure 5.4 : DCMU supplementation *in planta* decreases lesion development by *Botrytis cinerea*. Quantifications of **(A)** total soluble sugars and **(C)** glucose, fructose and sucrose content at midday after five days including 24 h of either mock or 100 μM of DCMU solution supplementation on detached leaflets in WL (grey bars) or WL+FR (red) light conditions. The same experimental procedure was performed prior to bioassay with *B. cinerea* spores. **(B)** Disease rating on tomato leaflets treated as for **(A)** and **(C)** at 3 days post inoculation. Data represent mean \pm SEM. Asterisks (Student's t-test, p -value < 0.05) and different letters indicate significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), $n = 7 - 8$ plants per treatment per experiment.

Local FR illumination in leaf 4 modulates sugar levels and immunity in leaf 3

Since FR light is rarely perceived homogeneously across the whole plant, we investigated the effect of spatially restricted supplemental FR illumination on sugar levels and immunity in a local and remote fashion. For that, we designed FR lamps that allow us to illuminate single tomato leaflets (fig. S5.2) and tested whether local FR enrichment (Local FR, hereafter) could affect whole plant responses compared to plants that did not receive any supplemental FR application taken as controls. We illuminated the top leaflet of the third leaf (L3) for five days (fig. 5.5A) and observed a strong stem elongation of the entire plant as well as petiole elongation of the illuminated leaf. These data indicate that even if FR enrichment is provided very locally, SAS is triggered throughout the plant (fig. S5.3). Upon local FR treatment on L3, we quantified soluble sugar content in the illuminated area (L3) and in another, younger leaf located above, corresponding to the fourth leaf (L4). We observed a large increase in soluble sugars in L3 but not in L4 (fig. 5.5B and C). The sugar levels were correlated with lesion area as local FR on L3 promoted lesion development in L3 but not L4 compared to WL control plants (fig. 5.5D). These data show that local FR enhances sugar levels and susceptibility locally. Next, we swapped leaves: we locally exposed L4 to supplemental FR (fig. 5.6A) and quantified soluble sugars and disease symptoms in this local L4, as well as in the now older, lower positioned L3. We now observed that both L3, located below, and L4 exhibit higher soluble sugar content as well as an increased susceptibility compared to WL (fig. 5.6B and C). These results clearly show a directional signal triggered by local FR in the illuminated leaflet, enhancing sugar levels and susceptibility in the local leaf and in an older leaf below. The signal thus seems to move from L4 to L3 but not in the opposite direction.

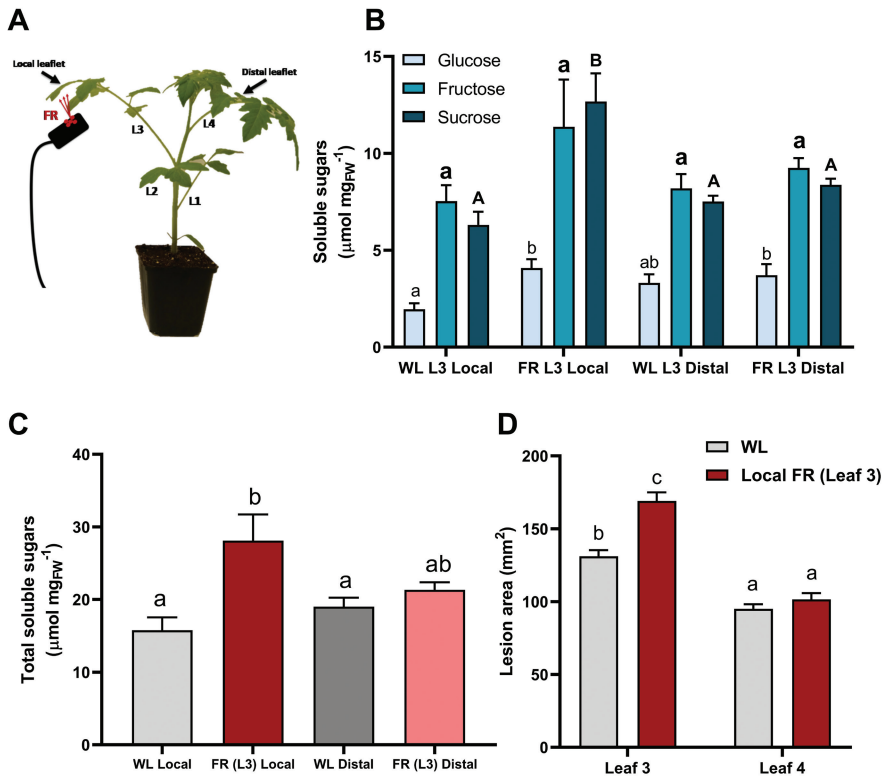


Figure 5.5 : Local far-red illumination on Leaf 3 increases sugar content and *Botrytis cinerea* lesion development locally. (A) Schematic overview of the local FR illumination procedure on leaf 3 (L3). Experiments were carried out on the illuminated leaflet (L3; Local) or a leaflet located on leaf 4 (L4; distal). After five days of local FR illumination (FR) or exposure to white light (WL) as a control, quantifications of (B) glucose, fructose and sucrose and (C) total soluble sugars content at midday on day 5. (D) Disease rating on detached tomato leaflets after WL or local FR illumination followed by inoculation with *B. cinerea* spores. Data show mean \pm SEM and different letters represent significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), $n = 7 - 8$ plants per treatment per experiment.

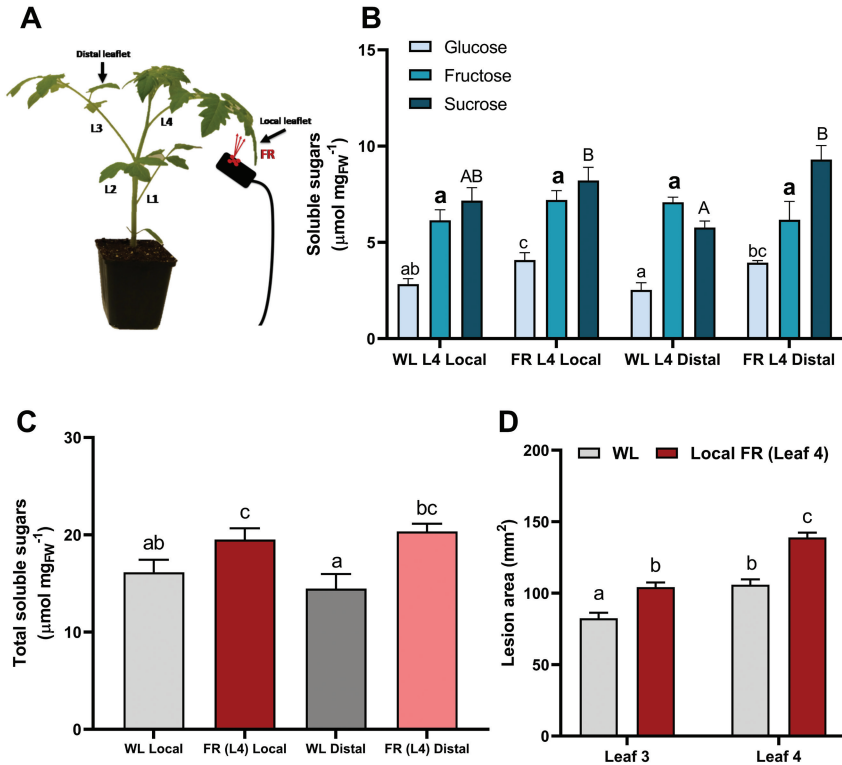


Figure 5.6 : Local far-red illumination on Leaf 4 increases sugar content and *Botrytis cinerea* lesion development in both leaf 4 and leaf 3. (A) Schematic overview of the local FR illumination procedure on leaf 4 (L4). Experiments were carried out on the illuminated leaflet (L4; Local) or a leaflet located on leaf 3 (L3; distal). After five days of local FR illumination (FR) or exposure to white light (WL) as a control, quantifications of (B) glucose, fructose and sucrose and (C) total soluble sugars content at midday on day 5. (D) Disease rating on detached tomato leaflets after WL or local FR illumination followed by inoculation with *B. cinerea* spores. Data show mean \pm SEM and different letters represent significant differences (ANOVA, Tukey's post-hoc test, p-value < 0.05), n = 7 - 8 plants per treatment per experiment.

Discussion

In this chapter, we show that tomato susceptibility to *B. cinerea* may involve a sugar-dependent mechanism. Low R:FR conditions led to an increase in soluble sugar content, mainly glucose and fructose that possibly results in increased lesion size compared to WL-treated plants (fig. 5.1). This hypothesis was further confirmed by glucose supplementation experiments on WL-treated plants that displayed similar lesion areas compared to WL+FR-treated plants alone (fig. 5.3). On the contrary, treating the plants with DCMU, a strong photosynthesis inhibitor, had the opposite effect and significantly reduced lesion areas compared to non-DCMU-exposed control leaves (fig. 5.4). These observations show that high sugar availability in plant tissue promotes tomato susceptibility, presumably by promoting *B. cinerea* growth. Interestingly, it has been reported in other studies that plants with high sugar availability show higher resistance to necrotrophic pathogens (*i.e.* Engelsdorf et al., 2013). Often, elevated glucose levels correlate with elevated secondary metabolite production (Miao et al., 2013) and many of these secondary metabolites promote plant defenses *i.e.* in the *Arabidopsis* / *B. cinerea* pathosystem (Kliebenstein et al., 2005). However, FR enrichment typically represses the biosynthesis of secondary metabolites (Cargnel et al., 2014), thus pairing high soluble sugars with low secondary metabolite levels. Furthermore, in our studies this has happened prior to infection. We believe, this makes the current study system particularly helpful to study a putative impact of soluble sugars on plant susceptibility without confounding impacts of secondary metabolites. Future studies might also study primary and secondary metabolite biosynthesis in WL and WL+FR-treated plants before and during the infection process to further disentangle these relative effects.

The relationship between FR signaling and the increased sugar levels in plant tissue is still not fully established. In **chapter 3**, we showed that FR conditions were able to downregulate photosynthesis-related processes. This observation would not directly explain the soluble sugar accumulation observed in fig. 5.1. However, also cell wall and glucan metabolic processes were downregulated by WL+FR compared to WL, which could mean the use of glucose as building blocks for cell wall components is reduced in WL+FR conditions resulting in the accumulation of free glucose and fructose molecules in leaf tissue. Also, we found that WL+FR strongly inhibited photosynthesis-related processes at the end of day (EOD). This might be explained by the fact that plants have enough supplies to survive nighttime in turn activating a

feedback loop signal inhibiting photosynthesis. Theoretically, all sugars produced and stored during the day are consumed during the night to sustain growth in darkness (Smith and Stitt, 2007; Stitt and Zeeman, 2012). By measuring the sugar content at the end of night (EON), we observed that WL-treated tissue had little carbohydrates left compared to WL+FR-treated samples that surprisingly still had rather high sugar concentration in leaf tissue (fig. S5.1). These leftover carbohydrates at EON were also observed in *phyBDE* mutants by (Yang et al., 2016) and could play a role in promoting lesion development. It would be interesting to study if these daily leftovers would add up over time, which would be consistent with the WL+FR-treated plants becoming more and more susceptible as the duration of the WL+FR pretreatment increases (**chapter 2**).

To investigate whether low R:FR affects local and/or systemic plant responses, we illuminated a single leaflet from L3 or L4 with FR lamps and measured the impact of such local treatments on sugar levels as well as immunity. We found that local FR treatment does not change sugar status and susceptibility to *B. cinerea* in younger leaves, but it does change both in older leaves (fig. 5.5. and fig. 5.6). These findings point toward a mobile signal that would be triggered at the illumination site and transported towards older leaves, possibly via the vasculature. Since the signal moves mostly down, not up, it is rather unlikely that the signal travels in the xylem. An obvious candidate for such long-distance transfer of information could be the soluble sugars themselves, that are typically transported via the phloem. Further research is needed to unravel the nature of the signal, and by using targeted gene expression analysis on known tomato sugar transporters we might at least be able to determine whether this signal could be sugar-related or not.

The FR-induced susceptibility in tomato seems to be associated with soluble sugar accumulation in the illuminated tissue that benefits *B. cinerea* growth and lesion development in plant tissue. As for the systemic effect of local FR on sugar levels and immunity, the signal still remains to be elucidated. The data presented here show that lowering sugar availability by increasing the R:FR in the canopy, for example by supplementing the light environment with R LEDs, would likely help to promote tomato resistance to *B. cinerea* while keeping high planting density in greenhouse systems.

Acknowledgements

We thank Dr. Hans van Veen and Dr. Putri Prasetyaningrum for help with soluble sugar quantifications. We also kindly thank all members from the LED it be 50% consortium for advice and useful feedback along the project. This work was funded by the Dutch Research Council, TTW Perspectief grant nr 14125 (LED it Be 50%) and supported by Signify B.V., WUR Greenhouse Horticulture and LTO Glaskracht.

Materials and methods

Plants growth conditions and light treatments

Tomato cv. Moneymaker were grown in long day conditions under WL LEDs as described in **chapter 2**. Whole plant supplemental FR treatment was performed on three-week-old plants treated for five days under WL supplemented with FR LEDs (WL+FR; R:FR = 0.2, PSS value = 0.5).

Local FR radiation was applied on the top leaflet of either the 3rd or 4th leaf of three-week-old plants by FR LEDs attached to a flexible arm shown in fig S5.2. Plants exposed to WL were taken as controls. Two lateral leaflets of the 3rd leaf were used for experiments.

Microbial growth conditions and bioassays

Botrytis cinerea (Bc 05.10) was maintained on half strength Potato dextrose agar medium and grown for two weeks under natural daylight conditions. Spores were harvested and used for bioassays as described in **chapter 2**.

In vitro mycelial growth assays

B. cinerea mycelium diameter measurements were performed by depositing a 5- μ l droplet of a 1.5×10^5 spores ml⁻¹ solution onto agar 1.5 % supplemented with WL- or FR-treated plant grinds (1 g FW ml⁻¹), or with glucose or fructose (10, 20 and 50 g L⁻¹). Inoculated plates were incubated for three days in WL. The newly grown mycelium diameter was measured by using a digital caliper.

