The Role of the Circadian Clock in the Control of Plant Immunity in *Arabidopsis thaliana*

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Abstract

The circadian clock regulates a wide range of biological processes, allowing plants to be prepared for predictable daily diurnal changes in environmental cues such as light and temperature. Recent studies have suggested that the circadian clock may also control plant immunity. The exact nature of the interaction between the circadian clock and plant pathogens remains unknown. Our focus in this study is on the elucidation of the role of the biological clock in plant immunity against the necrotrophic pathogen to *Botrytis cinerea*. In order to do this we tested the level of susceptibility to *B. cinerea* in *Arabidopsis thaliana* wild type and transgenic plants: *toc1*, *cca1/lhy*, *cca1/toc1*, *lhy/toc1*, *cca1/lhy/toc1*, *GLK1* OE, *GLK2* OE, *glk1, glk2*, and *glk1/glk2*. We demonstrated that the time of infection plays a role in susceptibility to *B. cinerea.* Specifically, we found that plants are more susceptible to infection in the subjective morning. We also found that genetic mutations in core clock components or in GLK genes leads to changes in susceptibility to *B. cinerea*. Our data suggests that clock genes are not solely responsible for plant immune responses to *B. cinerea* but rather the ways in which the biological clock system regulates outcome pathways. Furthermore, when we entrain the biological clock by changing the photoperiod (day length) in normal earth conditions LD 24h and SD 24h, we observed that short day plants had higher susceptibility to *B. cinerea* than long day plants. In addition, when we entrain the biological clock in different photoperiods, the LD 30h photoperiod plants displayed similar responses as those in the SD 24h photoperiod. The data indicates that day length is not responsible for the control of plant immunity; it is the ability of light to entrain the biological clock that is important. Together, the data strongly support the conclusion that the circadian clock plays a role in plant defense regulation.

Résumé

L'horloge interne régularise un grand nombre de processus biologiques, permettant aux plantes d'être préparées aux changements diurnes journaliers prévisibles dans les indicateurs environnementaux telle la lumière et la température. Quoique l'horloge interne joue de multiples rôles dans la vie des plantes, récemment, quelques études ont révélées que l'horloge interne contrôle l'immunité chez les plantes. La nature exacte des interactions entre l'horloge interne et les phytopathogènes n'est pas connue. Notre axe principal est l'éclaircissement du rôle de l'horloge interne dans l'immunité à *Botrytis cinerea*, un champignon inférieur nécrotrophique. Nous avons testé le niveau de sensibilité à *B. cinerea* chez des *Arabidopsis thaliana* de type sauvage ou les plantes transgéniques suivantes: *toc1*, *cca1/lhy*, *cca1/toc1*, *lhy/toc1*, *cca1/lhy/toc1*, *GLK1* OE, *GLK2* OE, *glk1, glk2*, and *glk1/glk2*. Les plantes ont été cultivées en utilisant différents cycles jour/nuit et infectées avec *B. cinerea* à l'un de deux temps : matin subjectif (AM) ou soir subjectif (PM). Nous avons montré que le moment de l'infection joue un rôle dans la sensibilité à *B. cinerea* : les plantes étaient plus sensible à l'infection lorsque inoculées le matin subjectif. Nous avons aussi trouvé que des mutations génétiques dans les éléments clé de l'horloge interne ou dans les gènes GLK mènent à un changement dans la sensibilité à *B. cinerea*. Nos données suggèrent que les gènes de l'horloge ne sont pas les seuls gènes responsables de la réponse immunitaire à *B. cinerea* chez les plantes ; leur responsabilité serait plutôt dans la façon dont le système biologique de l'horloge régularise les voies de résultats. Lorsque l'horloge interne fut modifiée en changeant le ratio jour/nuit à l`intérieur d'une photopériode normale de 24 h, LJ 24 h et CJ 24h, nous avons observé que les plantes cultivées en condition de jours courts (CJ) avaient une plus grande sensibilité à *B. cinerea* que les plantes cultivées en jour longs (LJ).

De plus, lorsque la photopériode de l'horloge interne fut modifiée, les plantes cultivées en photopériode LJ sur 30 h ont montrées une réponse semblable à celle observée à CJ 24 h. Nos données ont indiquées que la longueur du jour n'est pas le paramètre responsable du contrôle de l'immunité chez les plantes; ce serait la capacité de la lumière à entrainer l'horloge biologique qui serait importante. Pris ensemble, nos données supportent fortement un rôle pour l'horloge interne dans la régularisation de la défense chez les plantes, et indiquent comment l'activité de l'horloge interne module la réponse de défense à *B. cinerea*.

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List of Abbreviations

B. cinerea	*Botrytis cinerea*
CAB	CHLOROPHYLL A/B BINDING PROTEIN
CBS	CCA1-binding site
CCA1	*CIRCADIAN CLOCK ASSOCIATED 1*
CKB	*CASEIN KINASE II BETA SUBUNIT*
CRY	Cryptochrome
DAMPs	Damage-associated molecular patterns
EE	Evening Element
ELF3/4	*EARLY FLOWERING 3/4*
ET	Ethylene
ETS	Effector-triggered susceptibility
GLK	*Golden 2-like*
GI	*GIGANTEA*
hpi	Hour post-infection
HR	Hypersensitive response
JA	Jasmonates
LHY	*LATE ELONGATED HYPOCOTYL*
LD	Long Day
LL	Constant light
LUX	*LUX ARRYTHMO*
PAMPs	Pathogens associated molecular patterns
µl	Microliter
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PHY	Phytochrome
PRR	*PSEUDO-RESPONSE REGULATORS*
PRRs	Pattern recognition receptors
PTI	PAMP-triggered immunity
SA	Salicylic acid
SD	Short Day
TOC1	*TIMING OF CAB EXPRESSION*
ZT	Zeitgeber time
ZTL	*ZEITLUPE*

CHAPTER 1

1. INTRODUCTION

1.1. Circadian clock

1.1.1 Overview of the circadian clock

The name ‘circadian’ is derived from a Latin word that means “about a day” (Doherty & Kay, 2010). The circadian clock can be described as an internal time-keeping mechanism or oscillator, which possesses an approximate 24-hour period (Harmer, 2009). Most organisms possess a circadian clock (Schultz & Kay, 2003). This includes organisms such as bacteria and fungi as well as plants and animals (Young & Kay, 2001). The circadian clock regulates expression of a large percentage of genes. Michael and McClung (2003) found that in *A. thaliana,* 36% of the genome is controlled by the circadian system. This circadian regulation occurs in all species but has been extensively studied in mice, *Drosophila* (Ceriani et al., 2002), *Neurospora* (Nowrousian, Duffield, Loros, & Dunlap, 2003)*, Arabidopsis thaliana* (Salome & McClung, 2004) and rice (Filichkin et al., 2010).

Plants undergo endogenous rhythms in the presence or absence of environmental signals in tandem with the 24-hour rotation of the Earth. These have been named circadian rhythms (McClung, 2001). The circadian rhythms are the oscillation of metabolic, physiological and behavioural processes (Nakamichi, 2011). The first observation of circadian rhythms was observed by French astronomer De Mairan in 1729 (McClung, 2006). De Mairan noticed leaf movement in heliotrope plants and tested his theory by transferring the plants to dark conditions. De Mairan observed that the leaves maintained movement despite the absence of a light signal (McClung, 2006). Similarly, pigment of insects

(Harzsch, Dircksen, & Beltz, 2009) and daily activity in rats (Richter, 1922) were also ascribed to circadian rhythms.

Circadian rhythms also exist at the cellular and organismal level and maintain time regardless of the climate (Harmer, 2009). Circadian rhythms can be described mathematically as sinusoidal curves. These rhythms are depicted using mathematical terms such as period, phase and amplitude (See **Figure 1**) (McClung, 2006). Mutations in clock (oscillator) genes affect the period length of the clock under constant conditions (free-running conditions). This means that these genes are necessary to sustain the timing of the clock and that they are thus part of the clock itself, or serve as clock regulators (Schaffer et al., 2001).

1.1.2. Circadian system

The circadian system regulates several aspects of plant development such as flowering time, hypocotyl elongation (Schultz & Kay, 2003), seed germination, leaf movement, cellular processes, stomatal opening, photosynthesis, carbon fixation and stress response (Yakir, Hilman, Harir, & Green, 2007). The circadian system contains three conceptual components: the input (entrainment) pathways (light & temperature), the central oscillator (clock), and the output pathways (**Figure 2**) **(**Somers, 1999).

1.1.2.1. Input pathways: light and temperature

The circadian system contains two input pathways: light and temperature. The clock can be reset by changing of these two variables. The input pathways convey environmental information, which entrains the central oscillator (clock). In nature, over the day/night cycle, there are variations in temperature, light quality and light quantity.

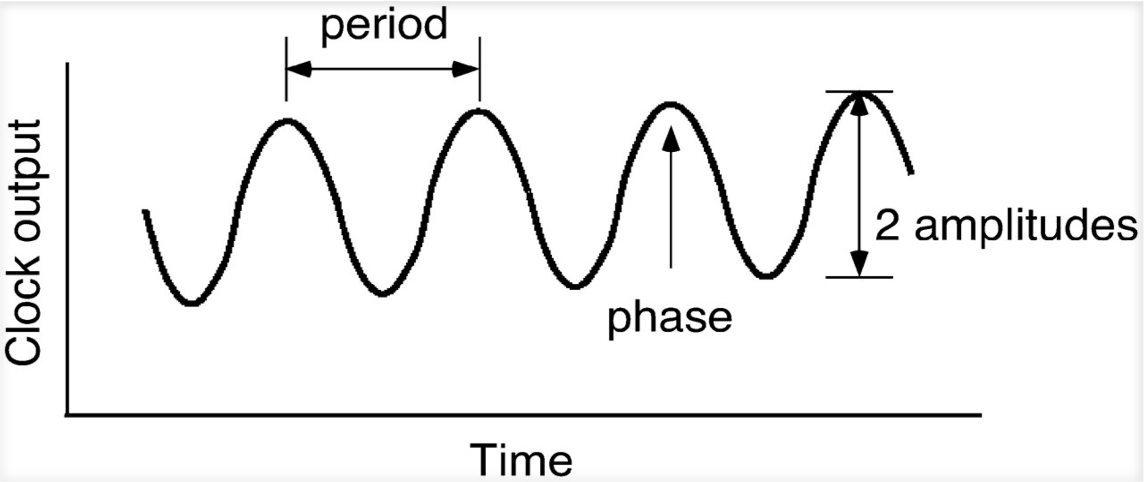


Figure 1. Description of circadian rhythms. Period represents the length of one cycle. Phase represents the peaked or troughed circadian rhythm oscillation. Amplitude represents half of the difference from peak to trough (Modified from McClung, 2006).

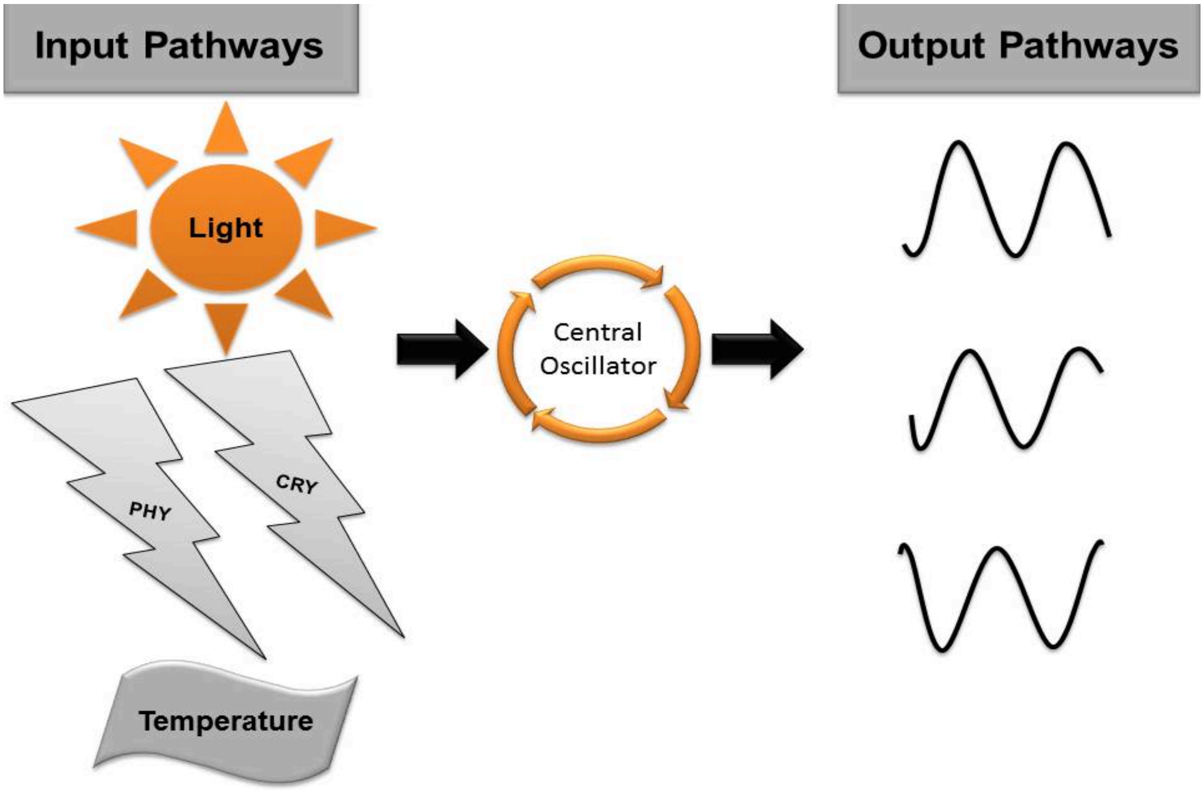


Figure 2. Model of a simple circadian system in *Arabidopsis thaliana*. The three conceptual parts include: input pathways, the central oscillator and output pathways. The input pathways include light and temperature. The input pathways then entrain the central oscillator. Light is mediated through plant photoreceptors *phytochromes* (*PHY*) and *cryptochromes* (*CRY*). The central oscillator contains a subset of proteins forming multiple feedback loops, controlling each other and generating a period of approximately 24h (refer to **Figure 3** for more detail). The output pathways contain several overt rhythms such as leaf movement, flowering time, stomatal opening, seed germination, nutrient uptake, and defense against pathogens (Modified from Somers, 1999).

In order to be in synchronicity with environmental rhythms, the endogenous timekeeping mechanism of the plant entrains the environmental information into the central oscillator, which keeps the circadian clock at approximately 24hrs (Millar, 2004). Entrainment is the process that regulates the period and phase of the circadian clock during the light-dark cycle. Therefore, entrainment of the circadian clock is an essential requirement in order to match the local environment. The circadian clock has the capacity to entrain the phase and period of the circadian system to match the phase and period of environmental changes (Doherty & Kay, 2010). What happens if the circadian clock is delayed or advanced? When the circadian clock is delayed, biological processes will occur later on and entrainment must advance the phase of the clock. In contrast, when the circadian clock is advanced, biological processes will occur too early and entrainment must delay the phase of the clock (Millar, 2004).

Long days in the summer and short days in the fall, winter and spring (identified as day length) provide the timing of the year. The Bünning hypothesis states that the circadian clock cycle of plants measures the day length via photoperiodic reactions (Bünning, 1960). The role of the circadian clock depends on light pulse and day length that is based on the Bünning hypothesis (Bünning, 1960). Light pulse doses not change the circadian rhythms during the day, but shifts them at night. Therefore, day length depends on two phases, photophilic (requiring abundant light) and skotophilic (thriving in darkness) (Bünning, 1960).

Light is critical for life on earth. For plants, light is essential for seed germination and is involved in the regulation of flowering time (Griebel & Zeier, 2008). Fifteen percent of all transcripts are light regulated (Fankhauser & Staiger, 2002). Plants have evolved using two photoreceptors which are capable of detecting light in different fluency rates and wavelengths (Mcwatters & Devlin, 2011). The plant photoreceptors are classified as phytochromes *(PHY*) and cryptochromes (*CRY*). Phytochromes absorb the red and far-red region of the spectrum and cryptochromes absorb blue light (Devlin & Kay, 2001).

Phytochromes are chromoproteins that contain linear tetrapyrrole chromophore (Quail, 2002). There are five phytochrome genes: *PHYA*, *PHYB, PHYC, PHYD* and *PHYE* (Clack, Mathews, & Sharrock, 1994). *PHYA* functions under low-fluence red and blue light, whereas *PHYB-D-E* functions at higher fluence rates of red light (Somers, Devlin, & Kay 1998). There are two cryptochrome genes: *CRY1* and *CRY2* (Devlin & Kay, 1999). *PHY* and *CRY* are sensory and respond to light signals that control clock components to reach entrainment in plants. The phytochromes *(PHY)* contain two forms. The Pr form absorbs red light and the Pfr form absorbs the far-red region of the light spectrum (Smith, 2000). Pr is biologically inactive and inhibits flowering whereas Pfr is biologically active and promotes flowering (Levy & Dean, 1998). The inactive Pr phytochromes exist in the cytosol and absorb red light. Once absorbed, Pr converts to the active Pfr form and moves from the cytosol to the nucleus where it absorbs far-red light.

The cryptochromes are flavin adenine dinucleotide (FAD) and pterinontaining chromoproteins (Mcwatters & Devlin, 2011). Cryptochromes are found in the nucleus of *Arabidopsis* plants in the light as well as the dark (Cashmore, Jarillo, Wu, & Liu, 1999; Kleiner, Kircher, Harter, & Batschauer, 1999). *CRY2* is responsive to low fluence rates of blue light, while *CRY1* is responsive to higher fluence rates of blue light (Lin et al., 1998). Studies have shown that a loss of function in specific phytochromes leads to period lengthening in red light, whereas a loss of function in cryptochromes leads to period lengthening in blue light under constant light (Devlin et al., 1999; Somers et al., 1998). This suggests that both photoreceptors are part of the light input components rather than the central oscillator.

How do oscillator (clock) components respond to light input in *Arabidopsis*? There are multiple genes located in the oscillator (clock). The oscillator contains morning genes (*CCA1* / *LHY*), evening gene (*TOC1)* and additional genes (*PRRs, ELF3-4, GI* and *LUX).* These genes modulate each other in multiple feedback loops (we will discuss them in more detail below). Some clock components are influenced by light. For example, the expression of the morning genes *CCA1* and *LHY* is influenced by the phytochrome input. Once the red light activates the Pfr form of PHYB, PHYB moves to the nucleus. PHYB then interacts with a transcription factor called PHYTOCHROME-INTERACTING FACTOR (PIF3), containing a basic helix-loop-helix (bHLH) motif (Ni, Tepperman, & Quail, 1998). PIF3 binds to the G box DNA sequence (CACGTG) found in various light-regulated promoters (Martínez-García, Huq, & Quail, 2000; Quail, 2000). These interactions between PIF3- PhyB then cause the activation of the expression of *CCA1* and *LHY* (Martínez-García, Huq, & Quail, 2000). MYB-related transcription factors CCA1 and LHY bind to their binding sites: CCA1-binding site (CBS*)*. CCA1 and LHY induce the expression of *CAB*, which is part of a photosynthesis related output pathway and peaks in the morning. CCA1 and LHY possibly repress glycine-rich *RNA-binding* protein *7, AtGRP7/CCR2 COLD CIRCADIAN RHYTHM 2* that peaks in the evening, and inhibit expression of *TOC1* (Fankhauser & Staiger, 2002). TOC1 in turn acts as positive regulator of *CCA1*, resulting in a negative-feedback loop within the central oscillator involving *CCA1/LHY* and *TOC1* (Alabadí et al., 2001; Quail, 2002).

ELONGATED HYPOCOTYL 5 (HY5) is a transcription factor with light signaling components. The HY5 protein has multiple roles including metabolism, energy, transcription, cellular transport, biogenesis of cellular components, subcellular localization, photosynthesis flowering time regulation and circadian rhythm regulation (Lee et al., 2007). Mutation of *HY5* inhibits hypocotyl elongation growth in all light conditions. This suggests that HY5 acts downstream of both phytochromes and cryptochromes (Ang et al., 1998; Koornneef, Rolff, & Spruit, 1980; Oyama, Shimura, & Okada, 1997; Ulm et al., 2004). HY5 also affects the expression of some circadian regulator genes such as *CCA1*, *LHY, TOC1* and *GI.* HY5 binds to the promoter of morning oscillator genes (*CCA1*/*LHY*) and evening oscillator genes (*TOC1* and *ELF4*). This suggests that HY5 binding is involved in the regulation of circadian rhythms (Lee et al., 2007).

There is evidence that the circadian clock controls the expression of phytochrome and cryptochrome genes in *Arabidopsis*. Under constant light, the expression of *PHYB*, *PHYC*, *PHYD*, *PHYE* and *CRY1* genes peak during the early hours of the day while *PHYA* and *CRY2* peak closer to dusk (Tóth et al., 2001).

Temperature is another environmental signal that resets the circadian clock, although the mechanism is less well understood (McClung, 2006). The clock genes *CCA1*, *LHY*, *TOC1* and *GI* are induced by temperature, although details of this process are unknown. These components alter amplitudes in different temperatures (Gould et al., 2006). As the temperature increases (from 17 to 27℃) so do the amplitude and peak levels of *TOC1* and *GI* RNA rhythms (Gould et al., 2006). For *CCA1* and *LHY* RNA rhythms, the amplitude and peak levels increase as the temperature decreases (from 17 to 12℃). In addition, the overt period length of *lhy* mutants is shorter than those of *cca1* mutants at high temperatures. The opposite occurs at low temperatures (Gould et al., 2006). This finding supports the role of *CCA1* and *LHY* in temperature response. The level of *LHY* mRNA decreases at higher temperatures, whereas the level of *CCA1* mRNA increases at lower temperatures (Gould et al., 2006). Furthermore, study has demonstrated that the *prr7 prr9* double mutant does not respond to temperature entrainment cycles. For example, the *prr7 prr9* double mutant fails to entrain to temperature cycles and exhibits long periods that unable entrainment to a 24h period, indicating that *PRR7* and *PRR9* play an important role in temperature response (Salome & McClung, 2005).

1.1.2.2. Central oscillator

The clock (central oscillator) generates a 24-hour rhythm, which regulates certain physiological processes of plants occurring at the optimal phase of the light–dark cycle (Fankhauser & Staiger, 2002). The central oscillator is the core of the circadian system and consists of multiple rhythmic behaviors controlling multiple physiological processes (Más, 2005). Although the clock occasionally stops functioning in constant light in *Drosophila* and *Neurospora* (Young & Kay, 2001), it still functions in plant oscillators under constant light or darkness (Strayer et al., 2000).

1.1.3. The components of the central oscillator in the circadian clock

The core of the central oscillator consists of three genes: *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*) and *TIMING OF CAB EXPRESSION 1* (*TOC1*) (Alabadí et al., 2001). *CCA1* and *LHY* are MYB- related transcription factors that contain similar DNA-binding domains (Dubos et al., 2010), a single MYB repeat of (R1/R2) types (Schaffer et al., 1998; Wang et al., 1997). MYB transcription factors comprise a large family of proteins and have become ubiquitous in all eukaryotes. Most MYB proteins contain a diverse number of MYB domain repeats (R) that are able to bind DNA (Dubos et al., 2010). Research has shown that single mutants of either *cca1* or *lhy* have a short period (Alabadí, Yanovsky, Más, Harmer, & Kay, 2002; Green & Tobin, 1999;

Mizoguchi et al., 2002) and that *cca1 lhy* double mutants show arrhythmic behavior and are unable to maintain the rhythms of the clock (Alabadí et al., 2002; Mizoguchi et al., 2002). This suggests that *CCA1* and *LHY* are functionally redundant (Lu, Knowles, Andronis, Ong, & Tobin, 2009). Studies have also demonstrated that overexpression of *CCA1/ LHY* results in long hypocotyl length, late flowering and loss of clock rhythms under constant light conditions (Schaffer et al., 1998; Wang & Tobin, 1998). Furthermore, CCA1 and LHY both bind to the same region of the promoter of light-harvesting chlorophyll a/b protein (*Lhcb)* (also called *CAB* (*CHLOROPHYLL A/B BINDING PROTEIN*) (Lu et al., 2009). Lu et al. (2009) also demonstrated that CCA1 and LHY are located in the nucleus and can form homodimer and heterodimer *in vitro* and *in vivo*. Homodimerization is essential for the control of clock function in other circadian systems (Dunlap, 1999).

CCA1/ LHY are expressed in the morning around dawn and are thus called dawn-phased genes or morning genes (Schaffer et al., 1998; Wang & Tobin, 1998). *CCA1/ LHY* promoters contain the CCA1-binding site (CBS) -AA(A/C)AATCT- and the expression of these genes peaks at mid-day (Wang et al., 1997). There is also an evening element (EE) -the nonamer AAAATATCT. EE is found in the promoter of dusk-phased genes or evening genes such as *TOC1*, *GI* and *LUX* (Harmer, 2009); their synthesis peaks at the end of the day. EE may play an essential role in the transcriptional loops of the central oscillator. The dawn-phased CCA1/LHY proteins bind to the EE in the *TOC1* promoter leading to the repression of the *TOC1* gene (Alabadí et al., 2001).

The *PSEUDO-RESPONSE REGULATOR (PRR)* family includes the following five genes: *PRR9*, *PRR7*, *PRR5*, *PRR3* and *PRR1* or *TOC1* (Matsushika, Makino, Kojima, & Mizuno, 2000). Members of the *Arabidopsis PRR* gene family contain a pseudo-receiver domain (PR) in the N terminus and a CCT domain in the C terminus, and these are present only in CONSTANS (CO), CO-like, and TOC1 proteins (Makino et al., 2000; Strayer et al., 2000). The *PRR* family genes lack the phosphor-accepting Aspartate (Asp) residue in the pseudo-receiver domain and thus, it is unlikely that these genes participate in the conventional His-to-Asp phosphorelay signal transduction pathway involved in cellular responses to environmental stimuli (Appleby, Parkinson, & Bourret, 1996; Makino et al., 2000; Strayer et al., 2000). The C terminus consists of a short motif containing 50 amino acids and appears to be responsible for nuclear localization (Makino et al., 2000). The C terminus also plays an essential role in DNA binding activity (Makino et al., 2000; Strayer et al., 2000). The *Arabidopsis PRR* gene family is expressed from early day until midnight (Makino et al., 2000). *TOC1* is expressed during the evening and its promoter contains an evening element (EE) (Stayer et al., 2000). TOC1 functions as a transcriptional regulator that binds DNA, which controls growth and development (Gendron et al., 2012). It is notable that the period of the *TOC1* circadian rhythm was shortened in the *toc1-1* mutant, demonstrating that TOC1 protein feeds back and controls its own expression (Strayer et al., 2000).