In planta modulation of sugar levels

Square Petri dishes containing Whatman® filter paper (bottom 1/3 cut out) soaked with 2.5 ml of tap water were placed on a metallic rack at a 45° angle. The bottom of the tilted plates were filled with 13 ml of 0.5 M glucose solution for glucose supplementation experiments or 100 μ M DCMU for sugar starvation experiments. Tap water was used as a control in mock conditions. Leaf n°3 lateral leaflets of three-week-old tomato plants were detached and place in the plates so that only the petiole was immersed in the solution. Plates were transferred to the growth cabinets either

in WL or FR conditions for 24 h prior to inoculation with *B. cinerea* spores or sugar quantifications.

Soluble sugars quantification

Tomato leaf discs (15 – 20 mg of fresh tissue) were harvested and snap frozen in liquid nitrogen. Samples were ground, supplemented with 125 μl of 0.83 M perchloric acid (HClO_4), vortexed and centrifuged for 15 min at 4 °C and 13000 rpm. The supernatant (110 μl) containing soluble sugars was transferred into a new tube and the pellet containing starch and proteins was kept at -80 °C for starch quantification analysis. The samples were mixed with 25 μl of 1 M Bicin and ~22 μl 4 M KOH solution to neutralize the pH to 7. All samples were centrifuged for 10 min at 4 °C and 13000 rpm and the supernatant was collected into a new tube for sugar quantifications that were performed using ©Megazyme Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG, ©Megazyme) with a few modifications. Per sample, 30 μl of sugar extract or of a dilution series from 2000 μM to 0 μM glucose solution (used as standards) were mixed with 185 μl of reaction mix containing 170 μl MilliQ, 10 μl of Buffer (solution 1) and NADP+/ATP (solution 2) in a transparent flat-bottomed 96-well plate. The absorbance at 340 nm ($A_{340\text{nm}}$) was measured at the start of the experiment as well as after a 15 min incubation at 37 °C (R_0 and R_1). All wells were supplemented with 2 μl of HXK/G6DPH (solution 3) prior to 15 min at 37°C and $A_{340\text{nm}}$ measurement (R_2). The procedure was repeated by adding 2 μl of PGI (solution 4) incubating for 15 min at 37 °C and measuring $A_{340\text{nm}}$ (R_3). The same incubation and $A_{340\text{nm}}$ measurements were performed after adding 2 μl of yeast invertase solution (100 mg ml^{-1} , Sigma-Aldrich) diluted in buffer (solution 1) were added to each well and incubated for 25 min at 37 °C before the final $A_{340\text{nm}}$ measurement (R_4). The glucose standard curve equation ($y = ax + b$) was used for soluble sugar quantifications. Formulas used to determine soluble sugar concentrations :

$$\begin{aligned} \text{Glucose} \left(\frac{\mu\text{mol}}{\text{mgFW}} \right) &= \frac{\left(\frac{(R_2 - R_1) - b}{a} * 135 \right)}{\text{mgFW}} \\ \text{Fructose} \left(\frac{\mu\text{mol}}{\text{mgFW}} \right) &= \frac{\left(\frac{(R_3 - R_1) - b}{a} * 135 \right)}{\text{mgFW}} - \text{Glucose} \\ \text{Sucrose} \left(\frac{\mu\text{mol}}{\text{mgFW}} \right) &= \frac{\left(\frac{(R_4 - R_1) - b}{a} * 135 \right)}{\text{mgFW}} - \text{Fructose} \end{aligned}$$

Supplemental data

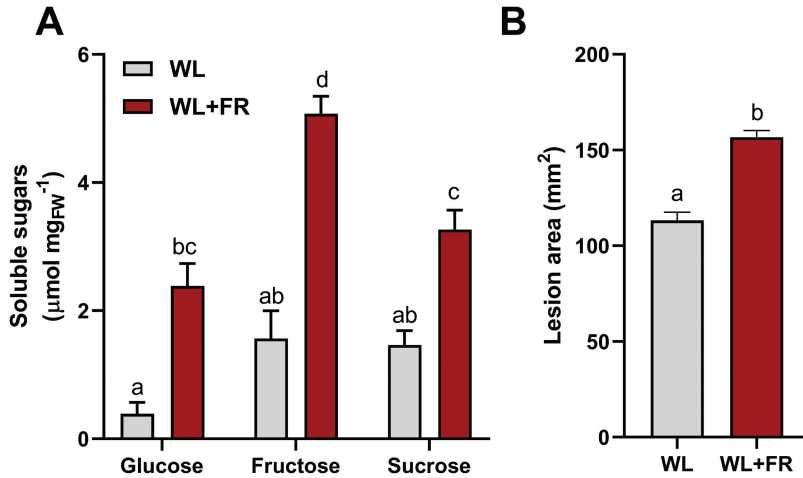


Figure S5.1 : Sugar quantifications and bioassay after a 3 h extension of the dark period. Three-week-old plants were pretreated for five days under white light (WL) or white light supplemented by FR LEDs (WL+FR). The first four days of treatment followed a normal photoperiod. The night period was extended for 3 h on the morning of day 5. **(A)** Soluble sugars quantifications and **(B)** bioassay with *Botrytis cinerea* were carried out before the light went on. Lesion area was measured at 3 dpi with the imageJ software. Data represent mean \pm SEM and different letters indicate significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), $n = 7 - 8$ plants per treatment per experiment.

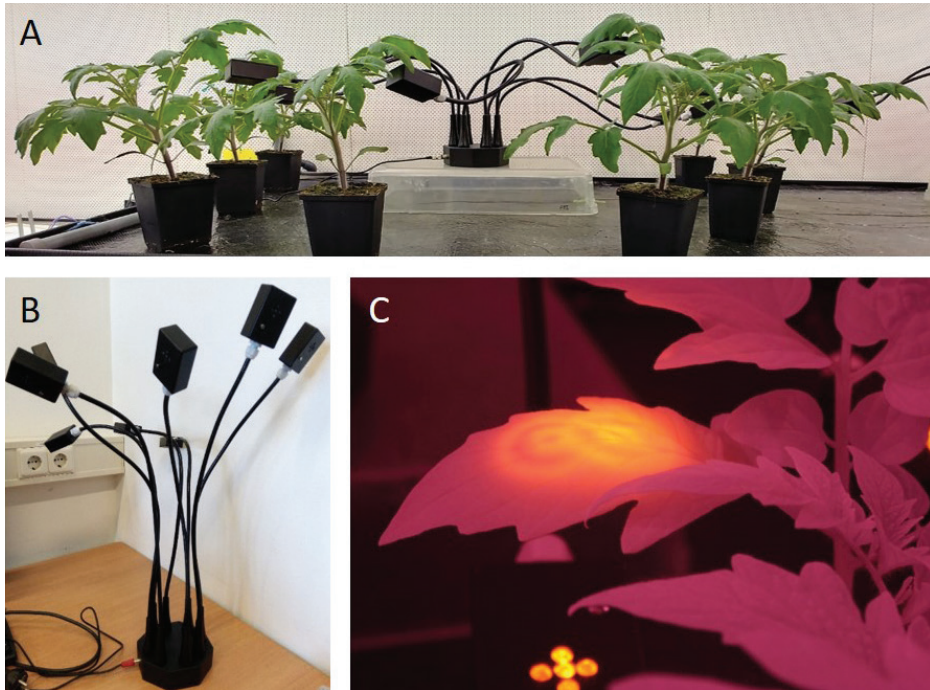


Figure S5.2 : Local far-red (FR) illumination setup. The top leaflet of leaf 3 was illuminated from the bottom (A) by using FR LEDs attached to flexible arms (B). Infrared pictures showing FR illumination of the center of the lamina (C).

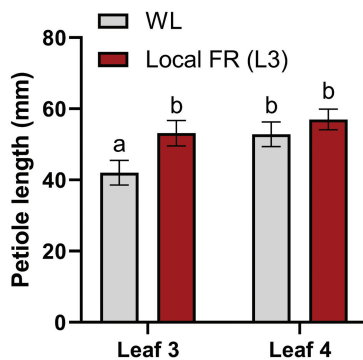
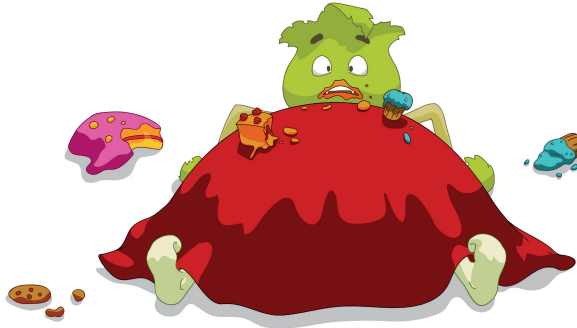


Figure S5.3 : Local FR enrichment triggers petiole elongation. Petiole elongation measurements of the third and fourth leaf of three-week-old tomato plants after five days of local FR illumination on the top leaflet of the third leaf. Measurements were performed at the start and after five days of WL or local FR treatment light treatment (grey and red bars, respectively) Data represent three replicates merged together ($n = 6 - 8$ plants). Plotted data correspond to mean \pm SEM. Different letters represent significant differences (ANOVA, Tukey's post-hoc test, p -value $< 0,05$).



Chapter

General Discussion

6

In this thesis, the effects of LED light environments on tomato growth and defense responses to the necrotrophic fungus *Botrytis cinerea* were investigated. We discovered that far-red (FR) radiation added to a white LED background (WL+FR) induces shoot elongation and modulates immune responses (**chapter 2**). The early perception of supplemental FR leads to transcriptional changes that are likely to be associated with the increased tomato susceptibility to *B. cinerea*, and hint at a delay in pathogen recognition, cell wall and primary metabolism readjustments and a dampening of JA-mediated defense responses (**chapter 3**). We show that the FR-induced susceptibility phenomenon in tomato is at least partly driven by jasmonic acid (JA) and glucose-dependent mechanisms (fig. 6.1). A group of six genes encoding *PROTEINASE INHIBITORS (PI)*, previously described as wound-responsive and regulated by JA and ethylene, were found to be induced upon *B. cinerea* infection in WL but not in WL+FR (**chapter 4**). In parallel, we found that additional FR perception allows plants to accumulate more soluble sugars (especially glucose and fructose) indirectly facilitating the pathogen colonization in leaf tissue (**chapter 5**). The association between dampened JA responses and elevated soluble sugar levels in WL+FR-exposed leaves could possibly explain the increased susceptibility (fig. 6.1). These proposed mechanisms by which FR enrichment would compromise tomato resistance were studied individually in **chapters 3-5**. Future experiments are needed to extend our knowledge of the FR-induced susceptibility and find interconnections between pathways affected by WL+FR. Some of the putative interconnections, especially between sugar and JA, will be discussed here. Ways to apply this knowledge in greenhouse crop cultivation will also be proposed in an attempt to contribute to enhancing tomato resilience to pathogen attacks in high density, commercial production systems.

Far-red enrichment downregulates defense signaling in tomato

Plants growing at high density compete with each other for optimal light capture. Shade avoidance is triggered by neighbor proximity signals that are generated by the selective absorption of red (R) and blue light for photosynthesis in addition to the selective reflection of FR towards other plants, thus perturbing the R:FR ratio within the canopy. Changes in R:FR are sensed by phytochromes, where phyB plays a dominant role (Franklin, 2008). At high density, plants experience a decrease in R:FR leading to the inhibition and restriction of phyB in the cytoplasm. This then prevents

phyB interaction with the bHLH transcription factors Phytochrome Interacting Factors (PIFs), thereby preventing PIF degradation by the 26S proteasome (Li et al., 2016). Upon phyB inactivation, PIFs can therefore accumulate and initiate downstream growth responses, largely via increased auxin synthesis and response (Hornitschek et al., 2012; Li et al., 2012; Pantazopoulou et al., 2017). In low R:FR conditions, gibberellic acid (GA) synthesis is also promoted leading to the degradation of the growth repressing DELLA proteins that would normally bind and inactivate PIFs. Elevated GA de-represses PIFs, in turn promoting elongation growth (Djakovic-Petrovic et al., 2007; Li et al., 2016). Under low R:FR conditions, plant responses to pathogen attacks are usually weakened (De Wit et al., 2013; Leone et al., 2014; Pieterse et al., 2014). Although the FR-induced susceptibility phenomenon has been well studied in the model plant *Arabidopsis thaliana*, it had hardly been investigated in crops, such as tomato (Izaguirre et al., 2006). In the context of the expanding human population and the resulting food demand, it is paramount to create resilient plants that allow growth at high density while being resistant to pathogens and other environmental stresses.

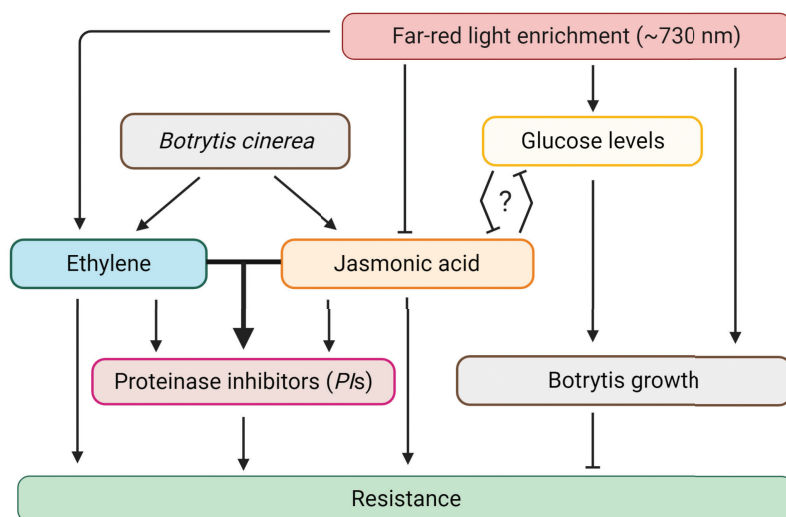


Figure 6.1 : Simplified overview of the effect of FR enrichment on tomato resistance either through differential hormonal regulation or via changes in primary metabolism based on results obtained in this thesis. Arrow-headed lines correspond to a positive effect and blunt arrows represent inhibitions. Bold lines represent stronger induction.

Far-red light affects glucose and fructose metabolism

In **chapter 2**, we studied the effect of an extended FR enrichment (five days) on tomato growth and immunity. We observed that plants respond quickly to the signal and give a visible stem elongation phenotype within the first 24 h. However, the effect of WL+FR on enhancing the plant susceptibility requires more than 24 h before effectively decreasing plant resistance. This hints at a different timing of events between the onset of shade avoidance and the increased susceptibility. Indeed, it has been previously shown that these two processes can be genetically uncoupled in *Arabidopsis* (Cerrudo et al., 2012; Moreno et al., 2009), and thus have (partially) specific signal transduction pathways. The discovery that supplemental FR exposure stimulates glucose and fructose accumulation in leaf tissue after five days implies an increased growth potential for *B. cinerea* on these sugar-enriched leaf materials. Indeed, lesion development upon *B. cinerea* infection is also promoted by artificially elevated glucose levels (**chapter 5**). However, in **chapter 5**, the sugar levels in FR-exposed plants were measured only after five days of additional FR, in the afternoon. When sugar levels were determined at the end of the night, where typically most sugars would be fully consumed, we could still observe large difference in sugar content between WL- and WL+FR-treated plants (**chapter 5**). It is possible that WL+FR-treated plants gradually accumulate monosaccharides in leaf tissue beyond levels that would be fully consumed in the dark period, and thus would gradually accumulate soluble sugars through the subsequent days. Sugar levels may at a certain point become high enough to promote *B. cinerea* development, thus increasing *B. cinerea* growth on tomato leaves after three days of WL+FR exposure and more (**chapter 2**). This hypothesis could be verified by following fluctuations in sugar content from the start of the FR enrichment until the inoculation starts. This would help identify the pattern of sugar accumulation through time, in order to explain the elevated levels observed at day 5. Sugars would thus have to be measured at the start and end of the light period every day to probe if they gradually accumulate following the suggestion made above.

Increased sugar levels were also observed in leaves that had only been locally exposed to FR supplementation for five days. Strikingly, a local FR illumination on just the top leaflet of leaf n°4 also led to increased sugar accumulation in an older leaf (leaf n°3) that had not even received any FR radiation itself, and these patterns strictly correlated with increased disease symptoms of these leaves. As sugars

are transported throughout the plant through the phloem, it would be interesting to study the effect of phloem transport inhibition on sugar levels upon local FR enrichment *i.e* by using transformed tomato plants expressing antisense sequences of sucrose transporter genes (Hackel et al., 2006). Also, ^{14}C labeling through exposure to radiolabeled CO_2 could be an option at the site of illumination, since it would allow for tracking newly formed carbohydrates to their final destination in the plant. Such approaches would help elucidate whether WL+FR promotes sugar transport from a leaf to another or only local sugar accumulation which, via a mobile signal (possibly in the phloem, but different than sugars themselves), would trigger sugar accumulation in systemic leaves. As FR enrichment has already been shown to promote biomass partitioning towards tomato fruits (Ji et al., 2019), it might be worth looking into carbohydrate levels and dynamics in adult fruiting plants as well.

It seems paradoxical that WL+FR would at the same time promote energy and carbon-requiring shoot elongation and soluble sugar accumulation in leaf tissue; one would expect sugars to be readily consumed to drive the elongation response. To better understand the involvement of primary metabolism in elongation growth and disease resistance in tomato it would be helpful to also include daily starch fluctuation measurements in sugar studies under different R:FR regimes. We are aware of one study showing that tomato plants are able to adjust their carbohydrate levels upon *B. cinerea* infection, indicating the importance of soluble sugars for the development of the fungus (Lecompte et al., 2017). As most pathogens target carbohydrate supplies to complete their life cycle, further understanding of the effect of WL+FR on sugar signaling, transport and metabolism could enable engineering of these processes in for example transgenic approaches. Such engineering could facilitate crop improvements towards more resilient plants against a broader array of pathogens than just *B. cinerea*.

Far-red affects hormonal regulation during immune response

In this thesis, the impact of the FR light enrichment on defense hormone regulation upon *B. cinerea* infection was investigated. We identified a set of six *PROTEINASE INHIBITOR* genes (*PI*) that are induced by *B. cinerea* at 12 hpi in WL conditions only (**chapter 3 and 4**). Further experimental work revealed that these genes require both active jasmonic acid and ethylene signaling for a full induction, and these hormone routes are likely to be altered in supplemental FR-exposed plants. We discovered that jasmonic acid and ethylene signaling were modulated in different ways by WL+FR where we saw an inhibition of JA-responsive genes and an increase in ethylene emission (**chapter 4**). Higher ethylene emission by supplemental FR could positively contribute to disease resistance as an impairment in ethylene perception or signaling was shown to increase plant susceptibility to necrotrophic pathogens (Knoester et al., 1998). We confirm this role of ethylene in **chapter 4** where we show that exogenous ethylene treatments promote plant resistance to *B. cinerea* while treatment with the ethylene receptor blocker 1-MCP had the opposite effect. This would predict that if supplemental FR would not have upregulated ethylene emission, the susceptibility of plants to *B. cinerea* in FR-enriched conditions would have been increased even more than observed already.

When testing the resistance phenotype of the JA-deficient mutants *def1* against *B. cinerea*, we obtained similar results as found for the Arabidopsis JA biosynthesis mutant *jar1-1*: Both exhibit increased susceptibility to *B. cinerea* (Cerrudo et al., 2012 ; **chapter 4**). In Arabidopsis, it has been proposed that reduced JA response in FR-enriched conditions is associated with increased expression of *JAZ10*, encoding a negative regulator of JA responsiveness (Cerrudo et al., 2012). The balance between JA and ethylene is probably compromised in FR-treated samples compared to WL, and this might lead to misregulation of the defense pathway. It would be interesting to investigate the importance of the balance between these hormones by altering the hormonal balance in WL-exposed plants in quantitatively similar manners as occurring in the FR supplementation experiments. The extent to which this then phenocopies the disease symptoms upon *B. cinerea* infection would help interpret the relative contribution of these pathways versus, for example, the sugar alterations that were observed in response to WL+FR.

In **chapter 5**, we conclude that local FR treatments induce local and systemic accumulation of monosaccharides accompanied with elevated susceptibility. As discussed in the previous section, sugars themselves could be transported systemically from the illuminated leaf to other leaves in a phloem-dependent manner. However, we cannot exclude the presence of a mobile signal other than sugars that would be transported to other leaves and trigger sugar accumulation and susceptibility in that location. In tomato, *PI* gene expression is induced upon mechanical wounding, insect feeding and also *B. cinerea* infection (El Oirdi et al., 2011; Farmer et al., 1992; Green and Ryan, 1972; Pearce et al., 1991). The induction of *PI* genes requires the cleavage of a small peptide called systemin (18 aa) from its precursor prosystemin (200 aa) present at the cell membrane of plant cells (Pearce et al., 1991). Upon wounding or pathogen attack, systemin is released from the membrane and induces *PI* gene expression in a JA-dependent manner (El Oirdi et al., 2011; Pearce et al., 1991). In addition to activating *PI* expression at the wounding site, systemin can also be transported to distal leaves and trigger *PI* induction in systemic leaves (McGurl et al., 1992). In parallel, wound-inducible prosystemin mRNA has been detected in all aerial organs of unwounded plants (but not in roots) suggesting that the constant presence of prosystemin mRNA in leaves helps plants to react quickly upon wounding or pathogen attacks (McGurl et al., 1992). As a local FR-enrichment increases susceptibility at the illuminated site and in the lower leaf (**chapter 5**), it could be possible that WL+FR would downregulate JA signaling by either affecting basal levels of prosystemin at the plasma membrane or interfere with prosystemin mRNA distribution throughout the plant compared to WL conditions. This would then lead to less *PI* induction upon *B. cinerea* infection in the remote leaves in FR-enriched conditions compared to control light, an alternative hypothesis to the sugar elevations.

Interplay between jasmonic acid and sugar levels

As discussed above, it is likely that supplemental FR interferes with tomato immunity through regulation of hormone-controlled defense pathways and a rather indirect gradual increase in soluble sugars. However, we had not investigated potential connections between hormone-controlled defense routes and primary metabolism. In **chapter 4**, we observed that *def1* mutants displayed a constitutive susceptibility to *B. cinerea* compared to the wild type. In a preliminary experiment, we measured the soluble sugar content in JA-deficient *def1* mutant plants after a WL or WL+FR treatment to study whether an alteration of JA signaling could also affect sugar levels in plant tissue. Interestingly, the sugar levels in tomato cv. Castlemart were not significantly increased by WL+FR, although trends were visible. Importantly, we observed a doubling of the glucose content in the *def1* mutant compared to its wild-type background (fig. 6.2), irrespective of the light conditions. These results suggest that JA inhibits glucose levels in tomato leaves. A negative correlation between JA and sugar levels has also been observed in other plant species than tomato such as *Nicotiana attenuata* (Machado et al., 2015): JA-deficient mutant tobacco plants contained higher levels of monosaccharides. However this was associated with increased resistance towards the chewing insect *Manduca sexta* (Machado et al., 2015); the opposite of what we describe here for WL+FR conditions, where elevated sugar levels occur together with reduced resistance against *B. cinerea*. The pathosystem and experimental conditions used by Machado et al. (2015) are hugely different from our study system, and it is therefore not possible to predict if the sugar-resistance relations in these studies contrast each other. Nevertheless, they both describe a negative relation between JA and soluble sugar levels. However, JA and sugar signaling pathways are not always negatively correlated. Studies in *Arabidopsis* have shown that glucose and JA can act synergistically to increase glucosinolates (GS) levels which have been shown to promote plant resistance towards *B. cinerea* in *Arabidopsis* (Buxdorf et al., 2013; Guo et al., 2013; Kliebenstein et al., 2005). Although GS are specific to Brassicaceae, it would be interesting to perform a metabolomic profiling in tomato upon *B. cinerea* infection to determine which secondary metabolites are associated with plant resistance to *B. cinerea* in WL conditions. The next step could be to study the effect of WL+FR or glucose supplementation on these metabolites, since it could reveal functional aspects of the increased susceptibility.

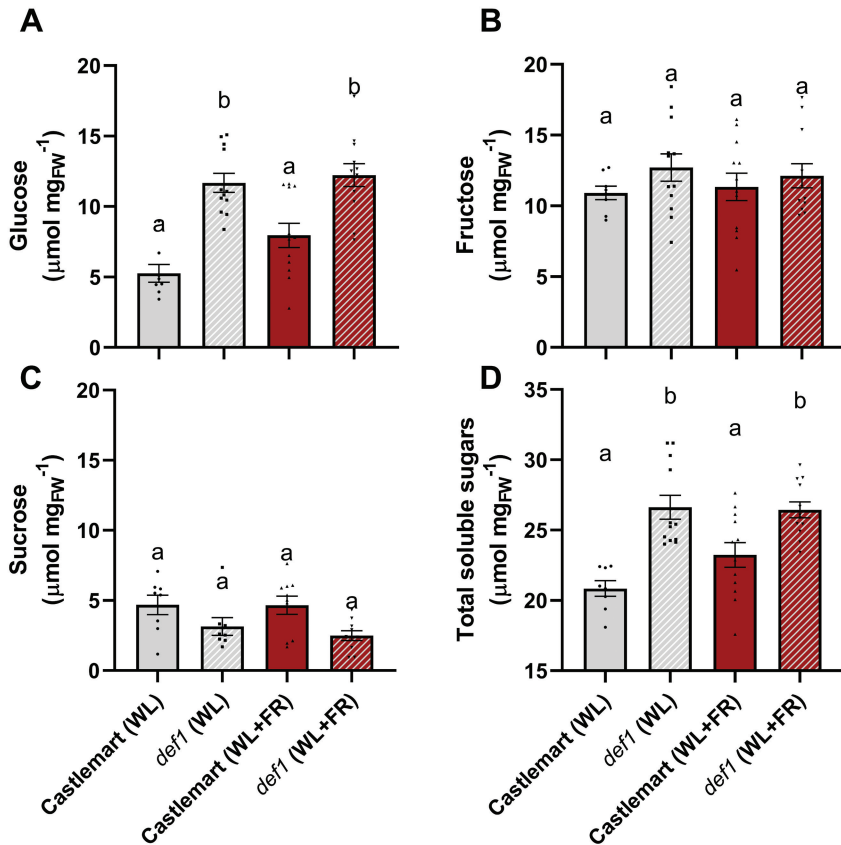


Figure 6.2 : Soluble sugar content is elevated in the JA-deficient *def1* tomato mutant. Tomato cv. Castlemart plants and JA biosynthesis mutants *def1* (*defenseless 1*) were treated either in white light (WL) or WL supplemented with far-red LEDs (WL+FR) conditions for five days. Glucose, fructose and sucrose levels were quantified from the first lateral leaflets of the third leaf (A-C) as well as the total soluble sugars (D) at midday on day 5. Data represent mean \pm SEM and different letters represent significant differences (ANOVA, Tukey's post-hoc test, p -value < 0.05), $n = 3 - 5$ plants.

Chapter 5 shows that glucose levels are increased in tomato leaves upon supplemental FR exposure. Interestingly, photosynthesis-derived glucose has been described to activate the serine/threonine kinase Target of Rapamycin (TOR) which plays a central role in the regulation of energy homeostasis, hormone regulation and growth (Xiong et al., 2013). In addition to being regulated by glucose (Xiong et al., 2013; Xiong and Sheen, 2012), TOR has been shown to influence JA signaling in Arabidopsis and cotton. Chemical inhibition of TOR reduced plant growth to a similar

extent as does exogenous MeJA treatment. Interestingly, inhibition of TOR promotes the expression of JA biosynthesis genes showing the negative effect of TOR on JA signaling (Song et al., 2017). Since FR enrichment leads to an increase in glucose levels and glucose activates TOR, in turn affecting JA signaling, one could speculate that TOR might play a regulatory role in the regulation of FR-induced susceptibility. Although glucose typically affects TOR protein activity, we also verified the expression profile of *TOR* (*Solyc01g106770.3*) in the RNA sequencing data presented in **chapter 3**. It was found that this gene was significantly upregulated in WL+FR-treated samples at 6 hpi compared to WL (fig. 6.3). It is possible that increased TOR abundance and/or activity in WL+FR-treated samples would result in the observed inhibition of JA response in tomato. This could represent a second pathway in addition to the earlier mentioned FR-induced expression of *JAZ10*, or *JAZ10* regulation could even be TOR-dependent. It would, however, be important to verify if glucose indeed affects *TOR* expression and/or TOR activity, for example by feeding supplemental glucose to WL-exposed leaflets. Constructing inducible *TOR* overexpression or mutant lines in tomato would also help to investigate the impact of *TOR* regulation by sugar levels and/or FR on susceptibility towards *B. cinerea*.