As mentioned above, *Arabidopsis* has a small family of *PRRs (PRR9, PRR7, PRR5, PRR3* and *PRR1* or *TOC1)*. All members of the *PRR* family show circadian rhythms at the level of transcription and are involved in the control of the circadian clock as a part of the central oscillator (Matsushika et al., 2000). The transcript accumulation of each *PRR* gene, in the order PRR9 *-PRR7 -PRR5 -PRR3*, peaks from shortly after dawn to approximately dusk (Matsushika et al., 2000).

Loss of function in any of the *PRR* genes changes circadian period phenotypes: *prr3* and *prr5* mutations have short periods, whereas *prr7* and *prr9* mutations have long periods (Eriksson, Hanano, Southern, Hall, & Millar, 2003; Yamamoto et al., 2003). In addition, studies have demonstrated that either single *prr7* or *prr9* mutants display a similar period lengthening of the clock and the double *prr7 prr9* mutant show extreme period lengthening, indicating that *PRR7* and *PRR9* genes are partially redundant within the clock mechanism (Farré et al., 2005; Nakamichi et al., 2005; Salome & McClung, 2005).

Three additional genes are also involved in the central oscillator system: *EARLY FLOWERING 3*(*ELF3*), *ELF4* and *LUX ARRYTHMO* (*LUX*). All three genes are known as evening genes and play an important role in controlling plant growth (hypocotyl growth) (Nusinow et al., 2011; Onai & Ishiura, 2005; Zagotta et al., 1996).

The ELF3 protein contains 695 amino acids and is rich in serine, proline and glutamine (Hicks et al., 1996). The ELF4 protein contains 111 amino acids (Doyle et al., 2002). The expression of the *ELF3* and *ELF4* genes peaks in the early night (Nusinow et al., 2011). Research has shown that mutations in these two proteins result in a decrease of the expression of *CCA1* and *LHY*. This would suggest that these genes are essential for *CCA1/LHY* expression (Doyle et al., 2002; Onai & Ishiura, 2005). The *ELF3/4* genes are also essential for the maintenance of circadian rhythms under constant light conditions (Doyle et al., 2002; Hazen et al., 2005; Hicks et al., 1996).

LUX, also known as PHYTOCLOCK 1-PCL1, is a GARP family transcription factor, a single-MYB domain in *A. thaliana*. It contains a single DNA-binding domain (type SHAQKYF), which is unique to plants (Nusinow et al., 2011; Hazen et al., 2005). The domain SHAQKYF has also been found in the morning genes *CCA1*/*LHY* and mediates binding to the evening element motif in the *TOC1* promoter, leading to the suppression of *TOC1* expression (Alabadí et al., 2001; Harmer et al., 2000). The *LUX* gene contains one evening element, thus expression peaks at dusk (Hazen et al., 2005). Mutations of the *LUX* gene cause arrhythmia in outputs such as hypocotyl elongation and flowering time (Nusinow et al., 2011).

The *GIGANTEA* (*GI*) complex acts as a light sensor (Kim et al., 2007). The transcript accumulation of *GI* oscillates in a circadian pattern, peaking in the late afternoon. *GI* controls the period length of the circadian clock. For example, the mutant *GI* has a short period at continuous light (Park et al., 1999).

The ZEITLUPE (ZTL) encodes a 609 amino acid polypeptide with an amino terminal PAS domain, an F-box and six Carboxy-terminal kelch repeats (Somers, Schultz, Milnamow, & Kay, 2000). The F-box protein has a light-regulated protein interaction domain called the LOV (Light, Oxygen, or Voltage) at its N terminus (Kim et al., 2007). Loss of function in any of the *ZTL* genes lengthens the circadian period (Baudry et al., 2010). *ZTL* is involved in the post-translational regulation of clock proteins. We will describe the function of this complex in more detail below (post-translation regulation).

1.2. Regulatory mechanisms of the central oscillator (clock)

There are many different mechanisms involved in the regulation of the central oscillator genes. These include transcriptional feedback loops, post-transcriptional regulation and post-translational regulation.

1.2.1. Transcriptional feedback loops

There is solid evidence that the core of the oscillator consists of negative and positive elements that form an auto-regulatory process controlling transcription, known as a feedback loop. The negative element of the loop increases its activity and the positive element inhibits its activity. In other words, physical interaction with the positive element leads to the activation of the transcription of the negative element, allowing the clock system to function. The circadian oscillator in *Arabidopsis* contains three interlocked feedback loops based on transcriptional regulations. These feedback loops are: the core loop, the morning loop and the evening loop. As seen in Figure 3, the core feedback loop is connected to the morning and evening loops (Pruneda-Paz & Kay, 2010).

The core feedback loop contains three components: two morning-phased Myb-related transcription factors *CCA1* and *LHY*, and the evening-phased and clock-regulated *TOC1* (Harmer, 2009). During the day, in the first loop (the core loop), the *CCA1* and *LHY* proteins bind to the *TOC1* promoter and inhibit the expression of *TOC1*. Thus, *CCA1* and *LHY* function as negative regulators (elements) of *TOC1*. Shortly before the evening, the levels of *CCA1* and *LHY* decrease and *TOC1* expression increases. At the end of the night, *TOC1* induces the expression of *CCA1* and *LHY*. *TOC1* thus functions as a positive regulator (element) of *CCA1* and *LHY* (Alabadí et al., 2001). There is evidence that *CCA1* and *LHY* in plants play roles similar to *PERIOD* (*PER*) and *TIMELESS* (*TIM*) respectively in *Drosophila* and *FREQUENCY* (*FRQ*) in *Neurospora* (Loros & Dunlap, 2001; McClung, Fox, Dunlap, 1989; Young & Kay, 2001). There is also evidence that *TOC1* in plants plays a similar role as *CLOCK* (*CLK*) and *CYCLE* (*CYC*) in *Drosophila* and *WHITE COLLAR 1* and 2 (*WC1* and *WC2*) in *Neurospora* (Loros & Dunlap, 2001; Young, 1998: Young & Kay, 2001).

Researchers have recently discovered that CHE, a *TCP* (*TB1, CYC, PCFs*) transcription factor, binds to the *CCA1* promoter (Pruneda-Paz, Breton, Para, & Kay, 2009). *CHE* is a clock component and is redundant with *LHY,* causing repression of *CCA1*. *CHE* and *CCA1* inhibit each other's expression, leading to the addition of a *CCA1/CHE* feedback loop to the *Arabidopsis* circadian network. In addition, the interaction of TOC1 and CHE proteins leads to binding to the *CCA1* promoter, which may prevent the repression of *CCA1* by the end of the night. *CHE* is thus a part of the *TOC1* regulation of *CCA1* expression (Pruneda-Paz et al., 2009).

In the second loop (the morning loop), *CCA1* and *LHY* increase the expression of two *TOC1*-related genes (*PRR7* and *PRR9*). These two genes repress the expression of *CCA1* and *LHY* in order to complete the second loop (Farré et al., 2005).

In the third loop (the evening loop), *GI* and *PPR5* function as positive regulators of *TOC1*, and *TOC1* inhibits *GI* and *PRR5* (Locke et al., 2005). Although the *CCA1/LHY/TOC1* transcription feedback loop is an important mechanism of the *Arabidopsis* oscillator, post-transcriptional and translational modifications are required to generate a day long cycle in the oscillator proteins as seen in Figure 3 (Pruned-paz & Kay, 2010).

1.2.2. Post-transcriptional regulation

It is a well-established fact that light and temperature are the major input pathways entraining biological clocks (Millar, 2004). Recent research suggests that the entrainment of biological clocks using temperature may be related to pre-mRNA splicing and alternative splicing of clock genes (James et al., 2012; Seo et al., 2012). Splicing is a mechanism that removes non-coding sequences (introns) and ligates coding sequences (exons) in most eukaryotic genes (Reddy, 2007). This process occurs through spliceosome, a multi-megadalton ribonucleoprotein (RNP) complex (Reddy, 2007). In alternative splicing, different combinations of exons are ligated together to generate multiple mRNA from a single gene in different selections of splice sites (alternative 5′and 3′ splice sites) (Reddy, 2007). There are multiple types of alternative splicing events in *Arabidopsis* including: exon skipping (ES), alternative 3′ splice site (3′ SS) and splice site 5′ (5′ SS) selection and Intron retention (IR). Exon skipping: exon can be removed in the mRNA. Alternative 3′ and 5′ splice site selection: splicing takes place in the intron 3′ SS or 5′ SS site. Intron retention: an intron can be retained in the mRNA (Reddy, 2007). IR is the most prevalent type of alternative splicing in plants (Barbazuk, Fu, & McGinnis, 2008). Alternative splicing in plants plays an essential role in modulating gene expression, plant form and plant function at the post-transcriptional level (Reddy, 2007).

Over the past decade, the alternative splicing of plant genes has been estimated at only 1.2%. This number is increased to over 61 % in *Arabidopsis* intron-containing genes (Marquez, Brown, Simpson, Barta, & Kalyna, 2012). This process is essential to increasing transcriptome plasticity and protein diversity in eukaryotes (Reddy, 2007). The regulation of clock gene expression using alternative splicing is not well established. Currently, the literature indicates that temperature regulates the alternative splicing of clock genes (Seo et al., 2012). Research has shown that alternative pre-mRNA splicing changes the function of *CCA1's* (Seo et al., 2012). The two CCA1 isoforms CCA1α and CCA1β are alternatively spliced versions (Seo et al., 2012). The *CCA1β* transcript is generated by the retention of the fourth intron. Although both CCA1 isoforms have the same dimerization domain, CCA1β lacks N-terminal MYB DNA binding (Seo et al., 2012). The same studies have shown that the alternative splicing of CCA1 is inhibited by cold temperature, inhibiting CCA1β production and reducing CCA1α activity. The end result is the suppression of clock function. Research also shows that the level of *CCA1α* transcript is increased under cold temperature, resulting in enhanced freezing tolerance. This data suggests that the *CCA1* gene plays a role in freezing tolerance (Seo et al., 2012). Furthermore, there is accumulating evidence that temperature affects the alternative splicing of *Arabidopsis* oscillator genes (*CCA1*, *LHY*, *TOC1*, *PRR3*, 5, 7, and 9), resulting in differentially spliced transcripts of these genes (James et al., 2012). Together, the data suggests that temperature controls the alternative splicing of clock genes at the post-transcriptional level.

1.2.3. Post-translational regulation

1.2.3.1. Ubiquitination and Degradation

Ubiquitination is part of the post-translational process and is a major mechanism of the modulation of protein stability leading to protein degradation (Moon, Parry, & Estelle, 2004). Protein degradation is mediated by ubiquitin, a small protein (about 76 residues) found in all eukaryotes. Ubiquitin is also important to plant lifecycle regulation. Ubiquitination is a complex process involving three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-ligase (E3). These enzymes allow the attachment of ubiquitin in a substrate protein (Sadanandom, Bailey, Ewan, Lee, & Nelis, 2012).

The E3s are the most varied proteins in the ubiquitination cascade. There are seven types of E3 ligases: Skp1-Cullin-F-box (SCF), VHL-ELONGIN-CUL2/5, Cullin 3 (CUL3)-Bric a brac, Tramtrack and Broad complex/Pox virus and Zinc finger (BTB/POZ), UV-Damaged DNA-Binding protein 1 (CUL4-DDB1) and Anaphase Promoting Complex (APC) (Sadanandom et al., 2012). The F-box protein family has more diversity in plants than any other eukaryotes. Over 700 of these proteins are encoded in the *Arabidopsis* genome (Gagne, Downes, Shiu, Durski, & Vierstra, 2002).

Researchers have recently discovered two *E3* ligases and three F-box proteins that promote the ubiquitination and degradation of some central oscillator components (Sadanandom et al., 2012). The E3s include the *CONSTITUTIVE PHOTOMORPHOGENESIS1* (*COP1*) and *SINAT5*, and the F-box proteins include the *ZEITLUPE (ZTL)*, *FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1)*, and *LOV KELCH PROTEIN2* (*LKP2*) genes (Baudry et al., 2010; Park et al., 2010; Yu et al., 2008).

The three F-box proteins contain three specific domains: the PAS domain, an F-box domain as a component of the SKP-Cullin-Rbx-F-box (SCF) complex, and a Kelch domain (Ito, Song, & Imaizumi, 2012). ZTL is the first component of the ubiquitin proteasome system (UPS). There is also evidence that there are physical interactions between *TOC1* and *ZTL* (Más, Kim, Somers, & Kay, 2003). For example, the *ztl-1* mutation eliminates the physical interaction between *TOC1* and *ZTL* and results in a constitutive level of TOC1 protein expression (Más et al., 2003). These findings suggest that the interaction between *ZTL* and *TOC1* is essential in the regulation of *TOC1* stability (Más et al., 2003). In addition, *TOC1* or its homolog (*PRR5*) interacts with the F-box protein in SCF^{ZTL} they are targeted for proteasomal degradation (Fujiwara et al., 2008).