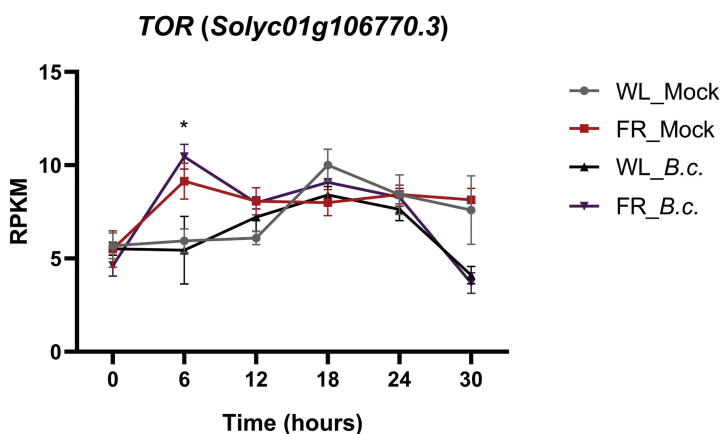


Figure 6.3 : TARGET OF RAPAMYCIN (*TOR*) is upregulated by WL+FR at 6 hpi and downregulated by *Botrytis cinerea* at 30 hpi. Expression patterns (RPKM) of *Solyc01g106770* encoding serine/threonine kinase TOR where each line corresponds to one of the four conditions tested in the RNA sequencing (see **chapter 3**). Tomato plants were either exposed to white light (WL_Mock, grey line) or WL + additional far-red LEDs (FR_Mock, red line) followed by *B. cinerea* infection (WL_B.c. in black or FR_B.c. in purple). Asterisk represent significant difference (Student's t-test, p-value < 0.05) at 6 hpi between FR-treated samples compared to WL.

Improving tomato growth and resistance to pathogens with LEDs

In the context of global climate change and the simultaneously increasing global food demand, novel agricultural approaches are needed to increase crop yield under challenging environmental conditions. One option to do so is to grow plants closer together, whilst maintaining yield and resilience. However, this thesis shows that high density elevates plant susceptibility to pathogenic microorganisms via changes in the overall R:FR ratio in the plant canopy. In the Netherlands, tall crop plants such as tomato, pepper or even cucumber are grown using modern greenhouse systems. The production levels are very competitive, but require immense energy usage, partly because of the required supplemental lighting. LED (Light Emitting Diode) lighting technology is much more energy-efficient than conventional lighting, such as HPS (High Pressure Sodium) or incandescent lamps. Some greenhouses are already equipped with LEDs. However, the effects of LEDs on plant growth are not fully understood and the light recipes as well as the illumination durations still have to be studied and optimized. Implementing LED lighting in greenhouse systems, in addition to sunlight, has many advantages over conventional lighting. In addition to energy saving, LEDs also allow for selection of specific wavelengths of light. It turns out that red and blue light are sufficient for photosynthesis, but their relative abundance, and that of FR light, have a profound impact on plant architecture and allocation. Furthermore, we observed an increase in pathogen susceptibility upon FR-enrichment even in a red and blue light background (**chapter 2**). This indicates that it is pertinent to increase the R:FR within the plant canopy to prevent the drop in resistance that occurs in low R:FR. Since R:FR will always drop due to light reflection and transmission through plant leaves in dense stands, this effect has far-reaching consequences in agricultural settings. Nevertheless, in greenhouse systems, applying supplemental light using LEDs is relatively straightforward and can be done through top-lighting and inter-lighting. Top-lighting allows for a better light quality on top of the canopy in addition to available sunlight and inter-lighting, between the rows of crops, increases light capture within the canopy. The latter is a good way to keep a rather high R:FR ratio inside the canopy which might improve plant resistance even at high density, assuming that supplemental red light would be applied as inter-lighting to counteract the naturally occurring drop in R:FR due to the absorption of red light by the crop stand. Our results indicate that FR-enrichment provokes a downregulation of JA-associated responses and an increase in sugar levels that

both lead to increased susceptibility to *B. cinerea* (**chapter 4 and 5**). The elevation of soluble sugars seen in **chapter 5** in WL+FR could possibly be controlled by increasing the R:FR ratio in the middle of the canopy in order to reduce the sugar-mediated susceptibility that occurs systemically in older, but not younger leaves than the FR-exposed ones. Higher in the canopy, there is still ample direct light from top lamps and outside to keep R:FR relatively high. Overall, increasing plant resistance at high density by using LED lighting seems possible and would partly solve the FR-induced susceptibility in tomato towards pathogens. Also, as sugar supplies are beneficial to most pathogens, we believe that balancing sugar levels *in planta* by smart LED lighting plans would rescue tomato susceptibility to other pathogens than *B. cinerea* as well. In a recent study, we observed a promotive effect of supplemental FR on tomato fruit development in a greenhouse, even though susceptibility to *B. cinerea* was also enhanced (Ji et al., 2019). Future studies on timing and spatial distribution of light quality all over a mature tomato stand are needed to understand if these positive effects of FR-enriched light can be achieved without having the negative effects on tomato pathogen resistance.

References

- AbuQamar, S., Chai, M.F., Luo, H., Song, F., Mengiste, T.** 2008. Tomato protein kinase 1b mediates signaling of plant responses to necrotrophic fungi and insect herbivory. *Plant Cell* **20**: 1964-1983.
- AbuQamar, S., Chen, X., Dhawan, R., Bluhm, B., Salmeron, J., Lam, S., Dietrich, R.A., Mengiste, T.** 2006. Expression profiling and mutant analysis reveals complex regulatory networks involved in Arabidopsis response to Botrytis infection. *Plant Journal* **48**: 28-44.
- Anders, S., Pyl, P.T., Huber, W.** 2015. HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**: 166-169.
- Anderson, J.P., Gleason, C.A., Foley, R.C., Thrall, P.H., Burdon, J.B., Singh, K.B.** 2010. Plants versus pathogens: An evolutionary arms race. *Functional Plant Biology* **37**: 499-512.
- Arnon, D.I., Whately, F.R., Allen, M.B.** 1954. Photosynthesis by Isolated Chloroplasts. II. Photosynthetic Phosphorylation, the Conversion of Light into Phosphate Bond Energy. *Journal of the American Chemical Society* **76**: 6324-6329.
- Ballaré, C.L.** 2014. Light regulation of plant defense. *Annual Review of Plant Biology* **65**: 335-363.
- Ballaré, C.L., Austin, A.T.** 2019. Recalculating growth and defense strategies under competition: key roles of photoreceptors and jasmonates. *Journal of Experimental Botany* **70**: 3425-3434.
- Ballaré, C.L., Pierik, R.** 2017. The shade-avoidance syndrome: Multiple signals and ecological consequences. *Plant Cell and Environment* **40**: 2530-2543.
- Berger, S., Sinha, A.K., Roitsch, T.** 2007. Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany* **58**: 4019-4026.
- Boller, T., Kende, H.** 1980. Regulation of wound ethylene synthesis in plants. *Nature* **286**: 259-260.
- Buxdorf, K., Yaffe, H., Barda, O., Levy, M.** 2013. The Effects of Glucosinolates and Their Breakdown Products on Necrotrophic Fungi. *PLoS One* **8**: e70771.
- Caarls, L., Pieterse, C.M.J., Van Wees, S.C.M.** 2015. How salicylic acid takes transcriptional control over jasmonic acid signaling. *Frontiers in Plant Science* **6**: 170.
- Cargnel, M.D., Demkura, P. V., Ballaré, C.L.** 2014. Linking phytochrome to plant immunity: low red: Far-red ratios increase Arabidopsis susceptibility to Botrytis cinerea by reducing the biosynthesis of indolic glucosinolates and camalexin. *New Phytologist* **204**: 342-354.
- Casal, J.J.** 2013. Photoreceptor Signaling Networks in Plant Responses to Shade. *Annual Review of Plant Biology* **64**: 403-427.

- Casal, J.J.** 2012. Shade avoidance. *The Arabidopsis Book* (American Society of Plant Biologists) **10**: e0157.
- Cerrudo, I., Caliri-Ortiz, M.E., Keller, M.M., Degano, M.E., Demkura, P. V., Ballaré, C.L.** 2017. Exploring growth-defence trade-offs in Arabidopsis: phytochrome B inactivation requires JAZ10 to suppress plant immunity but not to trigger shade-avoidance responses. *Plant Cell and Environment* **40**: 635-644.
- Cerrudo, I., Keller, M.M., Cargnel, M.D., Demkura, P. V., de Wit, M., Patitucci, M.S., Pierik, R., Pieterse, C.M.J., Ballaré, C.L.** 2012. Low red/far-red ratios reduce Arabidopsis resistance to Botrytis cinerea and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. *Plant Physiology* **158**: 2042-52.
- Chang, K.N., Zhong, S., Weirauch, M.T., Hon, G., Pelizzola, M., Li, H., Carol Huang, S.S., Schmitz, R.J., Urich, M.A., Kuo, D., Nery, J.R., Qiao, H., Yang, A., Jamali, A., Chen, H., Ideker, T., Ren, B., Bar-Joseph, Z., Hughes, T.R., Ecker, J.R.** 2013. Temporal transcriptional response to ethylene gas drives growth hormone cross-regulation in Arabidopsis. *eLife* **2**: e00675.
- Chen, L.Q., Hou, B.H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.Q., Guo, W.J., Kim, J.G., Underwood, W., Chaudhuri, B., Chermak, D., Antony, G., White, F.F., Somerville, S.C., Mudgett, M.B., Frommer, W.B.** 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* **468**: 527-532.
- Chen, Y.F., Shakeel, S.N., Bowers, J., Zhao, X.C., Etheridge, N., Schaller, G.E.** 2007. Ligand-induced degradation of the ethylene receptor ETR2 through a proteasome-dependent pathway in Arabidopsis. *Journal of Biological Chemistry* **282**: 24752-24758.
- Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nürnberger, T., Jones, J.D.G., Felix, G., Boller, T.** 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **448**: 497-500.
- Chong, J., Piron, M.C., Meyer, S., Merdinoglu, D., Bertsch, C., Mestre, P.** 2014. The SWEET family of sugar transporters in grapevine: VvSWEET4 is involved in the interaction with *Botrytis cinerea*. *Journal of Experimental Botany* **65**: 6589-6601.
- Coolen, S., Proietti, S., Hickman, R., Davila Olivas, N.H., Huang, P.P., Van Verk, M.C., Van Pelt, J.A., Wittenberg, A.H.J., De Vos, M., Prins, M., Van Loon, J.J.A., Aarts, M.G.M., Dicke, M., Pieterse, C.M.J., Van Wees, S.C.M.** 2016. Transcriptome dynamics of Arabidopsis during sequential biotic and abiotic stresses. *Plant Journal* **86**: 249-267.
- Cortés, L.E., Weldegergis, B.T., Boccalandro, H.E., Dicke, M., Ballaré, C.L.** 2016. Trading direct for indirect defense? Phytochrome B inactivation in tomato attenuates direct anti-herbivore defenses whilst enhancing volatile-mediated attraction of predators. *New Phytologist* **212**: 1057-1071.
- Cui, H., Tsuda, K., Parker, J.E.** 2015. Effector-Triggered Immunity: From Pathogen Perception to Robust Defense. *Annual Review of Plant Biology* **66**: 487-511.

- Das, D., St Onge, K.R., Voeselek, L.A.C.J., Pierik, R., Sasidharan, R.** 2016. Ethylene- and shade-induced hypocotyl elongation share transcriptome patterns and functional regulators. *Plant Physiology* **172**: 718-733.
- De Wit, M., George, G.M., Ince, Y.Ç., Dankwa-Egli, B., Hersch, M., Zeeman, S.C., Fankhauser, C.** 2018. Changes in resource partitioning between and within organs support growth adjustment to neighbor proximity in Brassicaceae seedlings. *Proceedings of the National Academy of Sciences of the United States of America* **115**: E9953-E9961.
- De Wit, M., Spoel, S.H., Sanchez-Perez, G.F., Gommers, C.M.M., Pieterse, C.M.J., Voeselek, L.A.C.J., Pierik, R.** 2013. Perception of low red: Far-red ratio compromises both salicylic acid- and jasmonic acid-dependent pathogen defences in Arabidopsis. *Plant Journal* **75**: 90-103.
- Demarsy, E., Fankhauser, C.** 2009. Higher plants use LOV to perceive blue light. *Current Opinion in Plant Biology* **12**: 69-74.
- Denby, K.J., Kumar, P., Kliebenstein, D.J.** 2004. Identification of *Botrytis cinerea* susceptibility loci in *Arabidopsis thaliana*. *Plant Journal* **38**: 473-486.
- Díaz, J., Ten Have, A., Van Kan, J.A.L.** 2002. The Role of Ethylene and Wound Signaling in Resistance of Tomato to *Botrytis cinerea*. *Plant Physiology* **129**: 1341-1351.
- Djakovic-Petrovic, T., Wit, M. de, Voeselek, L.A.C.J., Pierik, R.** 2007. DELLA protein function in growth responses to canopy signals. *Plant Journal* **51**: 117-126.
- Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R.** 2013. STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **29**: 15-21.
- Doidy, J., Grace, E., Kühn, C., Simon-Plas, F., Casieri, L., Wipf, D.** 2012. Sugar transporters in plants and in their interactions with fungi. *Trends in Plant Science* **17**: 413-422.
- El Oirdi, M., El Rahman, T.A., Rigano, L., El Hadrami, A., Rodriguez, M.C., Daayf, F., Vojnov, A., Bouarab, K.** 2011. *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *The Plant Cell* **23**: 2405-2421.
- Engelsdorf, T., Horst, R.J., Pröls, R., Pröschel, M., Dietz, F., Hückelhoven, R., Voll, L.M.** 2013. Reduced carbohydrate availability enhances the susceptibility of arabidopsis toward *Colletotrichum higginsianum*. *Plant Physiology* **162**: 225-238.
- Evans, J.R., Poorter, H.** 2001. Photosynthetic acclimation of plants to growth irradiance: The relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment* **24**: 755-767.
- Farmer, E.E., Johnson, R.R., Ryan, C.A.** 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. *Plant Physiology* **98**: 995-1002.
- Farmer, E.E., Ryan, C.A.** 1990. Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences of the United States of America* **87**: 7713-7716.

- Ferrari, S., Savatin, D. V., Sicilia, F., Gramegna, G., Cervone, F., De Lorenzo, G.** 2013. Oligogalacturonides: Plant damage-associated molecular patterns and regulators of growth and development. *Frontiers in Plant Science* **35**: 193-205.
- Franciosini, A., Rymen, B., Shibata, M., Favero, D.S., Sugimoto, K.** 2017. Molecular networks orchestrating plant cell growth. *Current Opinion in Plant Biology* **35**: 98-104.
- Franklin, K.A.** 2008. Shade avoidance. *New Phytologist* **179**: 930-944.
- Franklin, K.A., Lee, S.H., Patel, D., Kumar, S.V., Spartz, A.K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J.D., Wigge, P.A., Gray, W.M.** 2011. Phytochrome-Interacting Factor 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 20231-20235.
- Franklin, K.A., Quail, P.H.** 2010. Phytochrome functions in Arabidopsis development. *Journal of Experimental Botany* **61**: 11-24.
- Glazebrook, J.** 2005. Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens. *Annual Review of Phytopathology* **43**: 205-227.
- Gommers, C.M.M., Buti, S., Tarkowská, D., Pěnčík, A., Banda, J.P., Arricastres, V., Pierik, R.** 2018. Organ-specific phytohormone synthesis in two Geranium species with antithetical responses to far-red light enrichment. *Plant Direct* **2**: e00066.
- Gommers, C.M.M., Keuskamp, D.H., Buti, S., van Veen, H., Koevoets, I.T., Reinen, E., Voeselek, L.A.C.J., Pierik, R.** 2017. Molecular Profiles of Contrasting Shade Response Strategies in Wild Plants: Differential Control of Immunity and Shoot Elongation. *The Plant Cell* **29**: 331-344.
- Gommers, C.M.M., Visser, E.J.W., Onge, K.R.S., Voeselek, L.A.C.J., Pierik, R.** 2013. Shade tolerance: When growing tall is not an option. *Trends in Plant Science* **18**: 65-71.
- Govrin, E.M., Levine, A.** 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Current Biology* **10**: 751-757.
- Green, T.R., Ryan, C.A.** 1972. Wound-induced proteinase inhibitor in plant leaves: A possible defense mechanism against insects. *Science* **175**: 776-777.
- Guo, R., Shen, W., Qian, H., Zhang, M., Liu, L., Wang, Q.** 2013. Jasmonic acid and glucose synergistically modulate the accumulation of glucosinolates in *Arabidopsis thaliana*. *Journal of Experimental Botany* **64**: 5707-5719.
- Hackel, A., Schauer, N., Carrari, F., Fernie, A.R., Grimm, B., Kühn, C.** 2006. Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways. *Plant Journal* **45**: 180-192.
- Hamza, R., Pérez-Hedo, M., Urbaneja, A., Rambla, J.L., Granell, A., Gaddour, K., Beltrán, J.P., Cañas, L.A.** 2018. Expression of two barley proteinase inhibitors in tomato promotes endogenous defensive response and enhances resistance to *Tuta absoluta*. *BMC Plant Biology* **18**: 24.

- Han, L., Li, G.J., Yang, K.Y., Mao, G., Wang, R., Liu, Y., Zhang, S.** 2010. Mitogen-activated protein kinase 3 and 6 regulate *Botrytis cinerea*-induced ethylene production in *Arabidopsis*. *Plant Journal* **64**: 114-127.
- Hartman, S., Liu, Z., van Veen, H., Vicente, J., Reinen, E., Martopawiro, S., Zhang, H., van Dongen, N., Bosman, F., Bassel, G.W., Visser, E.J.W., Bailey-Serres, J., Theodoulou, F.L., Hebelstrup, K.H., Gibbs, D.J., Holdsworth, M.J., Sasidharan, R., Voeselek, L.A.C.J.** 2019. Ethylene-mediated nitric oxide depletion pre-adapts plants to hypoxia stress. *Nature Communications* **10**: 4020.
- Hogenhout, S.A., Bos, J.I.B.** 2011. Effector proteins that modulate plant-insect interactions. *Current Opinion in Plant Biology* **14**: 422-428.
- Hornitschek, P., Kohnen, M. V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I., Fankhauser, C.** 2012. Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *Plant Journal* **71**: 699-711.
- Hou, S., Liu, Z., Shen, H., Wu, D.** 2019. Damage-associated molecular pattern-triggered immunity in plants. *Frontiers in Plant Science* **10**: 464.
- Hou, X., Lee, L.Y.C., Xia, K., Yan, Y., Yu, H.** 2010. DELLAs Modulate Jasmonate Signaling via Competitive Binding to JAZs. *Developmental Cell* **19**: 884-894.
- Izaguirre, M.M., Mazza, C.A., Astigueta, M.S., Ciarla, A.M., Ballaré, C.L.** 2013. No time for candy: Passionfruit (*Passiflora edulis*) plants down-regulate damage-induced extra floral nectar production in response to light signals of competition. *Oecologia* **173**: 213-221.
- Izaguirre, M.M., Mazza, C.A., Biondini, M., Baldwin, I.T., Ballaré, C.L.** 2006. Remote sensing of future competitors: Impacts on plants defenses. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 7170-7174.
- Ji, Y., Ouzounis, T., Courbier, S., Kaiser, E., Nguyen, P.T., Schouten, H.J., Visser, R.G.F., Pierik, R., Marcelis, L.F.M., Heuvelink, E.** 2019. Far-red radiation increases dry mass partitioning to fruits but reduces *Botrytis cinerea* resistance in tomato. *Environmental and Experimental Botany* **168**: 103889.
- Jones, J.D.G., Dangl, J.L.** 2006. The plant immune system. *Nature* **444**: 323-329.
- Kamoun, S., Van West, P., De Jong, A.J., De Groot, K.E., Vleeshouwers, V.G.A.A., Govers, F.** 1997. A gene encoding a protein elicitor of *Phytophthora infestans* is downregulated during infection of potato. *Molecular Plant-Microbe Interactions* **10**: 13-20.
- Kegge, W., Ninkovic, V., Glinwood, R., Welschen, R.A.M., Voeselek, L.A.C.J., Pierik, R.** 2015. Red:far-red light conditions affect the emission of volatile organic compounds from barley (*Hordeum vulgare*), leading to altered biomass allocation in neighbouring plants. *Annals of Botany* **115**: 961-970.

- Kegge, W., Weldegergis, B.T., Soler, R., Eijk, M.V. Van, Dicke, M., Voeselek, L.A.C.J., Pierik, R.** 2013. Canopy light cues affect emission of constitutive and methyl jasmonate-induced volatile organic compounds in *Arabidopsis thaliana*. *New Phytologist* **200**: 861-874.
- Kendrick, M.D., Chang, C.** 2008. Ethylene signaling: new levels of complexity and regulation. *Current Opinion in Plant Biology* **11**: 479-485.
- Keuskamp, D.H., Pollmann, S., Voeselek, L.A.C.J., Peeters, A.J.M., Pierik, R.** 2010. Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 22740-22744.
- Kliebenstein, D.J., Rowe, H.C., Denby, K.J.** 2005. Secondary metabolites influence *Arabidopsis/Botrytis* interactions: Variation in host production and pathogen sensitivity. *Plant Journal* **44**: 25-36.
- Knoester, M., Van Loon, L.C., Van Den Heuvel, J., Hennig, J., Bol, J.F., Linthorst, H.J.M.** 1998. Ethylene-insensitive tobacco lacks nonhost resistance against soil-borne fungi. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 1933-1937.
- Kohnen, M. V, Schmid-Siegert, E., Trevisan, M., Petrolati, L.A., Sénéchal, F., Müller-Moulé, P., Maloof, J., Xenarios, I., Fankhauser, C.** 2016. Neighbor Detection Induces Organ-Specific Transcriptomes, Revealing Patterns Underlying Hypocotyl-Specific Growth. *The Plant Cell* **28**: 2889-2904.
- Kölling, K., Thalmann, M., Müller, A., Jenny, C., Zeeman, S.C.** 2015. Carbon partitioning in *Arabidopsis thaliana* is a dynamic process controlled by the plants metabolic status and its circadian clock. *Plant, Cell and Environment* **38**: 1965-1979.
- Küpers, J.J., van Gelderen, K., Pierik, R.** 2018. Location Matters: Canopy Light Responses over Spatial Scales. *Trends in Plant Science* **23**: 865-873.
- Lapin, D., Van den Ackerveken, G.** 2013. Susceptibility to plant disease: More than a failure of host immunity. *Trends in Plant Science* **18**: 546-554.
- Lecompte, F., Nicot, P.C., Ripoll, J., Abro, M.A., Raimbault, A.K., Lopez-Lauri, F., Bertin, N.** 2017. Reduced susceptibility of tomato stem to the necrotrophic fungus *Botrytis cinerea* is associated with a specific adjustment of fructose content in the host sugar pool. *Annals of Botany* **119**: 931-943.
- Leone, M., Keller, M.M., Cerrudo, I., Ballaré, C.L.** 2014. To grow or defend? Low red: Far-red ratios reduce jasmonate sensitivity in *Arabidopsis* seedlings by promoting DELLA degradation and increasing JAZ10 stability. *New Phytologist* **204**: 355-367.
- Li, K., Yu, R., Fan, L.M., Wei, N., Chen, H., Deng, X.W.** 2016. DELLA-mediated PIF degradation contributes to coordination of light and gibberellin signalling in *Arabidopsis*. *Nature Communications* **7**: 11868.

- Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U. V., Jung, H.S., Ecker, J.R., Kay, S.A., Chory, J.** 2012. Linking photoreceptor excitation to changes in plant architecture. *Genes and Development* **26**: 785-790.
- Livak, K.J., Schmittgen, T.D.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* **25**: 402-408.
- Lorenzo, O., Piqueras, R., Sánchez-Serrano, J.J., Solano, R.** 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *The Plant Cell* **15**: 165-178.
- Lorrain, S., Allen, T., Duek, P.D., Whitelam, G.C., Fankhauser, C.** 2008. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant Journal* **53**: 312-323.
- Lozano-Durán, R., Macho, A.P., Boutrot, F., Segonzac, C., Somssich, I.E., Zipfel, C.** 2013. The transcriptional regulator BZR1 mediates trade-off between plant innate immunity and growth. *eLife* **2**: e00983.
- Lozano-Durán, R., Zipfel, C.** 2015. Trade-off between growth and immunity: Role of brassinosteroids. *Trends in Plant Science* **20**: 12-19.
- Machado, R.A.R., Arce, C.C.M., Ferrieri, A.P., Baldwin, I.T., Erb, M.** 2015. Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*. *New Phytologist* **207**: 91-105.
- McGuire, R., Agrawal, A.A.** 2005. Trade-offs between the shade-avoidance response and plant resistance to herbivores? Tests with mutant *Cucumis sativus*. *Functional Ecology* **19**: 1025-1031.
- McGurl, B., Pearce, G., Orozco-Cardenas, M., Ryan, C.A.** 1992. Structure, expression, and antisense inhibition of the systemin precursor gene. *Science* **255**: 1570-1573.
- Miao, H., Wei, J., Zhao, Y., Yan, H., Sun, B., Huang, J., Wang, Q.** 2013. Glucose signalling positively regulates aliphatic glucosinolate biosynthesis. *Journal of Experimental Botany* **64**: 1097-1109.
- Moghaddam, M.R.B., Van Den Ende, W.** 2012. Sugars and plant innate immunity. *Journal of Experimental Botany* **63**: 3989-3998.
- Morelli, G., Ruberti, I.** 2000. Update on Light Signaling Shade Avoidance Responses. Driving Auxin along Lateral Routes. *Plant Physiology* **122**: 621-626.
- Moreno, J.E., Tao, Y., Chory, J., Ballaré, C.L.** 2009. Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. *Proceedings of the National Academy of Sciences* **106**: 4935-4940.
- Mou, Z., Fan, W., Dong, X.** 2003. Inducers of plant systemic acquired resistance Regulate NPR1 function through redox changes. *Cell* **113**: 935-944.

- Müller, M., Munné-Bosch, S.** 2006. Several plant hormones, such as ethylene. *Plant Physiology* **169**: 32-41.
- Nozue, K., Devisetty, U.K., Lekkala, S., Mueller-Moulé, P., Bak, A., Casteel, C.L., Maloof, J.N.** 2018. Network Analysis Reveals a Role for Salicylic Acid Pathway Components in Shade Avoidance. *Plant Physiology* **178**: 1720–1732.
- O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O., Bowles, D.J.** 1996. Ethylene as a signal mediating the wound response of tomato plants. *Science* **274**: 1914–1917.
- Oh, E., Zhu, J.Y., Bai, M.Y., Arenhart, R.A., Sun, Y., Wang, Z.Y.** 2014. Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl. *eLife* **3**: e03031.
- Pandey, D., Rajendran, S.R.C.K., Gaur, M., Sajeesh, P.K., Kumar, A.** 2016. Plant Defense Signaling and Responses Against Necrotrophic Fungal Pathogens. *Journal of Plant Growth Regulation* **35**: 1159-1174.
- Pandey, S.P., Somssich, I.E.** 2009. The role of WRKY transcription factors in plant immunity. *Plant Physiology* **150**: 1648-1655.
- Pantazopoulou, C.K., Bongers, F.J., Küpers, J.J., Reinen, E., Das, D., Evers, J.B., Anten, N.P.R., Pierik, R.** 2017. Neighbor detection at the leaf tip adaptively regulates upward leaf movement through spatial auxin dynamics. *Proceedings of the National Academy of Sciences* **114**: 7450-7455.
- Pearce, G., Strydom, D., Johnson, S., Ryan, C.A.** 1991. A Polypeptide from Tomato Leaves Induces Wound-Inducible Proteinase Inhibitor Proteins. *Science* **253**: 895-897.
- Pedmale, U. V, Huang, S.S.C., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A.B., Sridevi, P., Nito, K., Nery, J.R., Ecker, J.R., Chory, J.** 2016. Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue Light. *Cell* **164**: 233-245.
- Peng, Y., Van Wersch, R., Zhang, Y.** 2018. Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. *Molecular Plant-Microbe Interactions* **31**: 403-409.
- Penninckx, I.A.M.A., Thomma, B.P.H.J., Buchala, A., Métraux, J.P., Broekaert, W.F.** 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. *The Plant Cell* **10**: 2103-2113.
- Pierik, R., Cuppens, M.L.C., Voeselek, L. a C.J., Visser, E.J.W.** 2004. Interactions between ethylene and gibberellins in phytochrome-mediated shade avoidance responses in tobacco. *Plant Physiology* **136**: 2928-2936.
- Pierik, R., Djakovic-Petrovic, T., Keuskamp, D.H., de Wit, M., Voeselek, L.A.C.J.** 2009. Auxin and Ethylene Regulate Elongation Responses to Neighbor Proximity Signals Independent of Gibberellin and DELLA Proteins in Arabidopsis. *Plant Physiology* **149**: 1701-1712.