Studies have also demonstrated that the stability of the ZTL protein is regulated by the GI protein through a post-translational mechanism (Kim et al., 2007). Among the *GI* mutations*,* the *GI-OX* plant increases the ZTL protein level whereas the *GI* plant decreases the ZTL protein level (Kim et al., 2007). In addition, the *LHY* interacts with the E3 ligase DE-ETIOLATED1 (DET1) in the proteasomal degradation pathway (Song & Carre, 2005). The *lhy-1 det1-1* mutant accelerates the degradation of the LHY protein to approximately 7 minutes while the wild type degrades in approximately 25 minutes (Song & Carre, 2005). This information suggests that the wild-type DET1 protein functions as a repressor of LHY protein degradation (See **Figure 3**) (Song & Carre, 2005).

1.2.3.2. Phosphorylation

Phosphorylation is the most common mechanism in post-translational modification. The phosphorylation process involves the addition of a phosphate group by a protein kinase to specific amino acid residues such as serine, threonine, tyrosine and histidine (Hodges et al., 2013). In general, the phosphorylation cycle regulates protein activity, protein localization, protein interaction and protein stability (Hodges et al., 2013). This regulatory modification is essential for plants, as they must quickly adjust to environmental changes. Phosphorylation is required in the circadian oscillator components of *Arabidopsis*. Here, the phosphorylation cycle controls protein stability, protein–protein interactions and protein–DNA interactions (Kusakina & Dodd, 2012). CK2 is a serine-threonine (SerThr) protein kinase containing two catalytic (α) subunits and two regulatory (β) subunits in all eukaryotes (Daniel, Sugano, & Tobin, 2004). In the core oscillator component, CK2 is necessary for the formation of DNA-protein complexes such as CCA1 (Sugano, Andronis, Green, Wang, & Tobin, 1998).

In *Arabidopsis* there are four ɑ-subunits (A1–A4) and four β-subunits (B1–B4) (Salinas et al., 2006). CCA1 is phosphorylated by the protein kinase CK2 and the α -subunits of CK2 control CCA1 degradation (Sugano et al., 1998). For example, in *ckα1α2α3* triple mutant plants, the levels of *CK2* kinase are impaired and CCA1 phosphorylation is reduced, leading to increased CCA1 protein stability (Lu et al., 2011). The β -subunits of CK2 also plays an important role in circadian function. For example, the over-expression of *CASEIN KINASE II BETA SUBUNIT3* (*CKB3)* shortens the period of *CCA1* and *LHY* mRNAs and output genes (Daniel, Sugano, & Tobin, 2004). There is also evidence that the over-expression of *CKB4* leads to reduced binding of *CCA1* to the promoters of morning genes (*PRR7*, *PRR9*) and evening genes (*TOC1* and *LUX*) (Portolés & Más, 2010). This indicates that the phosphorylation of CCA1 by CK2 is essential to the normal functioning of the circadian clock in *Arabidopsis*.

Phosphorylation also regulates other components of the *Arabidopsis* circadian oscillator (Fujiwara et al., 2008). For example, the phosphorylation of TOC1 and PRR3 controls the interaction of TOC1–PRR3 resulting in the stabilization of the TOC1 protein (Fujiwara et al., 2008). PRR3 also inhibits the *TOC1/ZTL* interaction. The level of *TOC1* decreases in the absence of *PRR3* and increases in the presence of *PRR3* (Fujiwara et al., 2008). This information suggests that *PRR3* might delay or interfere with the TOC1/ZTL interaction. *PRR5* is the most highly phosphorylated of the PRR genes. *PRR5* and *TOC1* interact with *ZTL, which* then alters their proteolysis (See **Figure 3**) (Fujiwara et al., 2008).

In summary, the actions of clock genes are dictated by their particular mechanism (transcriptional, post-transcriptional and post-translational regulations). In the morning, clock proteins CCA1 and LHY inhibit evening-genes such as *LUX*, *TOC1* and *ELF4* at the transcriptional regulation level (Alabadí et al., 2001; Hazen et al., 2005; Li et al. 2011; Perales & Más, 2007). CCA1 and LHY also activate the day-phase genes *PRR9* and *PRR7* (Farré et al., 2005). From early on in the day until midnight, the *PRR9*, *PRR7* and *PRR5* genes repress the *CCA1* and *LHY* morning genes (Nakamich et al., 2009).

At the post-translational level, blue light activates the interaction between ZTL and GI. In the dark, the ZTL–GI complex is separated, allowing ZTL to promote the degradation of PRR5 (David, Armbruster, Tama, & Putterill; 2006; Más et al., 2003).

At the transcriptional regulation level, in the evening, LUX inhibits *PRR9* and *LUX* expression and *ELF4* and *ELF3* repress *PRR9* and *PRR7* (Dixon et al., 2011; Doyle et al., 2002; Hazen et al., 2005; Helfer et al., 2011). At the post-translational regulation level, *PRR5* boosts nuclear localization of TOC1 and PRR3 stabilizes TOC1. At night, ZTL promotes the degradation of PRR5 and TOC1 (Fujiwara et al., 2008). At the transcriptional regulation level, TOC1 activates CCA1 expression by antagonizing CHE on the CCA1 promoter (See **Figure 3**) (Pruneda-Paz et al., 2009).

1.1.2.3. Output pathways

Output pathways represent the different biological processes whose rhythms are controlled by the clock (Más, 2008). The biological processes include leaf movement (Millar et al., 1995), water stress response (Nakamichi et al., 2009), stress response (Kreps et al., 2002), flowering time (Levy & Dean, 1998), photosynthesis and carbon metabolism (Harmer et al., 2000; Schaffer et al., 2001), stomatal opening (Somers et al., 1998), seed germination (Zhong et al., 1998), nutrient uptake (Blaesing et al., 2005) and defense against pathogens (Wang et al., 2011). Output pathways such as flowering, photosynthesis, cold/salt/drought tolerance, hypocotyl growth**,** and nutrient uptake and metabolism are controlled by the following genes: *CCA1*, *LHY*, *TOC1*, *ELF3/4*, *LUX*, and *GI* (Pruneda-Paz & Kay, 2010). In general, *Arabidopsis* flowers earlier in long day (LD) conditions than short day (SD) conditions (Hicks, Albertson, & Wagner, 2001). There is evidence *toc-1* mutation shortens the period of rhythms that influence the photoperiodic regulation of floral induction. *toc-1* flowers earlier in LD than in SD when compared to wild type (Strayer et al., 2000). Mutants of *ElF3* flower earlier in both LD and SD conditions (Zagotta et al., 1996) while mutants of *ElF4* flower earlier in SD conditions than in LD conditions (Doyle et al., 2002). On the other hand, *GI* mutants show delayed flowering under LD, but no difference in SD (Mizoguchi et al., 2002). Furthermore, *prr9-prr7-prr5* triple mutants show cold/salt/drought tolerance, suggesting that *PRR9*, *PRR7* and *PRR5* regulate the tolerance of cold/salt/drought stress responses (Nakamichi et al., 2009).

The circadian oscillator enhances photosynthesis in *TOC1* and *CCA1* mutants (Dodd et al., 2005; Ni et al., 2009). *ELF3, ELF4* and *LUX* genes show abnormal hypocotyl growth under diurnal cycles, suggesting that these genes control the hypocotyl growth in diurnal conditions (Nusinow et al., 2011). Transcriptional regulation of nitrogen assimilation genes is directly controlled by *CCA1* and nitrogen nutrients generate phase shifts in *CCA1* expression (Gutiérrez et al., 2008). Nitrogen regulation of *CCA1* acts as input to influence the circadian clock (Gutiérrez et al., 2008).

Thus, data points to a robust circadian clock mechanism in which input pathways play a key role in clock regulation and output pathways control the functions of the elements in the circadian oscillator. There is a regulatory relationship between the circadian oscillator and the output pathways. If the circadian oscillator components are altered, the output pathways are also altered (Pruneda-Paz & Kay, 2010).

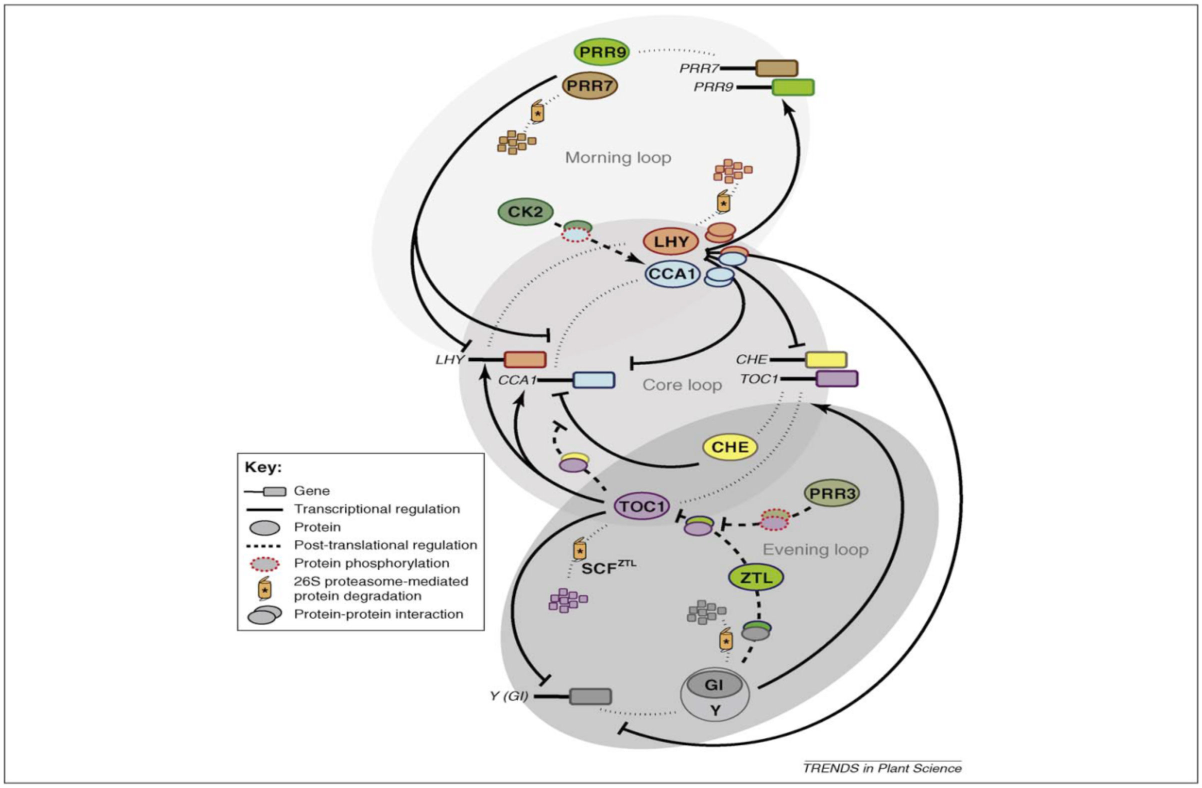


Figure 3. Model representing the circadian clock regulation mechanisms. The central oscillator genes are regulated via transcriptional and post-translational regulation. In transcriptional regulation, the central oscillator is comprised of three interlocked transcriptional feedback loops: the core loop, the morning loop and the evening loop. The core loop involves Myb transcription factors (*CCA1* and *LHY*) that negatively regulate a *PSEUDO-RESPONSE REGULATORS (PRR)* gene (*TOC1*). In the morning loop, *CCA1* and *LHY* activate the expression of *PRR 7/9.* In the evening loop, *TOC1* inhibits an unknown component called Y [GIGANTEA (GI)] that in turn activates the expression of *TOC1*. Post-translational regulation includes phosphorylation and ubiquitination. *CCA1* is phosphorylated by the protein kinase *CK2. TOC1* is degraded by the F-box protein ZEITLUPE (ZTL). Additional details regarding these regulatory mechanisms can be found throughout the text (Figure from Pruneda-Paz & Kay, 2010).

1.3. Overview of plant disease

Disease in plants is defined as a visible or invisible response of plant cells and tissues to a pathogenic or environmental factor that can cause a change in the form, function or integrity of the plant (Agrios, 2005). Three fundamental factors that are required for the development of a disease are a susceptible host, a virulent pathogen and a favorable environment. Disease does not occur if any of these three factors are absent (Agrios, 2005).

1.4. Plant defense mechanisms

Plants are attacked by insect herbivores and microbial pathogens such as fungi, bacteria and viruses (Agrios, 2005). In order to protect themselves from damage, plants have evolved a wide range of defenses including constitutive and inducible defenses (Agrios, 2005). The structural barriers in plant cells are one example of a constitutive defense and include multiple layers such as cuticles, epidermal, wax, pectin and cellulose (Agrios, 2005). This is the first line of defense against a pathogen attack. For the pathogen to enter the plant cell it must breach the structural barriers in the cell wall. Plants possess pattern recognition receptors (PRRs) able to recognize molecular signatures that recognize a broad group of microbes. These molecular signatures are known as pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Boller & Felix, 2009; Mengiste, 2012).