- Pieterse, C. M. J., Pierik, R., Van Wees, S.C.M.** 2014. Different shades of JAZ during plant growth and defense. *New Phytologist* **204**: 261-264.
- Pratt, L.H., Cordonnier-Pratt, M.M., Hauser, B., Caboche, M.** 1995. Tomato contains two differentially expressed genes encoding B-type phytochromes, neither of which can be considered an ortholog of Arabidopsis phytochrome B. *Planta* **197**: 203-206.
- Pré, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C.M.J., Memelink, J.** 2008. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. *Plant Physiology* **147**: 1347-1357.
- Quail, P.H.** 1994. Photosensory perception and signal transduction in plants. *Current Opinion in Genetics and Development* **4**: 652-661.
- Rai, M.I., Wang, X., Thibault, D.M., Kim, H.J., Bombyk, M.M., Binder, B.M., Shakeel, S.N., Schaller, G.E.** 2015. The ARGOS gene family functions in a negative feedback loop to desensitize plants to ethylene. *BMC Plant Biology* **15**: 157.
- R core Team.** 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rizzini, L., Favory, J.J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schäfer, E., Nagy, F., Jenkins, G.I., Ulm, R.** 2011. Perception of UV-B by the arabidopsis UVR8 protein. *Science* **332**: 103-106.
- Roig-Villanova, I., Martínez-García, J.F.** 2016. Plant responses to vegetation proximity: A whole life avoiding shade. *Frontiers in Plant Science* **7**: 236.
- Ryan, C.A.** 1990. Protease Inhibitors in Plants: Genes for Improving Defenses Against Insects and Pathogens. *Annual Review of Phytopathology* **28**: 425-449.
- Salisbury, F.J., Hall, A., Grierson, C.S., Halliday, K.J.** 2007. Phytochrome coordinates Arabidopsis shoot and root development. *Plant Journal* **50**: 429-438.
- Sasidharan, R., Hartman, S., Liu, Z., Martopawiro, S., Sajeev, N., Van Veen, H., Yeung, E., Voesenek, L.A.C.J.** 2018. Signal dynamics and interactions during flooding stress. *Plant Physiology* **176**: 1106-1117.
- Scalschi, L., Sanmartín, M., Camañes, G., Troncho, P., Sánchez-Serrano, J.J., García-Agustín, P., Vicedo, B.** 2015. Silencing of OPR3 in tomato reveals the role of OPDA in callose deposition during the activation of defense responses against *Botrytis cinerea*. *Plant Journal* **81**: 304-315.
- Schrager-Lavelle, A., Herrera, L.A., Maloof, J.N.** 2016. Tomato phyE is required for shade avoidance in the absence of phyB1 and phyB2. *Frontiers in Plant Science* **7**: 1275.
- Schumacher, J.** 2017. How light affects the life of Botrytis. *Fungal Genetics and Biology* **106**: 26-41.

- Smith, A.M., Stitt, M.** 2007. Coordination of carbon supply and plant growth. *Plant, Cell and Environment* **30**: 1126-1149.
- Song, Y., Zhao, G., Zhang, X., Li, L., Xiong, F., Zhuo, F., Zhang, C., Yang, Z., Datla, R., Ren, M., Li, F.** 2017. The crosstalk between Target of Rapamycin (TOR) and Jasmonic Acid (JA) signaling existing in Arabidopsis and cotton. *Scientific Reports* **7**: 45830.
- Stitt, M., Lunn, J., Usadel, B.** 2010. Arabidopsis and primary photosynthetic metabolism - More than the icing on the cake. *Plant Journal* **61**: 1067-1091.
- Stitt, M., Zeeman, S.C.** 2012. Starch turnover: pathways, regulation and role in growth. *Current Opinion in Plant Biology* **15**: 282-292.
- Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J., Dong, X.** 2008. Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. *Science* **321**: 952-956.
- Tanpure, R.S., Barbole, R.S., Dawkar, V. V., Waichal, Y.A., Joshi, R.S., Giri, A.P., Gupta, V.S.** 2017. Improved tolerance against *Helicoverpa armigera* in transgenic tomato over-expressing multi-domain proteinase inhibitor gene from *Capsicum annum*. *Physiology and Molecular Biology of Plants* **23**: 597-604.
- Tikhonov, A.N.** 2014. The cytochrome b6f complex at the crossroad of photosynthetic electron transport pathways. *Plant Physiology and Biochemistry* **81**: 163-183.
- Townsley, B.T., Covington, M.F., Ichihashi, Y., Zumstein, K., Sinha, N.R.** 2015. BrAD-seq: Breath Adapter Directional sequencing: A streamlined, ultra-simple and fast library preparation protocol for strand specific mRNA library construction. *Frontiers in Plant Science* **6**, 366.
- Turner, J.G., Ellis, C., Devoto, A.** 2013. The Jasmonate Signal Pathway. *The Plant Cell* **14** Suppl: S153-S164.
- Underwood, W.** 2012. The plant cell wall: A dynamic barrier against pathogen invasion. *Frontiers in Plant Science* **3**: 85.
- van Gelderen, K., Kang, C., Paalman, R., Keuskamp, D., Hayes, S., Pierik, R.** 2018. Far-Red Light Detection in the Shoot Regulates Lateral Root Development through the HY5 Transcription Factor. *The Plant Cell* **30**: 101-116.
- van Veen, H., Mustruph, A., Barding, G.A., Vergeer-van Eijk, M., Welschen-Evertman, R.A.M., Pedersen, O., Visser, E.J.W., Larive, C.K., Pierik, R., Bailey-Serres, J., Voeselek, L.A.C.J., Sasidharan, R.** 2013. Two Rumex Species from Contrasting Hydrological Niches Regulate Flooding Tolerance through Distinct Mechanisms. *The Plant Cell* **25**: 4691-4707.
- van Wees, S.C.M., Van Pelt, J.A., Bakker, P.A.H.M., Pieterse, C.M.J.** 2013. Bioassays for assessing jasmonate-dependent defenses triggered by pathogens, herbivorous insects, or beneficial rhizobacteria. *Methods in Molecular Biology* **1011**: 35-49.

- Velthuys, B.R.** 1981. Electron-dependent competition between plastoquinone and inhibitors for binding to photosystem II. *FEBS Letter* **126**: 277-281.
- Wang, H., Wu, G., Zhao, B., Wang, B., Lang, Z., Zhang, C., Wang, H.** 2016. Regulatory modules controlling early shade avoidance response in maize seedlings. *BMC Genomics* **17**: 269.
- Wasternack, C., Hause, B.** 2013. Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of Botany* **111**: 1021-1058.
- Wasternack, C., Strnad, M.** 2018. Jasmonates: News on occurrence, biosynthesis, metabolism and action of an ancient group of signaling compounds. *International Journal of Molecular Sciences* **19**: 2539.
- Whalen, M.C., Innes, R.W., Bent, A.F., Staskawicz, B.J.** 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *The Plant Cell* **3**: 49-59.
- Windram, O., Madhou, P., McHattie, S., Hill, C., Hickman, R., Cooke, E., Jenkins, D.J., Penfold, C.A., Baxter, L., Breeze, E., Kiddle, S.J., Rhodes, J., Atwell, S., Kliebenstein, D.J., Kim, Y.-S., Stegle, O., Borgwardt, K., Zhang, C., Tabrett, A., Legaie, R., Moore, J., Finkenstadt, B., Wild, D.L., Mead, A., Rand, D., Beynon, J., Ott, S., Buchanan-Wollaston, V., Denby, K.J.** 2012. *Arabidopsis* defense against *Botrytis cinerea*: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. *The Plant Cell* **24**: 3530-3557.
- Xiong, Y., McCormack, M., Li, L., Hall, Q., Xiang, C., Sheen, J.** 2013. Glucose-TOR signalling reprograms the transcriptome and activates meristems. *Nature* **496**: 181-186.
- Xiong, Y., Sheen, J.** 2012. Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. *Journal of Biological Chemistry* **287**: 2836-2842.
- Xu, H., Fu, Y. nan, Li, T. lai, Wang, R.** 2017. Effects of different LED light wavelengths on the resistance of tomato against *Botrytis cinerea* and the corresponding physiological mechanisms. *Journal of Integrative Agriculture* **16**: 106-114.
- Yang, D., Seaton, D.D., Krahmer, J., Halliday, K.J.** 2016. Photoreceptor effects on plant biomass, resource allocation, and metabolic state. *Proceedings of the National Academy of Sciences* **113**: 7667-7672.
- Yang, D.L., Yao, J., Mei, C.S., Tong, X.H., Zeng, L.J., Li, Q., Xiao, L.T., Sun, T.P., Li, J., Deng, X.W., Lee, C.M., Thomashow, M.F., Yang, Y., He, Z., He, S.Y.** 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America* **109**: E1192-E1200.
- Zhang, X., Zhu, Z., An, F., Hao, D., Li, P., Song, J., Yi, C., Guo, H.** 2014. Jasmonate-activated MYC2 represses ETHYLENE INSENSITIVE3 activity to antagonize ethylene-promoted apical hook formation in *Arabidopsis*. *The Plant Cell* **26**: 1105-1117.

- Zheng, Z., Qamar, S.A., Chen, Z., Mengiste, T.** 2006. Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. *Plant Journal* **48**: 592-605.
- Zhu, Z., An, F., Feng, Y., Li, P., Xue, L., A, M., Jiang, Z., Kim, J.M., To, T.K., Li, W., Zhang, X., Yu, Q., Dong, Z., Chen, W.Q., Seki, M., Zhou, J.M., Guo, H.** 2011. Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 12539-12544.
- Zipfel, C.** 2014. Plant pattern-recognition receptors. *Trends in Immunology* **35**: 345-351.
- Ziv, C., Zhao, Z., Gao, Y.G., Xia, Y.** 2018. Multifunctional roles of plant cuticle during plant-pathogen interactions. *Frontiers in Plant Science* **9**: 1088.

Summary

Our generation is challenged by a climate crisis and a large increase in world population associated with higher food demand. In parallel, soil degradation leads to the loss of considerable arable land areas. To sustain the current food demand, it is paramount to create and maintain proper food security measures by producing more yield per area. One option to address these issues is to grow crops plants at higher density in order to produce more yield per hectares of land. In the Netherlands, efficient greenhouse systems are now used and allow for competitive yield production of worldwide crops such as tomato in limited spaces. However, the energy usage and CO₂ emissions of those structures remain too high and environmental-friendly measures to reduce energy consumption while conserving crop yield need to be optimized. The work presented in this thesis is part of the “LED it be 50%” consortium aiming at reducing energy usage by 50% in greenhouse systems by 2050. This could be achieved partly by replacing conventional high pressure sodium (or mercury) lamps by Light Emitting Diodes (LED). In this thesis, we investigated the effect of LED on tomato growth and resistance towards the necrotrophic pathogen *Botrytis cinerea*, a major tomato pathogen worldwide.

Light is essential for plant growth. Red and blue light are the major wavelength which are absorbed by leaf tissue and used for photosynthesis while others, *i.e* yellow and green light are reflected giving the plants their natural color. Far-red light, which is invisible to the human eye, is transmitted by green plant tissues and constitute an important signal for plants to sense neighboring vegetation. At high planting density, the absorption of red light and the reflection of far-red light lead to a decrease in the ratio between red and far-red light (R:FR). Upon detection of a low R:FR, plants elongate and move their leaves upwards (hyponasty) in order to outcompete their neighbors and capture more light above the canopy. These changes in plant architecture called “shade avoidance” have been shown to enhance plant susceptibility to herbivores and pathogens including *B. cinerea*. In this thesis, we unraveled how low R:FR can manipulate defense responses of tomato plants towards *B. cinerea*. In **chapter 2**, we found that the addition of FR LEDs to a white light background or a red and blue light background could change tomato morphology leading to stem and petiole elongation; typical traits of shade avoidance where plants attempt to grow taller than neighboring plants. The addition of FR also led to an increase in susceptibility to *B. cinerea* when perceived before the inoculation

occurred. This phenomenon referred to as “FR-induced susceptibility” was studied at the gene expression level in **chapter 3** where we performed an RNA sequencing analysis to investigate how a FR pre-exposure (prior to the inoculation) interferes with the plant’s capacity to defend itself against *B. cinerea* following a 30 h time course. We found that the increased susceptibility caused by FR-enrichment relied on a delay in pathogen detection and in plant defense activation compared to control conditions. Plant defense against *B. cinerea* are known to be mainly based on the production of plant defense hormones, namely jasmonic acid and ethylene. Our data revealed that FR delayed the induction of a set of six genes known to be regulated by those two hormones. Also, we observed that FR could affect sugar metabolism which might be the cause of increased susceptibility upon *B. cinerea* infection. **Chapter 4** focuses on the group of six genes which were inhibited by FR in our dataset. These genes, known as *PROTEINASE INHIBITOR (PI)* genes, have been studied in the context of herbivory and found to interfere with insects’ digestive system affecting their feeding behavior and promoting plant resistance. We managed to validate that jasmonic acid and ethylene are both needed for the full induction of *PI* which did not occur after FR exposure. The lack of *PI* induction in FR-treated plants points at a decrease in hormone sensitivity through which occurred a delay in plant defense activation against *B. cinerea*. From the RNA sequencing results, we hypothesized that FR might cause changes in the sugar status of the plant indirectly promoting the growth of the fungus in plant tissue. In **chapter 5**, we found that plants experiencing FR light accumulated more monosaccharides (glucose and fructose) than in control conditions which was correlated with an increase susceptibility. As glucose is the main food source for *B. cinerea* and glucose levels were elevated in FR-treated leaves, we managed to experimentally modulate glucose levels in tomato leaves and could associate the monosaccharide accumulation with a faster development of the fungus leading to higher plant susceptibility. This sugar-mediated susceptibility was also observed when we only exposed a single leaflet with FR. Soluble sugar levels were increased at the site of illumination but also in older leaves (located below) which did not receive FR themselves. Interestingly, wherever we observed an increase in soluble sugars, we also observed an increase in plant susceptibility which suggests that soluble sugars play a major role in the FR-induced susceptibility in tomato.

To summarize, FR light enhances tomato susceptibility towards *B. cinerea* by delaying pathogen recognition and jasmonic acid-mediated plant defense activation possibly through *PI* induction. Also, FR light leads to a rather indirect glucose and fructose

accumulation promoting *B. cinerea* growth inside the leaves. The combination of lower hormonal defense and higher carbohydrate levels in FR-exposed plants could very well explain how the FR-induced susceptibility is regulated in tomato plants. We propose that the negative effect of FR on plant susceptibility could be counteracted in tomato greenhouse systems by applying additional red light in between the rows of crops to increase the R:FR ratio. This way, the effect of FR reflection within a tomato canopy grown in a greenhouse would not have such a strong effect on plant susceptibility but would still increase yield and increase sustainable vegetable production.

Résumé

Notre génération est confrontée à une crise climatique ainsi qu'à un essor dramatique de la population menaçant la sécurité alimentaire mondiale. Parallèlement à cela, l'appauvrissement des sols par l'agriculture intensive entraîne une perte considérable de surfaces cultivables. Afin de répondre à la demande alimentaire mondiale, il est nécessaire de développer des moyens efficaces pour augmenter les rendements sur des surfaces agricoles de plus en plus réduites. La culture en serre adoptée par les Pays-Bas permet (de produire) des rendements compétitifs en augmentant le nombre de plantes cultivées par mètre carré. Cependant, la consommation d'énergie et les émissions de CO₂ de ces structures restent trop élevées. Des mesures respectueuses de l'environnement afin de réduire la consommation d'énergie tout en conservant le rendement des cultures doivent être optimisées. Le travail présenté dans cette thèse fait partie d'une collaboration entre plusieurs universités Néerlandaises visant à réduire la consommation d'énergie de 50% dans les serres d'ici 2050. Cela pourrait être réalisé en partie en remplaçant les systèmes d'éclairage classiques par des diodes électroluminescentes (DEL) dans les serres.

Pour se développer, les plantes requièrent des nutriments, de l'eau mais aussi une source de lumière. Tandis que les lumières rouge et bleue sont les principales longueurs d'ondes absorbées par les feuilles et utilisées comme substrat pour la photosynthèse, d'autres comme les lumières jaune et verte sont réfléchies donnant aux plantes leur couleur naturelle. Bien qu'invisible à l'œil nu, une autre longueur d'onde appelée « rouge lointain » est réfléchiée par les plantes et constitue un signal important pour détecter la végétation environnante. Lorsque la végétation est dense, l'absorption de rouge et la réflexion de rouge lointain par les plantes entraînent une diminution du ratio rouge/rouge lointain (R/RL) dans l'environnement. Une plante qui détecte un déclin du ratio R/RL, synonyme de compétition avec d'autres plantes, adopte une stratégie « d'évitement de l'ombre » qui consiste à pousser plus haut que les plantes voisines pour capturer plus de lumière au-dessus de celles-ci. Bien que la stratégie d'évitement de l'ombre permette aux plantes de mieux se développer dans une végétation dense, elles deviennent plus sensibles à des pathogènes. Dans cette thèse, nous avons étudié l'effet des DEL sur la croissance et la résistance des tomates contre le champignon pathogène *Botrytis cinerea*, causant d'importants dégâts dans les cultures de tomate dans le monde.

Dans le **chapitre 2**, nous avons constaté que le rouge lointain, ajouté à de la lumière blanche ou un mélange de rouge et de bleu, provoque une élongation de la tige et des pétioles, caractéristiques de l'évitement de l'ombre. L'ajout de rouge lointain, lorsqu'il est perçu avant l'infection par le pathogène, conduit à une forte augmentation de la sensibilité à *Botrytis* chez la tomate. Ce phénomène, appelé «sensibilité induite par le rouge lointain», a été étudié au niveau génétique dans le **chapitre 3** où nous avons effectué une analyse de séquençage d'ARN (acide ribonucléique) pour étudier comment la perception de rouge lointain avant l'inoculation pouvait moduler la capacité de la plante à se défendre contre *Botrytis*. Nous avons constaté que la sensibilité accrue causée par l'enrichissement en rouge lointain reposait sur un retard dans la détection du pathogène et dans l'activation des défenses par rapport au contrôle. Les défenses des plantes contre *Botrytis* sont connues pour être principalement basée sur la production d'hormones de défense, à savoir l'acide jasmonique et l'éthylène. Dans nos données, nous avons constaté que la perception de rouge lointain affecte l'induction de six gènes connus pour être régulés par ces deux hormones. Le **chapitre 4** se concentre sur ces six gènes connus sous le nom de *PROTEINASE INHIBITOR (PI)* favorisant la résistance des plantes contre les herbivores en inhibant le système digestif des insectes. Nous avons réussi à montrer que l'acide jasmonique et l'éthylène sont tous deux nécessaires pour l'induction complète de *PI* pour promouvoir la résistance des plantes. Cet effet n'a pas été observé en présence de rouge lointain indiquant une diminution de la sensibilité des plantes à l'acide jasmonique et l'éthylène associé à un retard dans l'activation des défenses des plantes. À partir des résultats de séquençage, nous savons que la perception de rouge lointain provoque des changements dans le métabolisme carboné chez la plante favorisant indirectement la croissance du champignon dans les tissus végétaux. Dans le **chapitre 5**, nous avons constaté que les plantes soumises au rouge lointain contenaient plus de monosaccharides (glucose et fructose) que dans des conditions témoins, probablement corrélé à une sensibilité accrue, étant donné que le glucose est la principale source de nourriture pour *Botrytis*. Cette sensibilité régulée par les sucres a également été observée lorsqu'une seule feuille était exposée au rouge lointain. En effet, les niveaux de glucose étaient plus élevés au niveau du site d'illumination mais aussi dans les feuilles plus âgées (situées en dessous) qui n'ont pas directement été exposées au rouge lointain. En d'autres termes, une augmentation des niveaux de glucose dans les feuilles est toujours synonyme d'une sensibilité accrue au pathogène signifiant que les sucres jouent un rôle majeur dans la sensibilité induite par le rouge lointain chez la tomate.

Pour résumer, le déclin du ratio R/RL ou l'omniprésence de rouge lointain perçus par la plante retardent la détection du pathogène ainsi que l'activation des défenses via l'acide jasmonique et l'éthylène. De plus, la présence de rouge lointain conduit à une accumulation plutôt indirecte de glucose et de fructose dans les feuilles favorisant la croissance de *Botrytis* à l'intérieur de la plante. L'association entre la réduction des défenses et l'augmentation des niveaux de glucose par le rouge lointain pourrait très bien expliquer pourquoi la sensibilité est augmentée. Nous pensons que ce phénomène pourrait être neutralisé dans les cultures de tomates en serres en appliquant de la lumière rouge entre les plantes pour compenser celle qui est absorbée par les plantes et augmenter le ratio R/RL. De cette façon, la réflexion du rouge lointain n'aurait plus tellement d'effet sur la sensibilité des plantes contre *Botrytis*, augmenterait le rendement et résoudrait en partie les problèmes de sécurité alimentaire mondiale.

Samenvatting

De wereldwijde voedselzekerheid staat ernstig onder druk vanwege de gevolgen van klimaatverandering en een toenemende bevolkingsgroei. Tegelijkertijd leidt intensieve landbouw tot uitputting van de bodem en een aanzienlijk verlies van geschikte landbouwgrond. Om aan de wereldwijde vraag naar voedsel te kunnen voldoen, is het daarom noodzakelijk om effectieve en duurzame technologieën te ontwikkelen om de opbrengst per landbouwoppervlak te verhogen. De Nederlandse glastuinbouw draagt hier aan bij door een enorm hoge productie per vierkante meter te realiseren. Echter, het energieverbruik en de CO₂-uitstoot van de glastuinbouw zijn nog altijd aanzienlijk, en er zijn milieuvriendelijke maatregelen nodig om het energieverbruik te verminderen terwijl de gewasopbrengst gewaarborgd blijft. Het werk dat in dit proefschrift wordt gepresenteerd, is onderdeel van een samenwerking tussen Nederlandse universiteiten en bedrijven om het energieverbruik in kassen tegen 2050 met minimaal 50% te verminderen. Dit kan gedeeltelijk worden bereikt door conventionele verlichtingsystemen in kassen te vervangen door energie-efficiënte Light Emitting Diodes (LED's).

Planten gebruiken bepaalde golflengtes van het zichtbare licht om optimaal te groeien. Zo zijn rood en blauw licht de belangrijkste golflengten die worden geabsorbeerd door de bladeren en worden gebruikt als energiebron voor fotosynthese. Daarentegen worden andere kleuren licht zoals geel en groen relatief meer gereflecteerd, waardoor planten hun natuurlijke groene kleur krijgen. Een andere golflengte, genaamd “ver-rood” (“Far-red”, FR), is onzichtbaar voor het menselijk oog, wordt gereflecteerd door planten en is een belangrijk signaal voor het detecteren van de omringende vegetatie. Wanneer de vegetatie een hogere dichtheid heeft, leidt de absorptie van rood en de reflectie van ver-rood licht door de planten tot een verlaging van de verhouding rood ten opzichte van ver-rood (R:FR-ratio) licht in de omgeving. Wanneer een plant deze afname van de R:FR-ratio waarneemt (een schaduwsignaal van naastgelegen planten), versnelt het de groei van bladstelen en stengels om zo de concurrentiestrijd met buurplanten om licht te kunnen winnen. Hoewel deze schaduwvermijdingsstrategie het mogelijk maakt dat planten kunnen concurreren en overleven in dichte vegetatie, worden ze tegelijkertijd ook vatbaarder voor ziekteverwekkers. De pathogene schimmel *Botrytis cinerea* is zo'n ziekteverwekker en richt wereldwijd grote schade aan bij gewassen. In dit proefschrift is het effect van rood en ver-rood (LED) licht op de groei van tomatenplanten (*Solanum lycopersicum*) en de resistentie tegen *Botrytis* bestudeerd.

In **hoofdstuk 2** is beschreven dat ver-rood de groei van de stengel en bladstelen versterkt in tomatenplanten. Daarnaast leidde een voorbehandeling met ver-rood licht tot een sterke toename van de gevoeligheid voor Botrytis in tomaten. Dit fenomeen, “ver-rood-geïnduceerde gevoeligheid” genoemd, werd op het genetische niveau bestudeerd in **hoofdstuk 3**, door middel van een analyse van RNA-sequencing om te onderzoeken hoe de perceptie van ver-rood vóór inoculatie met de schimmel Botrytis de afweer van de plant beïnvloedt. De verhoogde gevoeligheid van tomatenplanten blijkt verklaard te kunnen worden door zowel een vertraagde detectie van de ziekteverwekker als door de vertraagde activatie van de ziekteafweer. Eerder onderzoek heeft aangetoond dat de afweer van planten tegen Botrytis voornamelijk gebaseerd is op de productie en activatie van de afweerhormonen jasmonzuur en ethyleen. De hier gepresenteerde resultaten laten zien dat ver-rood de activatie van zes genen remt, waarvan bekend is dat ze door deze twee plantenhormonen worden aangestuurd. **Hoofdstuk 4** richt zich op deze zes *PROTEINASE INHIBITOR (PI)* genen, die de weerstand tegen insecten en ziekteverwekkers bevorderen. We laten zien dat jasmonzuur en ethyleen beide noodzakelijk zijn voor volledige activatie van *PI* genen om zo de weerstand van de plant te bevorderen. De activatie van deze genen werd niet waargenomen in planten die waren behandeld met ver-rood licht. Deze bevindingen duiden op een afname van de gevoeligheid van planten voor jasmonzuur en ethyleen onder deze omstandigheden en geven zo een verklaring voor de vertraagde en verminderde afweer van deze planten.

Uit de resultaten van de moleculaire analyse bleek ook dat het metabolisme van koolhydraten in ver-rood behandelde planten verstoord was, en dit zou ook de groei van Botrytis in plantenweefsel kunnen bevorderen. **Hoofdstuk 5** laat inderdaad zien dat planten die aan ver-rood werden blootgesteld meer monosacchariden (glucose en fructose) bevatten. Deze verhoogde suiker niveaus zorgden ervoor dat de Botrytis beter groeide in de ver-rood behandelde bladeren, mogelijk omdat glucose een belangrijke voedselbron is voor Botrytis. Deze door suiker gereguleerde gevoeligheid voor Botrytis werd ook waargenomen wanneer een enkel blad werd blootgesteld aan ver-rood. De glucosegehalten waren niet alleen verhoogd op de (ver-rood) belichte plek, maar ook in de oudere bladeren (hieronder gelegen), die niet direct werden blootgesteld aan ver-rood. Met andere woorden, een toename van glucosespiegels in de bladeren door (lokale) ver-rood belichting leidt altijd tot verhoogde gevoeligheid voor de ziekteverwekker. Deze resultaten suggereren dat suikers een belangrijke rol spelen in de verhoogde ziekte gevoeligheid die wordt veroorzaakt door ver-rood in tomatenplanten.

Samenvattend laat dit proefschrift zien dat de daling van de R:FR-ratio in een dichte tomatenplant vegetatie (zoals in de glastuinbouw) leidt tot zowel een vertraagde herkenning van de ziekteverwekker *Botrytis* als een vertraagde activatie van de afweer door de hormonen jasmonzuur en ethyleen. Bovendien leidt de aanwezigheid van ver-rood licht tot een ophoping van glucose en fructose in de bladeren die de groei van *Botrytis* in de plant bevordert. Het verband tussen verminderde afweer en verhoogde glucosewaarden verklaart waarom de gevoeligheid voor ziekteverwekkers in een vegetatie is verhoogd. Dit effect kan wellicht worden bestreden in tomatenplanten in de kas door rood licht LEDs tussen de planten aan te brengen om zo de R:FR-verhouding te verhogen. Op deze manier wordt het effect van ver-rood licht op een duurzame manier binnen de vegetatie geneutraliseerd en zal het waarschijnlijk niet langer de vatbaarheid van planten voor *Botrytis* verhogen.

Acknowledgements

Here we are, at the end of an incredible journey. Not an easy one though. This book does not only represent a milestone and four and a half years of PhD, it also marks the end of an amazing life chapter in the Netherlands. Looking back, I realize how much I learned about myself. I cannot believe how many new people I met over the years who contributed to making me a better person. Thanks to those people, I became a scientist who enjoys the thrills of discoveries and the excitement of sharing them with the entire community! But of course, as a PhD thesis is never a one-person job, I would like to take a few pages to acknowledge the people who took an important part in this adventure.