PAMPs are conserved molecules that are present in a broad class of microbes. DAMPs are signals generated by the plants in responding to pathogen damage (Boller & Felix, 2009; Mengiste, 2012). There are several DAMPs including oligogalacturonides (OGAs), cutin monomers, chitin and peptides (Boller & Felix, 2009). OGAs that act as endogenous elicitors have of a variety of defense responses (Boller & Felix, 2009). It is also known that cuticular wax regulates fungal cutinase gene expression, leading to the generation of cutin monomers from the plant cuticle (Boller & Felix, 2009). The fungal cell walls are composed of chitin and peptides (Boller & Felix, 2009).

The plant innate system is consists of two types of induced defenses. These two types are defined as PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones & Dangl, 2006). PTI is the first type of induced defense depending on the PRR-mediated recognition of PAMPs and DAMPs. PTI can prevent further microbial colonization of the plant. Successful pathogens deliver effectors, which suppress PTI, resulting a phenomenon known as effector-triggered susceptibility (ETS) (Jones & Dangl, 2006). The second type of induced defense is ETI. ETI is dependent on direct or indirect interaction of pathogen effectors and the products of plant resistance (R) genes according to the gene-for-gene theory. ETI is stronger response than PTI and is often associated with a hypersensitive response at the infection site (Jones & Dangl, 2006).

Microbial pathogen lifestyles can be divided into two categories: biotrophic and necrotrophic (Glazebrook, 2005). Biotrophs obtain nutrients from living tissue and feed on the host cell while necrotrophs kill the host and obtain nutrients from dead tissue (Glazebrook, 2005). Hemi-biotrophs feed on both living and dead tissue (Glazebrook, 2005). Necrotrophs are further divided into host-specific necrotrophs (HSNs) such as *Cochliobolus carbonum,* and broad host-range necrotrophs (BHNs) such as *Botrytis cinerea*. HSNs generate host specific toxins, which are essential for their pathogenicity and virulence in their hosts. BHNs, specifically *B. cinerea*, generates cell wall degrading enzymes (CWDEs), necrosis and ethylene inducing proteins (NEPs), oxalic acid and other toxins (Boller & Felix, 2009).

1.5. Overview of *Botrytis cinerea*

Botrytis cinerea (teleomorph: Botryotinia fuckeliana) belongs to the phylum Ascomycota, family Sclerotiniaceae and is classified as a necrotrophic fungal pathogen. It is an airborne pathogen known as “grey mould fungus” and a causal agent of grey mould disease (van Kan, 2005). Because of the availability of molecular genetic tools, *B. cinerea* has become the most broadly studied necrotrophic fungal pathogen (Williamson, Tudzynski, Tudzynski, & van Kan, 2007).

1.5.1. Life cycle of *Botrytis cinerea*

The life cycle of *B. cinerea* contains multiple stages. In the overwintering stage, *B. cinerea* survives in the soil and in greenhouses on plant debris in the form of mycelium and sclerotia (Agrios, 2005). While the fungus does not infect the seed, the seed can be contaminated by the sclerotia of the fungus (Agrios, 2005). The sexual development of *B. cinerea* evolves in sclerotia (Schumacher & Tudzynski, 2012; Williamson et al., 2007). Sclerotia germinate in the early spring and produce mycelia. Mycelia then produce conidiophores which have large masses of conidia (spores) (Williamson et al., 2007). The conidia are the primary source of *B. cinerea* infection. The conidia can move via wind or rain-splash to cause new infections in susceptible host tissue. The disease cycle starts with a conidium. The conidium will attach itself to the surface of a leaf or flower petal. Once the conidium breaches the cell tissue via a short germ tube, the infected tissues begin to disintegrate. The tissue becomes soft and rotten, causing grey mould disease. The cycle begins again with the following crop (See **Figure 4**) (Agrios, 2005).

Researchers have discovered that the life cycle of *B. cinerea* B05.10 *in vitro* occurs at a different time of day. The germination of the conidium on the primary leaf (French bean

Phaseolus vulgaris) takes a few hours, while the penetration of the cell tissue takes 24 hours. Two days post-inoculation, the symptoms of the disease appear as small necrotic spot (primary lesions). Four days post-inoculation, the lesions grow to diameters of 25 mm (secondary lesion) (Schumacher & Tudzynski, 2012).

1.5.2. Host range and symptoms

Phylogenetic analysis of the genus *Botrytis* indicates that there are 22 species (Staats, van Baarlen, & van Kan, 2005). *B. cinerea* is a ubiquitous necrotrophic pathogen with an extensive host range, infecting over 200 crop species worldwide (Williamson et al., 2007). The *Botrytis* infection of host plants appears on plant parts at both the pre and post-harvest stages (Williamson et al., 2007). In addition, the infection can occur on bulbs, seeds and other propagation materials (Williamson et al., 2007). *B. cinerea* has the ability to infect several plant organs such as flowers, leaves, fruits (grape, strawberry, raspberry, and blackberry), vegetables (cabbage, lettuce, broccoli, and beans) and orchard crops (Droby & Lichter, 2007). Grey mould disease has a large geophraphical distribution. It has been reported in Argentina, Australia, Bangladesh, Canada, Chile, Colombia, India, Myanmar, Nepal, Pakistan, Spain, Turkey, and the USA. The cost of anti-Botrytis products has recently been estimated at 15-25 million US dollars (Droby & Lichter, 2007). The most typical symptoms of grey mould disease on leaves and soft fruits are soft rot with collapse and water soaking of parenchymal tissues and grey masses of conidia (Droby & Lichter, 2007). The dark water-soaking only appears after cutting thick-skinned fruits such as kiwifruits (Williamson et al., 2007). Hot, wet and humid weather facilitates the spread of the grey mould disease (Williamson et al., 2007).

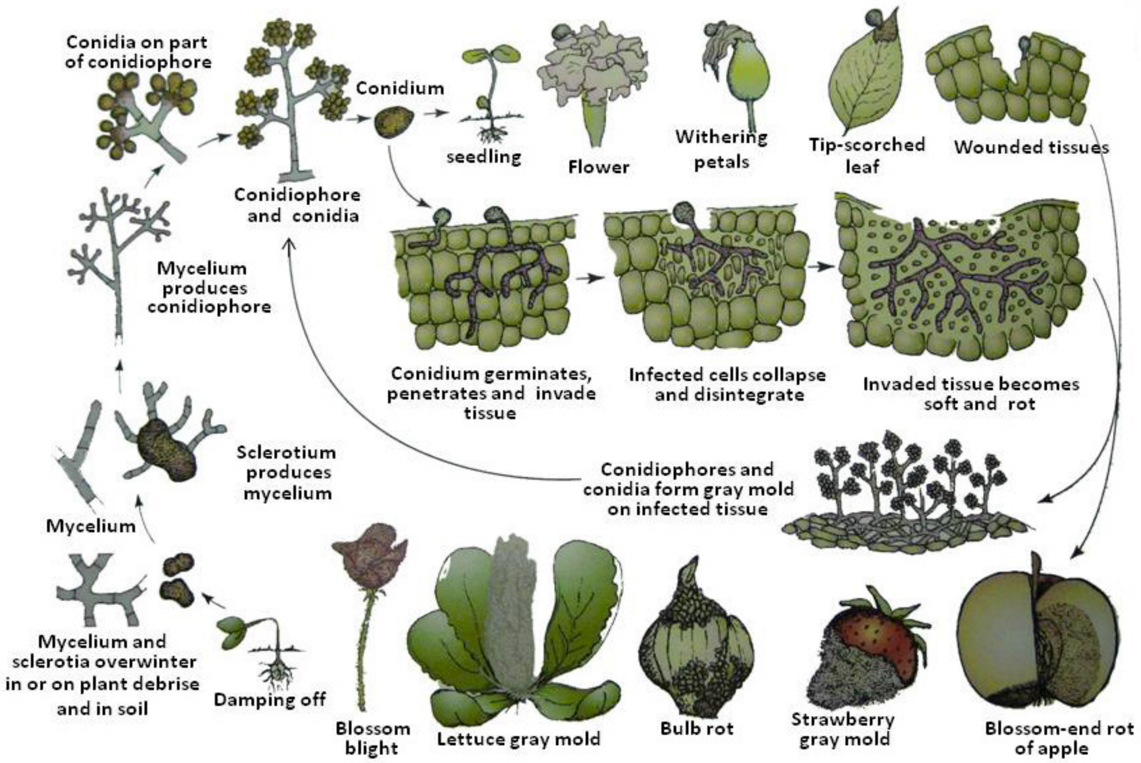


Figure 4. The life cycle of *Botrytis cinerea*, grey mould disease. In the overwintering stage, *B. cinerea* develops sclerotia and mycelia in dead plant tissue and soil. Conidia can then travel via wind or rain-splash. The disease cycle of grey mould begins when a conidium lands on a host tissue. In our work we used conidial suspensions inoculated on *Arabidopsis* leaves and measured the diameter of the lesions from the time the conidium penetrates and infects the tissue (Figure from Agrios, 2005).

1.5.3. Strategies of infection

In general, *B. cinerea* is able to penetrate through wounds, stomata and other natural openings (Gudesblat, Torres, & Vojnov et al., 2009). The *B. cinerea* infection process occurs in stages: penetration of the host tissue, killing of the host tissue/primary lesion formation, and lesion expansion/ tissue maceration and sporulation (van Kan, 2006).

The first stage of *B. cinerea* infection involves the penetration of the plant surface. Once *B. cinerea* (a conidium) lands on the leaf it must penetrate the host surface, which is made up of cutin covered with wax. To do so, *B. cinerea* develops appressoria, a specialized infection structure cell (van Kan, 2006). The *B. cinerea* appressoria excrete the enzymes cutinase and lipase used to penetrate the plant surface. A penetrating peg breaches the host cuticle and produces H_2O_2. As a substrate for oxidases, peroxide may aid in the modification of the cuticle (van Kan, 2006). Upon breaching the host cuticle, a penetrating peg grows into the underlying anticlinal wall, which is rich in pectin. *B. cinerea* then secretes pectinases, a degradation enzyme with breaks down the epidermal layer of the cells in the plant (van Kan, 2006).

The second stage of *B. cinerea* infection involves killing the host tissue using fungal toxins, reactive oxygen species (ROS) and oxidative burst (van Kan, 2006). Killing the host cell requires the contribution of both the pathogen and the host itself (van Kan, 2006). *B. cinerea* can produce botrydial. Botrydial is phytotoxic metabolite of low molecular weight found in infected plant tissue. It is still unanswered whether these phytotoxic metabolites are inducers of programmed cell death rather than toxins causing necrosis (Williamson et al., 2007). *B. cinerea* also generates oxidative burst during cuticle penetration and primary lesion formation. Oxidative burst occurs in many plant-pathogen interactions during the hypersensitive response. The fungus then begins forming masses of outgrowth leading to rapid maceration and sporulation in order to produce inoculum for the next infection (van Kan, 2006).

1.5.4. *Botrytis cinerea* and the hypersensitive response

The hypersensitive response (HR) is one of plant defense mechanism in response to pathogen attack. This response is involved in form of programmed cell death (PCD). It is characterized by the rapid death of plant cell at the site of pathogen infection. In general, HR is identified by the presence of brown and dead cells at the infection site (Heath, 2000). HR is typically triggered upon recognition of a pathogen-encoded avirulence protein by a resistance (R) protein. R protiens are characterized by nucleotide binding (NB) and leucine rich repeat (LRR) domains (Jones & Dangl, 2006). A phenomenon is known as gene-for-gene interaction. These interactions are between the host plant R genes and pathogen avirulence (Avr) genes (Jones & Dangl, 2006). When both the R gene and corresponding Avr genes are present, recognition occurs that resulting to active resistance of the plant and avirulence of the pathogen. The outcome of these interactions will be an incompatible reaction and resistance. If either Avr gene in the pathogen or R gene in the host is absent no recognition will occur and outcome will be a compatible reaction and disease (Glazebrook, 2005). HR is initiated following the interaction between Avr/R genes (Heath, 2000; Morel & Dangl, 1999).

Although HR is frequently contributed to resistance to biotrophic pathogens, the HR induced the virulence of necrotrophic pathogens (Govrin & Levine, 2000; Kliebenstein & Rowe, 2008). Research has shown that *B. cinerea* activates the hypersensitive response. Specifically, Govrin and Levine (2000) proposed that cell death induced by *B. cinerea* is a form of HR and utilizes the cell death as a virulence factor. Another support study that shows

B. cinerea activates the expression of the two plant signaling components EDS1 and SGT1, which are required for HR dependent resistance (El Oirdi & Bouarab, 2007). Thus, HR appears to be one of the strategies used by *B. cinerea* altering the immune system of the plant.