I could not start this section without thanking my promotor **Ronald**. I remember the day of my job interview in Utrecht like it was yesterday. I am so glad you trusted me and gave me the opportunity to work on this project. It was not always easy but your patience and guidance all along the way contributed to the motivated scientist I am today. It was really good to learn from you and I cannot thank you enough for that. **Saskia**, my co-promotor, I am thankful for the time and energy that you invested in me and this thesis. I will always consider you and Ronald as my academic parents. **Rens**, your door was always open for me when I needed it. Thank you for that. I really enjoyed spending almost five years of my life in the group and I learned a lot from you. I will never forget the amazing barbecues as well as some Christmas dinners at your place. You and Carla are such wonderful hosts. **Kaisa**, you were always here to discuss either professional or personal matters and you made me a RNA-seq library pro! Thank you for sharing your knowledge and your enthusiasm along the project, I learned a lot. **Rashmi**, thank you for communicating your joviality at coffee breaks and for all the input you gave at the work discussions.

The work presented in this thesis would not have been possible without the help of the talented EvP technical staff. **Ankie, Rob, Diederik, Yorrit, Dominique** and **Liao**, thank you for sharing your energy, knowledge and advice with me in the lab or in the phytotron. **Sara**, you are one of the few who started before me and will still stay after I leave, it shows your dedication and tenacity to your work which is amazing. Thank you for your help. **Emilie**, you were a true leader, I consider you more as a friend than a colleague and our many chats helped me to canalize my feelings when things were

going sideways. I will miss your awesome jokes, amazing cakes and simply the fact of having someone to gossip with.

From day one to the end, I could not have been more happy with all the awesome officemates I had in Z303. **Lot**, our time together was relatively short but I really enjoyed it. You were the one who made me feel comfortable in this new group. The energy and the *gezelligheid* that you brought in our office was truly amazing. I am also grateful that you accepted to take part of my defense committee and hope we can keep in touch. **Scott**, I cannot believe how smart and talented you are, thank you for helping me and sometimes challenging my English skills! It was hard to see you leave the group but I'm sure our paths will cross again. **Franca**, I really enjoyed our time together in the office. Your interest for other projects, else than your own, was a great example and taught me a lot. Thanks to you, I am not afraid of not being the expert and ask simple curiosity questions. I wish you the best in China. **Chiakai**, thank you for bringing joy to the office. I miss your sense of humor and your incredible stories about the virtues of adding coconut oil to your coffee or smear ethanol on your skin to cool down during hot summers. **Jesse**, you are the best officemate and a friend above all. I had the best time with you in the office. Thank you for being the one who helped me through the PhD dips and during the PhD life in general. Thank you for communicating your energy and curiosity all along the project! You are such a great scientist and I wish you and Mo all the best and a bright future together wherever you go. **Martina**, when you joined our office, I thought: "Finally a *normal person* who chose to work on crops rather than Arabidopsis!" Thank you for the good discussions we had. Your dedication for your project is amazing to see. **Tom**, the first non-shady in Z303. Thank you for bringing fun in the office. I really enjoyed your company and I wish you the best for the rest of your PhD. **Mariana**, your time in Z303 was short but I learned a lot from our discussions and you helped me a lot during the writing process of this thesis. Thank you for that! **Melissa**, we did not have time to properly get to know each other as the Corona virus outbreak got between us but I wish you all the best for your post-doc.

Of course, the rest of the group also greatly contributed to this thesis. **Chrysa**, thank you for your all thoughtful advice you gave me throughout my time in Utrecht. You made my PhD life a little sweeter and I thank you for that. Thank you for your help and good luck for the future. **Elaine**, it was good to have you around during all these years and I hope we get to see each other soon again. **Linge**, it was a pleasure to help

you start up your tomato work. Your motivation is immense and you evolved a lot since you started. Good luck for the rest of your PhD. **Kasper**, thank you for all the fun you brought at the borrels and for your feedback and input in my project. **Valérie**, I enjoyed sharing stories with you around a drink or simply in the corridor. You are a great scientist and I am sure you will have an awesome thesis, HY5 to that! **Lisa**, thank you for saving my life (well, my painful feet) at EPSR 2019, I still owe you one! **Nicole**, you chose wisely by joining EvP rather than staying in PMI ;). I will miss your energy and your awesome sense of humor and I wish you the best for your PhD. **Shanice**, your creativity is beyond words! It was extremely hard to see you leave the group so soon but I am glad you found your dream job and that we are still in touch. Thank you for making me laugh and for teaching me amazing dancing moves at all the parties we have been to. I wish you and Thomas a very bright future together. **Zeguang**, I learned so much about the Chinese history and traditions thanks to you. Thank you for distracting me from my project during coffee break or a hotpot evening at your place. Good luck in Geneva! **Hans**, you are the master of bioinformatics and your energy is without limits. Thank you for your help in the lab but also during meetings. I wish you all the best. **Thijs**, thank you for bringing *paaseitjes* every single year. **Angelica**, thank you for always being up for a sushi evening in Zeist or a last minute Muse concert, it was a lot of fun. **Justine**, I wish you the best of luck for your last year. **JZ**, keep your energy and I wish you good luck for your PhD. **Yaron**, you played a wonderful Kasper in Sjon's movie. Good luck as being the new Kasper lookalike of EvP.

Nikita, Putri and **Jana**, even though you were not part of EvP for long, you were totally included in the group. Nikita, I had so much fun with you and your crazy stories. I am glad that you found your happy place in Wageningen and wish you and Gilles the best for your PhD. And watch your keys! Putri, thank you for bringing your energy and positivity to the group and for bunny-sitting Yolo during our trip to Thailand. Jana, it was great to get to know you and have you in Zeist sometimes when you joined the PhD defenses.

I would also like to thank the students who participated to this project. **Qiqi, Pierre-Olivier, Sanne** and **Emma**, I learned a lot from all of you and would like to thank you for your trust and the motivation that you all showed during your internships. Sanne, you did an amazing job in starting up and optimizing the sugar-related experiments. Your energy and positivity helped me to overcome a rather difficult period of my PhD. Thank you for that! Emma, even though you joined the project when I was writing

my thesis, you were able to take initiatives and work on your own. I am sure we will publish a great paper together.

I would also like to thank former and current PMI members. **Corné** and **Guido**, I am grateful for your help and guidance during the half-year reports and throughout my PhD. I would also like to thank **Miek** and **Kim** (oops, I mean Sinterklaas) for the great presents I got every year! I am also thankful to the members of Saskia's group, **Irene**, **Lotte**, **Marciel**, **Richard**, **Niels** and **Dharani** for helping me in the lab and for the good discussions we had during meetings. **Hans** and **Anja**, I annoyed you more than once when I was looking for someone or something but you were always willing to help with a smile, I really felt part of the group thanks to you. **Manon**, my evil twin sister, thank you for all the fun we had over the years and for not stealing my boyfriend when you had the chance at the Lunteren meeting. I wish you all the best for finishing your PhD. **Hao**, you are the sweetest person I have ever met, and you are a master when it comes to preparing the delicious hotpot evenings we had together at your place. **Tijmen**, we are forever bound by that amazing sticky toffee pudding that makes me drool just by thinking about it. I am really glad we became friends and hope we can keep contact and catch many more Pokémon in the future. I wish you the very best. **Pim**, your sense of humor is truly amazing but I never want to be near you holding a banana again. Best of luck for finishing your PhD. **Sietske**, thank you for making our trip to Edinburg and Glasgow such a fabulous journey, I do not see rhododendrons the same way anymore. **Merel**, you are such a good and thoughtful friend, I am glad that we got closer and keep on seeing each other in Zeist after you left the group. Your cocktail parties were always a success and a lot of fun. And last but not least, **Pauline**, je suis tellement contente que nous nous soyons rencontrées et rapprochées au cours de nos années à Utrecht. Je me souviendrai longtemps de nos pauses café et de nos soirées filles qui m'ont permis de réduire le stress lié à la thèse. Merci pour tout ce que tu m'as appris qui m'a permis d'en arriver jusque-là.

Nathalie and **Peter**, thank you for inviting me to the E&B coffee breaks, borrels and events, I felt very welcome and had a lot of fun. Nathalie, I wish you all the best for your PhD and hope we can keep in touch. Peter, thank you for keeping me fit and being my boxing partner. Without you, I would be enormous. **Erini**, I enjoyed our coffee breaks and discussions so much. I really hope you find your dream job and I wish you the best of luck for the future.

This project would not have been possible without the funding provided by the TTW perspectief grant “LED it be 50%” and the contribution of Signify, WUR Greenhouse Horticulture and LTO Glaskracht. I wish to thank the entire LED it be 50% consortium for their precious help and for the lively discussions during the project meetings and lunches. I would especially like to thank **Leo** for putting this program together and organizing all meetings and outings so well. **Ep**, I really appreciated your interest for the project but also for my professional and personal development, thank you for that. Lastly, I would like to thank the “LED it Beer” PhDs and post-docs who made my PhD journey much more enjoyable. **Yongran, Haris, David, Wouter, Kiki, Victor, Priscilla, Dália, Rachel** and **Aina**, I am happy that we became almost like a family. You were always here to listen, help and willing to discuss science. I had a lot of fun working with you all and wish you the very best for the future. Yongran, thank you for taking amazing initiatives to go for dinner and gather everyone together. You also organized the greatest scientific trip ever to Croatia that I will never forget.

Maud, nos années ensemble à Antibes semblent déjà si loin mais me rappellent tellement de bons souvenirs. Merci mille fois de t’être faite embarquer dans mon aventure. Tes illustrations sont parfaites et apportent la légèreté et l’humour dont la science a de plus en plus besoin. Ta créativité est débordante et je n’ai aucun doute sur ta future réussite dans la vulgarisation scientifique. **Isabelle**, une de mes plus belles rencontres et ma *bestie* depuis tant d’années. Je me souviendrai toujours de nos périple à Toulouse, à Majorque où en Thaïlande. Merci d’être toujours là pour moi. Je ne te remercierai jamais assez pour toutes ces années d’amitié et je suis sûre que l’on ne va pas s’arrêter là.

Enfin ma famille, **Papa, Maman** et **Johan**, merci de m’avoir fait confiance et de m’apporter tant de bonheur au quotidien. Papa, Maman, vous êtes les meilleurs parents que l’on puisse imaginer, je suis contente de pouvoir partager cette étape de ma vie avec vous. Je sais que je peux toujours compter sur vous pour m’aider et m’encourager, je vous aime de tout mon cœur. **Tatie** et **Tonton**, merci pour toutes ces sessions Skype qui m’ont permis de me changer les idées et de découvrir de jolis livres pour m’évader un peu de toute cette science! Merci pour vos conseils et votre soutien, je vous dois beaucoup. Mes grands-parents, **Papys, Mamies, Gilbert** et **Geneviève** qui m’ont tant appris, merci pour tous les petits paquets et jolis courriers que vous m’avez envoyés pendant mes années à l’étranger. Merci au reste de la famille pour m’avoir soutenue dans tout ce que j’ai entrepris jusqu’ici.

My family in law, **Annemiek, Iris, Patrick, Maijkel, Jessica** and **Thomas**, thank you for your support and understanding. I felt immediately welcome to the family and I am very grateful for that, it means a lot to me. **Yolo**, you were always here to keep me warm and cheer me up on dark and cold winter evenings. **Twiggy** and **Groentje**, thanks for being the entertainment at every dinner and parties we hosted.

Lastly, **Sjon**, thank you for being who you are. I could not have imagined a better life partner than you, you are truly one of a kind. I would have never made it through my PhD without your support and unconditional love. You were here for me and always used the right words. I am so happy we found each other and I am looking forward to starting a new life chapter together.

Sarah

About the author



Sarah Courbier was born on the 11th of May 1991 in Brive-la-Gaillarde, France. Sarah received her primary and secondary education in Brive-la-Gaillarde. In July 2010, she obtained her general baccalaureate certificate and moved to Toulouse to study Biology at the Paul Sabatier University. Then, she enrolled in professional bachelor studies at the National Agronomy School (ENFA, Auzeville-Tolosane, France). During her bachelor, Sarah did a short term internship (4 months) in the plant-oomycete interactions group under the supervision of Dr. Agnès Attard at the

Sophia Agrobiotech institute (Sophia Antipolis, France) working on characterizing susceptibility genes of *Arabidopsis* in response to *Phytophthora parasitica*. In 2013, Sarah got her BSc degree and subsequently started her master in plant biology at the University of Montpellier (UM2) for the first year and at Paul Sabatier University for the second year. During her master, Sarah did a 4-month followed by a 6-month internship in the plant-microbe interactions group at the Plant Research Laboratory (LRSV, Toulouse, France) where she was supervised by Prof. Bernard Dumas and Dr. Elodie Gaulin and studied a new class of effector proteins of the legume pathogen *Aphanomyces euteiches*. Shortly after receiving her MSc, Sarah started her PhD at the plant ecophysiology group under the supervision of Prof. Ronald Pierik and Dr. Saskia C.M. Van Wees in November 2015. During her PhD, Sarah was also involved in organizing the 10th European plant science retreat (EPSR) in 2018 to promote interactions between PhD students working in plant science within Europe. For a year, she also took part of the organizing committee of monthly PhD meetings within the institute of Environmental biology (IEB) of Utrecht University. This thesis is the result of her PhD research.

List of publications

- Courbier, S., Pierik, R.** 2019. Canopy light quality modulates stress responses in plants. *iScience* **22**: 441-452.
- Ji, Y., Ouzounis, T., Courbier, S., Kaiser, E., Nguyen, P.T., Schouten, H.J., Visser, R.G.F., Pierik, R., Marcelis, L.F.M., Heuvelink, E.** 2019. Far-red radiation increases dry mass partitioning to fruits but reduces *Botrytis cinerea* resistance in tomato. *Environmental and Experimental Botany* **168**: 103889.
- Horst, S., Butselaar, T., Zhang, H., Vismans, G., Steenbergen, M., Courbier, S., Neilen, M., Küpers, J.J.** 2019. Bringing together Europe's young plant scientists. *New Phytologist* **222**: 29-32.
- Gaulin, E., Pel, M.J.C., Camborde, L., San-Clemente, H., Courbier, S., Dupouy, M.A., Lengellé, J., Veyssiere, M., Le Ru, A., Grandjean, F., Cordaux, R., Moumen, B., Gilbert, C., Cano, L.M., Aury, J.M., Guy, J., Wincker, P., Bouchez, O., Klopp, C., Dumas, B.** 2018. Genomics analysis of *Aphanomyces* spp. identifies a new class of oomycete effector associated with host adaptation. *BMC Biology* **16**: 43.



Moo