1.5.5. Plant signaling against *Botrytis cinerea*

There are several signaling pathways against pathogens in plant defense. These include: jasmonates (JA), salicylic acid (SA), ethylene (ET) and abscisic acid (ABA) (Bari & Jones, 2009). JA plays a key role in the defense response against insect and necrotrophic pathogen attacks (Penninckx et al., 1996; Rojo, Solano, & Sánchez-Serrano, 2003). Jasmonates also play an important role in plant life, regulating physiological and developmental processes such as root growth tuberization, fruit ripening, senescence, tendril coiling and pollen development (Turner, Ellis, & Devoto, 2002). Several studies have shown that resistance to *B. cinerea* depends on JA signaling (Glazebrook, 2005). Plant mutations that block JA synthesis or the signaling pathway are more susceptible to necrotrophic pathogens than wild type plants (Vijayan, Shockey, Levesque, Cook, & Browse et al., 1998).

There is also evidence that SA, Abscisic acid (ABA) and ethylene (ET) levels increase in response to infection by *B. cinerea* (El Oirdi et al., 2011; Hernandez-Blanco et al., 2007; Lloyd et al., 2011). A recent study has shown that *B. cinerea* manipulates the antagonism between SA and JA to cause the disease development in tomatoes (El Oirdi et al., 2011). The same study also discovered that *B. cinerea* generates an exopolysaccharide called β-(1,3)(1,6)- D glucan which induces SA accumulation (El Oirdi et al., 2011).In *Arabidopsis*, mutations in ABA1 and ABA2 lead to enhanced resistance to *B. cinerea*

(Hernandez-Blanco et al., 2007). ET plays essential role in resistance against *B. cinerea* via the modification of the cell wall (Lloyd et al., 2011).

1.6 Biological clock and plant defense

There is growing evidence that the circadian clock is involved in animal immune responses (Bryant, Trinder, & Curtis, 2004). In *Drosophila*, circadian alternations in the resistance to *Pseudomonas aeruginosa* have been documented (Shirasu-Hiza et al., 2007). Recent studies have revealed that the circadian clock also controls immunity in plants. Wang et al. (2011) found that clock mutants *cca1*, ztl-4, *lhy* and *CCA1 OE* affect the defense response to *Hyaloperonospora arabidopsidis* (*HPA) Emwal*. Specifically, *CCA1 OE* causes an increase in plant resistance to *HPA Emwal*, whereas *cca1* and *ztl-4* cause a decrease in plant resistance to *HPA Emwal* (Wang et al., 2011). They also found that *cca1* shows more resistance at dawn and that there was no further increase in susceptibility when inoculation occurred at dusk. These findings suggest that the resistance and susceptibility of plants to pathogens may vary depending on the time of day (Wang et al., 2011). In their research, Bhardwaj, Meier, Petersen, Ingle, & Roden (2011) found that the circadian clock controls the defense response to *Pseudomonas syringae Pst* DC3000 and that susceptibility is dependent on the time of infection. Specifically, the researchers found that *Arabidopsis* wild type displays increased resistance during the morning inoculation (Bhardwaj et al., 2011).

Recent research has shown that gene expression profiles are altered during *B. cinerea* infection (Mulema & Denby, 2012; Windram et al., 2012). According to Mulema and Denby (2012), gene expression changes depending on time of infection and lesion size. A large number of genes are differentially up- and down-regulated after 12 or 24 hpi when comparing lesion sizes ranging from 0 to 6 mm and 6 to 12 mm. Studies have also discovered that *B. cinerea* infection of *Arabidopsis* leaves influences the expression of the core clock genes (Windram et al., 2012). Windram et al. (2012) observed the inhibition of the rhythms of clock expression for the *CCA1*, *LHY*, *TOC1*, *PRR5-7-9*, *GI*, *ELF4*, and *LUX* genes at different times following *B. cinerea* infection.

Golden 2-like (*GLK*) genes belong to the GARP superfamily and are MYB transcription factors (Riechmann et al., 2000). The GLK proteins contain two pairs of genes (*GLK1* and *GLK2*). *GLK1* and *GLK2* have been implicated in photosynthetic development, organic nitrogen and brasinosteroid signaling, and disease defense responses against different pathogens (Gutiérrez et al. 2008; Murmu et al., 2014; Savitch et al., 2007; Waters et al., 2009).

Several studies have documented the role of *GLK* in plant defense. Interestingly, alterations in *GLK* expression lead to altered pathogen susceptibility. For example, the overexpression of *AtGLK1* in *Arabidopsis* results in the transcription of a number of defence-related genes and conferred resistance to *Fusarium graminearum* (Savitch, et al., 2007). Studies have also shown that *GLK1* and *GLK2* plants increase the resistance against biotrophic pathogen oomycete pathogen *Hyaloperonospora arabidopsidis* (Hpa) Noco2 while *AtGLK1* plants decrease the resistance (Murmu et al., 2014). The same study found that *GLK1* and *GLK2* plants show more susceptibility to the necrotrophic fungal pathogen *B. cinerea*, while *AtGLK1* plants are more resistant (Murmu et al., 2014).

Light influences *GLK* proteins. For example, *AtGLK1* transcript accumulation levels increase in samples harvested during the 16 h light period compared to samples harvested during the dark period. This finding suggests that both genes are regulated by endogenous circadian mechanisms and by light (Fitter, Martin, Copley, Scotland, & Langdale, 2002).

According to our lab, *GLK* genes may work in the regulation of circadian clocks (Sprott et al. 2010, unpublished). Transcript analysis of *Arabidopsis GLK* mutants has indicated that *GLK1* and *GLK2* are involved in the circadian clock through the regulation of morning genes with strong transcriptional and post-transcriptional co-regulation. *GLK1 OE* and *GLK1 KO* suppress the amplitude of expression of endogenous *GLK2*. In contrast *GLK2 OE* does not affect the amplitude of expression of endogenous *GLK1*, but shifts the circadian rhythmicity of *GLK1* by 3h into the late afternoon hours.

Monitoring the circadian rhythmicity of major clock related genes has shown that *GLK1 OE*, *GLK2 OE* and *GLK1 KO* affect the diurnal amplitude or time of appearance of *CCA1*, *LHY*, *APRR3*, *APRR5*, *APRR7*, *APRR9*, *TOC1*, *GI*, *PCL1*, *ELF3* and *CHE*. This would indicate that both the overexpression of *GLK1* and *GLK2* and the deletion of *GLK1* perturb circadian clocks in *Arabidopsis* and that observed changes in circadian rhythmicity might be due to the combinatorial effects of perturbations in the *GLK* loci. Moreover, alterations in *GLK* loci impact diurnal changes in transcription of photosynthesis related genes, pathogenesis and defense related genes, cold-response related genes and major flowering locus related genes (Sprott et al. 2010, unpublished).

1.7 Hypotheses and research objectives

The primary objective of this research was to investigate whether or not the biological clock plays a role in plant immune response (susceptibility or resistance) to the necrotrophic pathogen *Botrytis cinerea* in the model plant *Arabidopsis thaliana.* As previously mentioned, input pathway (light and temperature) signals entrain the plant circadian clock and the clock output pathways modulate the function of the circadian oscillator. The biological clock in *A. thaliana* regulates output pathways, creating different responses.

Our hypotheses are (i) That input pathway signals influence the infection process; and (ii) That clock mutations influence the infection process. In order to test these hypotheses we performed the infection of the *Arabidopsis* leaves at two different times (subjective morning AM and subjective evening PM) using two different approaches: modification of light and modification of the central oscillator.

We first modified light, which is a key player in the circadian system. We used entrainment methodology by growing the wild type in normal earth conditions: long day (LD) 24h (16 h: light 8h: dark) and short day (SD) 24h (8h: light 16h: dark) in order to determine whether or not length of the day/night controls susceptibility to the *B. cinerea* infection. In order to confirm whether light entrainment of the biological clock or length of the day/night control plant immune response, we grew the plants in different cycle photoperiods: LD 21h (14h:light 7h:dark), LD 30h (20h:light 10h:dark), SD 21h (7h:light 14h:dark) and LL (constant light: 24). The literature suggests growing plants under different photoperiods affects the regulation of flowering time (the response of the output pathways) (Strayer et al., 2000). We should thus expect to obtain different output pathways (differences in the susceptibility to *B. cinerea*) in different photoperiods.

Second, we investigated the role of the central oscillator (which is part of the circadian system) in plant defense with mutants in the core loop genes. Transcriptional regulation of the central oscillator involves three feedback loops. In our experiment we modified core feedback loop genes *CCA1/LHY/TOC1* as these genes are connected to the morning and evening feedback loops and stop the entire cycle of the central oscillator. We used the single knockout of the evening gene *toc-1,* known to control the morning genes *CCA1/LHY* and the double knockout of the morning genes *cca1/lhy*. We also used the following combinations: double knockouts *cca1/toc-1*, *lhy/toc-1* and triple knockout *cca1/lhy/toc-1*. These examinations will allow us to determine whether or not the deletion of core genes affects susceptibility to *B. cinerea*. We expect to obtain different output pathways (differences in the susceptibility to *B. cinerea*) in all mutants.

Previous studies have shown that *GLK* genes play a role in pathogen resistance as well as the biological clock (Murmu et al., 2014; Savitch et al., 2007; Sprott et al., 2010, unpublished) and we also studied the role of *GLK* using the same experimental approach.

We believe that this study will help us better understand the link between the biological clock and plant immune responses to *B. cinerea.*

CHAPTER 2

2. MATERIALS AND METHODS

2.1 Plant materials and growth conditions

A. thaliana ecotype Columbia (Col-0) plants were grown in a controlled growth condition (Conviron and SANYO versatile environmental test chamber) in trays containing ProMix soil mixture under a light intensity of 150 µlmol $m^{-2}s^{-1}$ and 22°C/20°C temperature day/night cycle and relative humidity of 70%. *A. thaliana* mutants: *toc-1* (N3756), *cca1/lhy* (N9862), *cca1/ toc*-1 (N9863), *lhy*/ *toc-1* (N9860), and *cca1/lhy/toc-1* (N9861), *glk1* (N9805), *glk2* (N9806) and *glk1/glk2* (N9807) were obtained from The European Arabidopsis Stock Centre (NASC). *GLK* line overexpressors *GLK1 OE* (At2g20570) and *GLK2* OE *(*At5g44190) were obtained from Dr. Jas Singh, ECORC (Savitch et al., 2007).

Plants were entrained to the following photoperiod conditions: long day - LD 24h contains 16 h: light followed by 8h: dark; LD 30h contains 20h: light followed by 10h dark; LD 21h contains 14h: light followed by 7h: dark. Short day - SD 24h contains 8 h: light followed by 16h: dark; SD 21h contains 7h: light followed by 14h dark; and LL constant light contains a 24 h light period. The photoperiod conditions can be seen in Figure 5.

2.2 The growth of *Botrytis cinerea*

The *Botrytis cinerea* strain MEE B191 (B191) was obtained from the Canadian Collection of Fungal Cultures (Agriculture and Agri-Food Canada, Ottawa, Canada). *B. cinerea* was grown on potato dextrose agar (PDA: potato starch 4 g/L, dextrose 20 g/L, agar 17 g/L) and incubated for three days at 22°C in complete darkness.

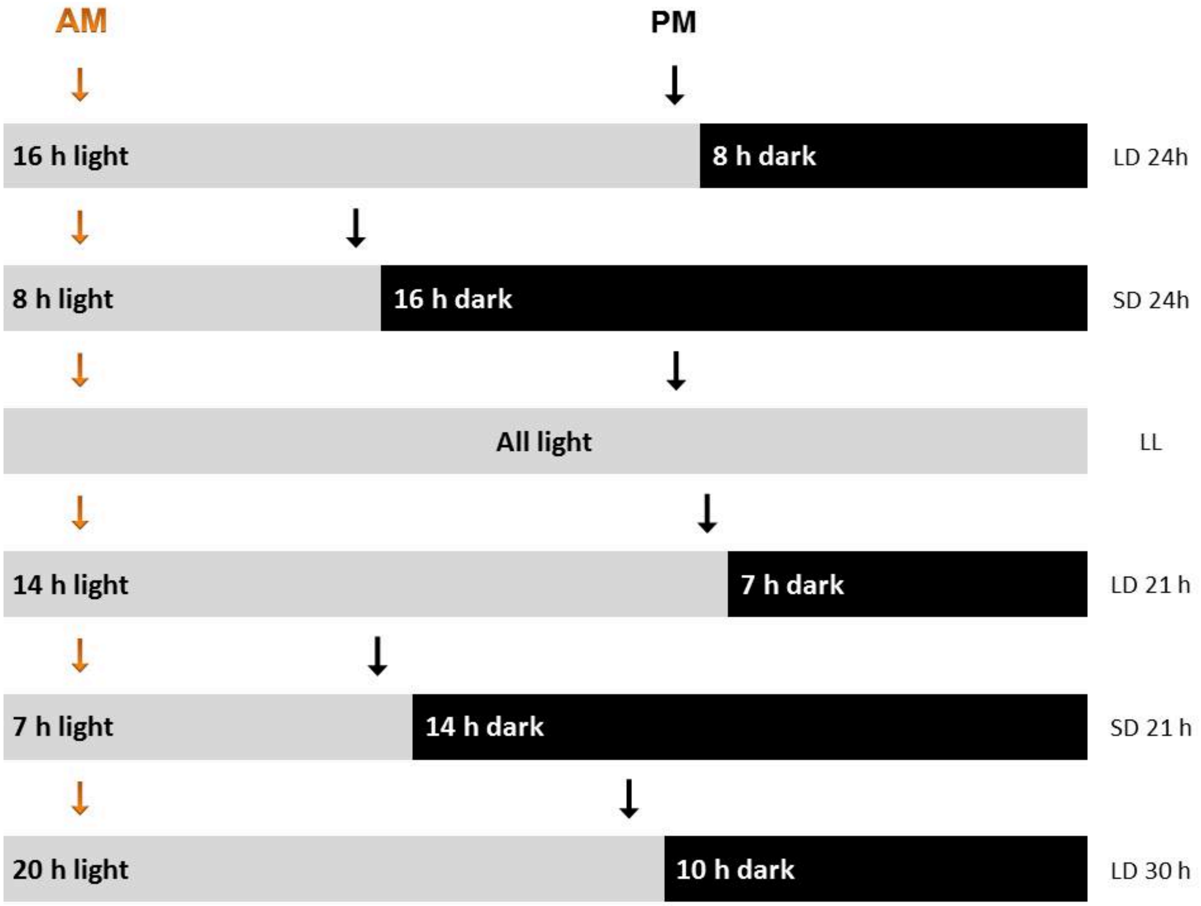


Figure 5. Simple diagram of photoperiod conditions and time of inoculations. Different photoperiod conditions are presented in this digram in gray (day time) and black (night time). Inoculations are carried out at two different times of the day: in the subjective morning (AM) and the subjective evening (PM). The inoculation times vary in all photoperiod conditions according to the cycle time of the day. An orange arrow indicates the time of morning inoculation at ZT4 (4h after the lights were turned ON). A black arrow indicates the time of evening inoculation at ZT1 (1h before the lights were turned OFF).

To induce sporulation, plates of *B. cinerea* were kept for three days at 22°C with 14 h darkness and 10 h of light. Plates of *B. cinerea* were scraped using a glass spatula into 10 ml sterile distilled H_2O. The conidia were filtered in four layers of sterile cheesecloth and washed three times in sterile water by centrifugation (3 min, 500 g). The conidial pellets were suspended into a potato dextrose broth (PDB) medium. The spores were diluted in PDB and counted using a hemocytometer under the microscope (El Oirdi & Bouarab, 2007). The concentration of spores used for all experimental replicates was 10^{6} spores/ml.

2.3 Plant-inoculation assays

Three leaves from each wild type and transgenic plant were gently washed using sterile water and then dried. Detached leaves of 4-week-old long day plants or 8-week-old short day plants were laid on Petri dishes under wet sterile Whatman filter paper. We chose different age groups for the short and long day plants to keep the flowering time and amount of leaves the same.

Inoculations were carried out at two different times of day (morning and evening). Each leaf was inoculated with a 6-µl spore suspension 10^6 spores/ml. After inoculation the leaves were placed in sealed Petri dishes and incubated in the same conditions as the plants were grown (long day (24h-21h-30h), short day (24h-21h), and constant light (LL24h).

The inoculation time was set using Zeitgeber time (ZT). ZT is the standardized notation for the time during an entrained circadian cycle. In our experiments, AM inoculations were performed 4hrs after the lights were turned ON and PM inoculations were performed 1h before the lights were turned OFF. The ZT settings of the inoculations were varied in all photoperiod conditions. For LD 24h and LL24h, time of morning inoculation was set for 10 am (ZT4) and the time of evening inoculation was set for 6 pm (ZT12). For the short day, the time of morning inoculation was set for 10 am (ZT4) and the time of evening inoculation was set for 2 pm (ZT8). The morning and evening inoculation times were different in the following growth conditions: LD 21h, LD 30h and SD 21h (according to the cycle time of the day). The necrotic areas were measured on each leaf. The lesion (necrosis) diameter (mm) was measured by a ruler under microscope and digital microscope (Dino-Lite) 24hrs post infection (hpi). Differences were observed between 52 and 72 hpi.

2.4 Statistical analysis

All experiments were performed three to six times on newly propagated plants (independent biological replicates) with 10 to 20 technical replicates for each individual experiment (inoculated leaves at each biological replicate). The error bars in figures represent standard error of the mean of a minimum of three biological replications. N value represents a minimum of 30 technical replicates.

Statistical analysis was performed using R version 3.0.2. For multiple means we used a One-way ANOVA followed by a post hoc analysis using the 95% simultaneous confidence intervals for specified linear combinations identified by the Tukey's test. Pairwise mean comparison between morning and evening infections were conducted in Microsoft Excel using Student’s t-test and 95% and 99% confidence intervals. Key statistical parameters are given in Tables with the Appendix.

CHAPTER 3

3. RESULTS

3.1 The role of the photoperiod, circadian oscillator genes, and time of infection on the susceptibility to *Botrytis cinerea* in *Arabidopsis thaliana*

The purpose of this research is to identify the ways in which a plant 's biological clock controls the defense response to B. cinerea. Evidence suggests that time of infection influences plant immune responses (Bhardwaj et al., 2011). In order to test this, we performed the infection of Arabidopsis leaves at two different times of day ((subjective morning (AM) and subjective evening (PM)). We would thus expect to obtain different susceptibility to *B. cinerea* depending on the time of infection.

In addition to changing the time of infection, we also used two other approaches. First we modified the light (an input pathway), a key player in the circadian system. We used the following photoperiod conditions: normal earth condition, long day (LD) 24h (16h: light 8h: dark), and short day (SD) 24h (8h: light 16h: dark). We also designed different cycle photoperiods based on previous studies (Strayer et al., 2000): LD 21h (14h: light 7h: dark), LD 30h (20h: light 10h: dark), SD 21h (7h: light 14h: dark), and LL (constant light: 24).

Second, we used plants with mutations in *CCA1/LHY* and *TOC1* (**Figure 6**), which are involved in the core loop. In order to examine the role of the central oscillator of biological clocks in the control of plant immunity, we used the single knockout of the evening gene *toc-1* and the double knockout of the morning genes *cca1/lhy*. Since studies have shown that the deletion of *CCA1/LHY* and *TOC-1* genes affects the period of circadian rhythms (Green & Tobin, 1999; Strayer et al., 2000), we also used combinations of these core loop genes: double knockouts *cca1/ toc-1* and *lhy/ toc-1* and triple knockout *cca1/lhy/toc-1.*

Since we entrained the photoperiod (light) and changed the central oscillator (core clock genes), we predicted that the output pathway would behave differently. These two approaches allowed us to understand how the biological clock alters plant defense to *B. cinerea* in *A. thaliana*. Similarities and differences described in this section are supported by the statistical analyses given in the Appendix.

We compared the level of susceptibility of *Arabidopsis* wild type plants in normal earth conditions (LD 24h and SD 24h photoperiods) 52 h post-infection (hpi). Morning inoculations resulted in significantly higher susceptibility of the wild type plant in both the SD 24h and LD 24h photoperiods (**Fig. 7A; Appendix Table 1**). Independent of the time of inoculations (AM or PM), wild type plants grown at SD 24h photoperiod were always more susceptible to the *B. cinerea* infection than wild type plants grown at LD 24h photoperiod (**Fig. 7A; Appendix Table 2**). In addition, wild type plants grown at either the LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* in AM inoculations (**Fig. 7A; Appendix Table 2**). These results would suggest that the length of day and night controls the susceptibility to *B. cinerea* infection.

In order to prove that it is the light entrainment of the biological clock and not the day/night length which controls plant immune responses, we compared the susceptibility of wild type plants in different cycle photoperiods: LD 21h and LD 30h (**Fig. 7A**). Growth of wild type plants at LD 21h photoperiod (14h: light 7h: dark) diminished the differences previously observed between AM and PM inoculations in plants grown at either LD 24h (16h: light 8h: dark) or SD24h (8h: light 16h: dark) (**Fig. 7A; Appendix Table 1**).

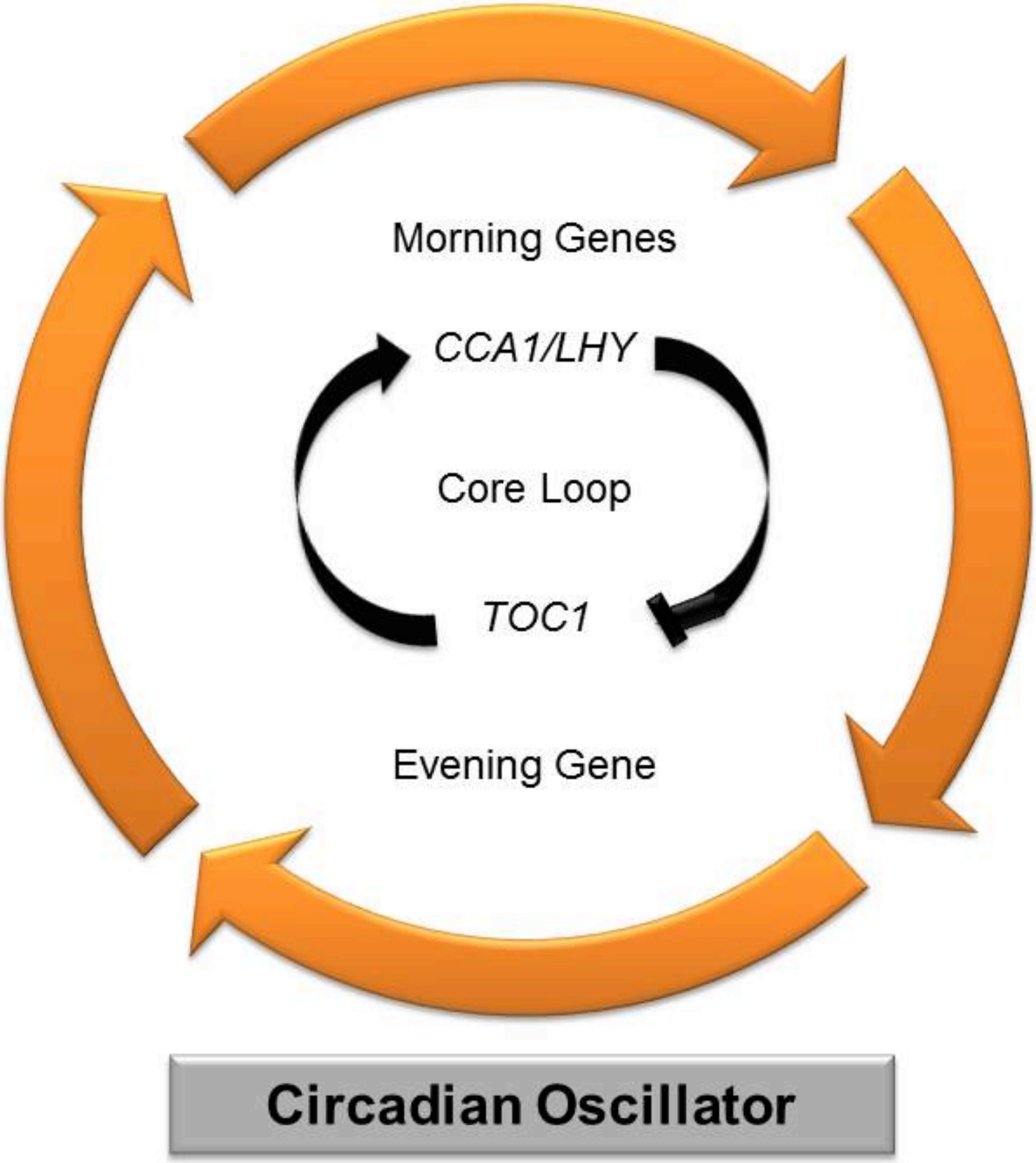


Figure 6. Diagram of the core loop of the circadian oscillator in *Arabidopsis*. The first loop on the core of the central oscillator consists of three genes: two morning genes (*CCA1* and *LHY*), and one evening gene (*TOC1*). The orange arrow indicates the circadian oscillator an auto-regulatory negative-feedback loop. The black arrow indicates activation and the T shape indicates inhibition (Modified from Pruneda-Paz & Kay, 2010).

The PM inoculations of LD 21h grown plants increased susceptibility to *B. cinerea* infection compared to the PM inoculation of LD 24h grown plants. The AM inoculation of either LD 21h or LD 24h grown plants led to similar levels of susceptibility to the *B. cinerea* infection (**Fig. 7A; Appendix Table 2**). In contrast to the response observed in LD 21h grown plants, the LD 30h (20h: light 10h: night) grown plants retained the differences between AM and PM inoculations and showed similar susceptibility to *B. cinerea* infection as the SD24h (8h:light 16h:dark) grown plants. The results presented strongly suggest that entrainment of the biological clock by light rather than length of the day or night controls the susceptibility to the B. cinerea infection in leaves of *A .thaliana.*

To evaluate the role of the central oscillator of biological clocks in the control of plant immunity we used mutants of the core loop of the circadian oscillator in *Arabidopsis* (See **Figure 6**). We used the single knockout of the evening gene *toc-1*, double knockout of the morning genes *cca1/lhy*, double knockouts *cca1/toc-1* and *lhy/toc-1*, and triple knockout *cca1/lhy/toc-1.* First, we compared the responses of mutants to the responses of wild type plants grown at either LD 24h or SD 24h to *B. cinerea* infection. This was done to evaluate the role of the core loop of the circadian oscillator as well as single genes in the control of plant immunity in normal earth conditions. Second, we examine the ability of light to entrain biological clocks by growing plants in LD 21h and LD 30 h photoperiods and comparing mutant responses to those of wild type plants.

When we compared *toc-1* and wild type plants with respect to their response to *B. cinerea* infection in normal earth condition. For the LD 24h photoperiod in contrast to the wild type plants, *toc-1* plants displayed similar levels of susceptibility to *B. cinerea* infection during AM and PM inoculations. *toc-1* showed difference in susceptibility between morning and evening inoculations in SD 24h conditions which is similar to the response observed in wild type plants (**Fig. 7A & B; Appendix Table 1)**. Independent from the time of inoculations (AM or PM), *toc-1* plants grown at SD 24h photoperiod were more susceptible to *B. cinerea* infection than *toc-1* plants grown at LD 24h photoperiod (**Fig. 7B; Appendix Table 2**). In addition, *toc-1* plants grown at either LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* for the AM inoculation (**Fig. 7B; Appendix Table 2**).

In order to evaluate the ability of light to entrain the biological clock of mutants we compared the level of susceptibility of the *toc-1* plant to the wild type plant in the LD 21h and LD 30h cycle photoperiods. In contrast to the wild type plants, *toc-1* showed differences in susceptibility to *B. cinerea* between morning and evening inoculations for plant grown in LD 21h conditions (**Fig. 7A & B; Appendix Table 1**). In both the AM and PM inoculations, the LD 21h photoperiod significantly increased susceptibility to *B. cinerea* when compared with the LD 24h photoperiod (**Fig. 7B; Appendix Table 2**). For the LD 30h photoperiod in contrast to the wild type plants, *toc-1* plants displayed similar levels of susceptibility to *B. cinerea* infection during AM and PM inoculations (**Fig. 7A & B; Appendix Table 1**). In both the AM and PM inoculations, the LD 30h photoperiod significantly increased the susceptibility to *B. cinerea* when compared to the LD 24h photoperiod (**Fig. 7B; Appendix Table 2**).

Next, we used double knockout of morning genes *CCA1* and *LHY*. In contrast to wild type, we did not observe any differences in the susceptibility to *B. cinerea* between morning and evening inoculations in *cca1/lhy* plant grown under LD 24h and SD 24h (**Fig. 7 A & C; Appendix Table 1**). The *cca1/lhy* mutant, when grown in the SD24h photoperiod, showed significantly more susceptibility in both the AM and PM inoculations than when grown in the LD24h photoperiod (**Fig. 7C; Appendix Table 2**). In addition, *cca1/lhy* plants grown in either the LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* for the AM inoculation (**Fig. 7C; Appendix Table 2**).

For the LD 30h photoperiod in contrast to the wild type plants, *cca1/lhy* plants displayed similar levels of susceptibility to *B. cinerea* infection during AM and PM inoculations (**Fig. 7 A & C; Appendix Table 1**). When compared to the LD 24h photoperiod, the LD 30h photoperiod significantly increased susceptibility to *B. cinerea* in both the AM and PM inoculations (**Fig. 7C; Appendix Table 2**). Similarly to wild type plants, *cca1/lhy* plants displayed no observable differences to *B. cinerea* infection during AM and PM inoculations under LD 21h (**Fig. 7 A & C; Appendix Table 1**). There was no observable difference between the LD 21h and LD 24h photoperiod conditions (**Fig. 7C; Appendix Table 2**).

In contrast to wild type, susceptibility to B. cinerea in *cca1/toc-1* mutant was similar between AM and PM inoculations in both normal earth conditions (**Fig. 7 A & D; Appendix Table 1**). When compared to the LD 24h photoperiod, we observed that the SD 24h photoperiod significantly increased susceptibility to *B. cinerea* in both AM and PM (**Fig. 7D; Appendix Table 2**). Moreover, *cca1/toc-1* plants grown at either LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* in AM inoculations (**Fig. 7D; Appendix Table 2**). *cca1/toc-1* plants grown in the LD 30h photoperiod showed difference in susceptibility between morning and evening inoculations which is similar to the response observed in wild type plants (**Fig. 7 A & D; Appendix Table 1**). When compared to the LD 24h photoperiod, the LD 30h photoperiod significantly increased susceptibility to *B. cinerea* in both the AM and PM inoculations (**Fig. 7D; Appendix Table 2**). In contrast to wild type, *cca1/toc-1* plants grown in the LD 21h photoperiod showed difference in susceptibility between morning and evening inoculations with greater susceptibility observed after evening inoculations (**Fig. 7A & D; Appendix Table 1**). The PM inoculations of LD 21h grown plants increased susceptibility to the *B. cinerea* infection when compared to the PM inoculation of LD 24h grown plants. AM inoculation of either LD 21h or LD 24h grown plants led to similar levels of susceptibility to the *B. cinerea* infection (**Fig. 7D; Appendix Table 2**).

The response of *lhy/toc-1* plants to the AM and PM inoculations was similar to the response observed in wild type plants when grown at LD 24h. Comparing to the response to the wild type, *lhy/toc-1* plants grown in SD 24h showed opposite response in susceptibility to the *B. cinerea* between AM and PM inoculations (**Fig. 7A & E; Appendix Table 1**). The *lhy/toc-1* mutant, grown in the SD24h photoperiod, showed significantly more susceptibility than in the LD24h photoperiod for both AM and PM inoculations (**Fig. 7E; Appendix Table 2**). In addition, *lhy/toc-1* plants grown in either the LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* in AM inoculations (**Fig. 7E; Appendix Table 2**).

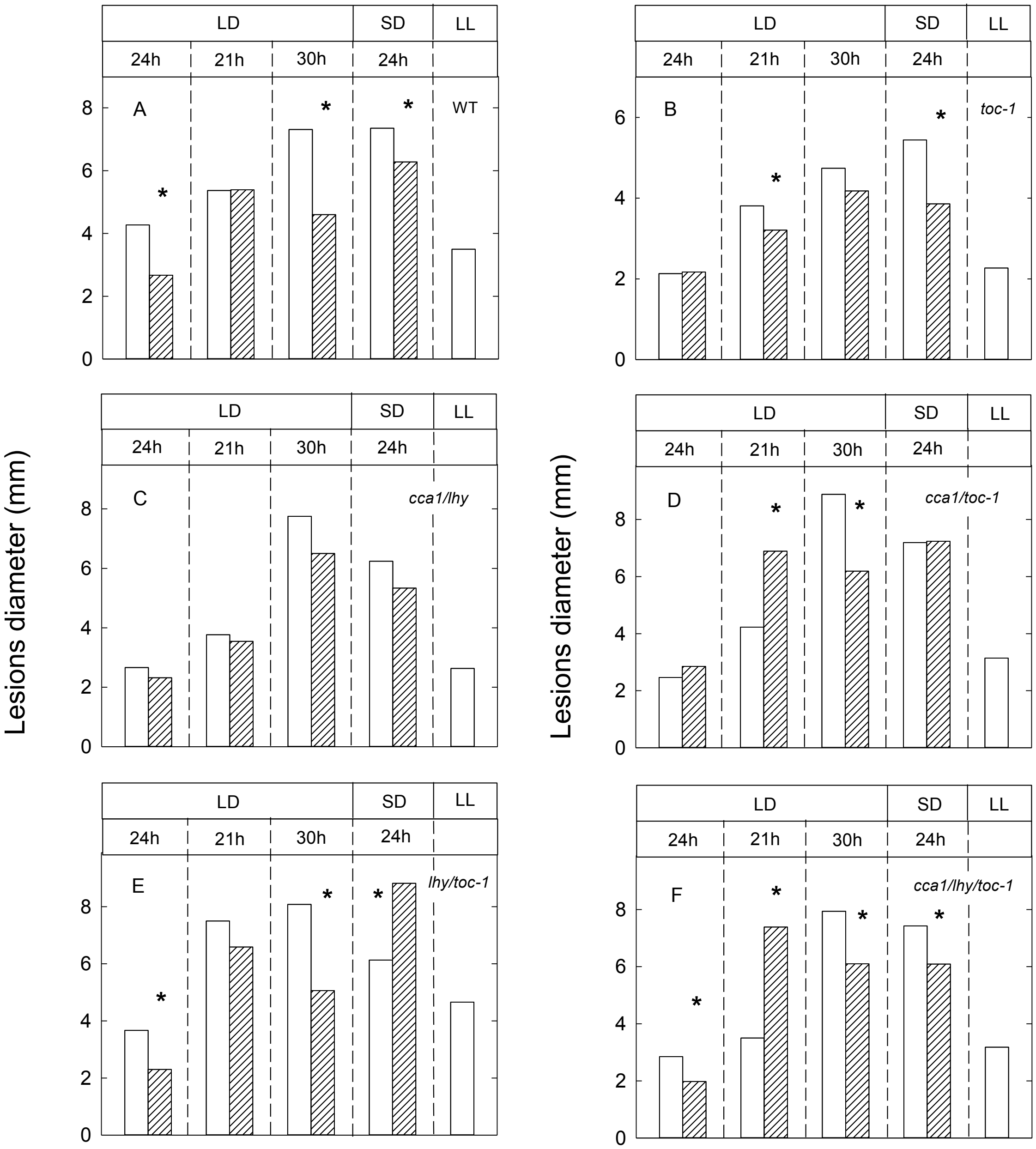
Similarly to the response of wild type, *lhy/toc-1* mutants showed no differences in susceptibility to *B. cinerea* infections in response to AM and PM inoculations when grown at LD 21h condition and drastic differences when grown at LD 30h (**Fig. 7A & E; Appendix Table 1**). The *lhy/toc-1* mutant, grown in the LD 21h photoperiod, showed significantly more susceptibility than in the LD24h photoperiod in both the AM and PM inoculations (**Fig. 7E; Appendix Table 2**). When compared with the LD 24h photoperiod, the *lhy/toc-1* mutant grown in the LD 30h photoperiod showed significantly greater susceptibility in both the AM and PM inoculations (**Fig. 7E; Appendix Table 2**).

Similarly, both the triple knockout mutant *cca1/lhy/toc-1* and wild type plant showed significant differences in susceptibility between AM and PM inoculations in both normal earth conditions (**Fig. 7A & F; Appendix Table 1**). When compared to the LD 24h photoperiod, we found a significant increase in susceptibility to *B. cinerea* for the SD 24h photoperiod in both the AM and PM inoculations (**Fig. 7F; Appendix Table 2**). Moreover, *cca1/lhy/toc-1* plants grown at either LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* in the AM inoculations (**Fig. 7F; Appendix Table 2**).

In contrast to wild type, differences between the AM and PM inoculations were observed in the *cca1/lhy/toc-1* plants grown under LD 21h photoperiod (**Fig. 7A & F; Appendix Table 1**). Compared to the LD 24h grown plants, the PM inoculations of LD 21h grown plants increased susceptibility to *B. cinerea* infection. AM inoculations of either LD 21h or LD 24h grown plants led to similar levels of susceptibility to *B. cinerea* infection (**Fig. 7F; Appendix Table 2**). Growth of *cca1/lhy/toc-1* plants in the LD 30h photoperiod led to the significant differences in susceptibility in response to morning and evening inoculations, which is similar to the response observed in wild type plants (**Fig. 7A & F; Appendix Table 1**). When compared to the LD 24h photoperiod, *cca1/lhy/toc-1* mutants grown in the LD 30h photoperiod showed significantly more susceptibility to *B. cinerea* in both AM and PM inoculations (**Fig. 7F; Appendix Table 2**).

Our results indicate that there is a difference in susceptibility to *B. cinerea* in clock mutants when using the single knockout of the evening or double knockout of morning genes or some combination of these genes (**Fig. 7 B, C, D, E, and F; Appendix Table 1)**. This indicates that biological clock regulation, and not day/night length, controls susceptibility to *B. cinerea*. These results suggest that the circadian clock manipulates susceptibility to *B. cinerea* in the wild type leaves of *Arabidopsis* and clock mutants under different photoperiods and at different times of the day at 52hpi.

Figure 7. The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* in leaves of *A. thaliana* wild type and circadian clock mutants. The susceptibility of *A. thaliana* leaves to *B.cinerea* infection was evaluated in wild type (**A**) and circadian clock mutant plants: *toc-1* (**B**), *cca1/lhy* (**C**), *cca1/toc-1* (**D**), *lhy/toc-1* (**E**), *cca1/lhy/toc-1* (**F**). Wild type (WT) and circadian clock mutants were grown under different photoperiod conditions long day (LD) 24h (16h: light 8h: dark), LD 21h (14h: light 7h: dark), LD 30h (20h: light 10h: dark), short day (SD) 24h (8h: light 16h: dark), and LL constant light (24:light). Detached leaves were inoculated with (6µl) spore suspension (1 x 10^6 spores/ml). Inoculations were performed in the AM (open bar) and PM (Shaded bar) and vary in time in all photoperiod conditions. Lesion areas were measured 52h post-infection (hpi). The star represents significant differences between morning and evening inoculations within different photoperiods at $P \leq 0.05$ (Student's t-test).


LD
SD
LL
24h
21h
30h
24h
A
WT
B
toc-1
C
cca1/lhy
D
cca1/toc-1
E
lhy/toc-1
F
cca1/lhy/toc-1
*
Lesions diameter (mm)
0
2
4
6
8

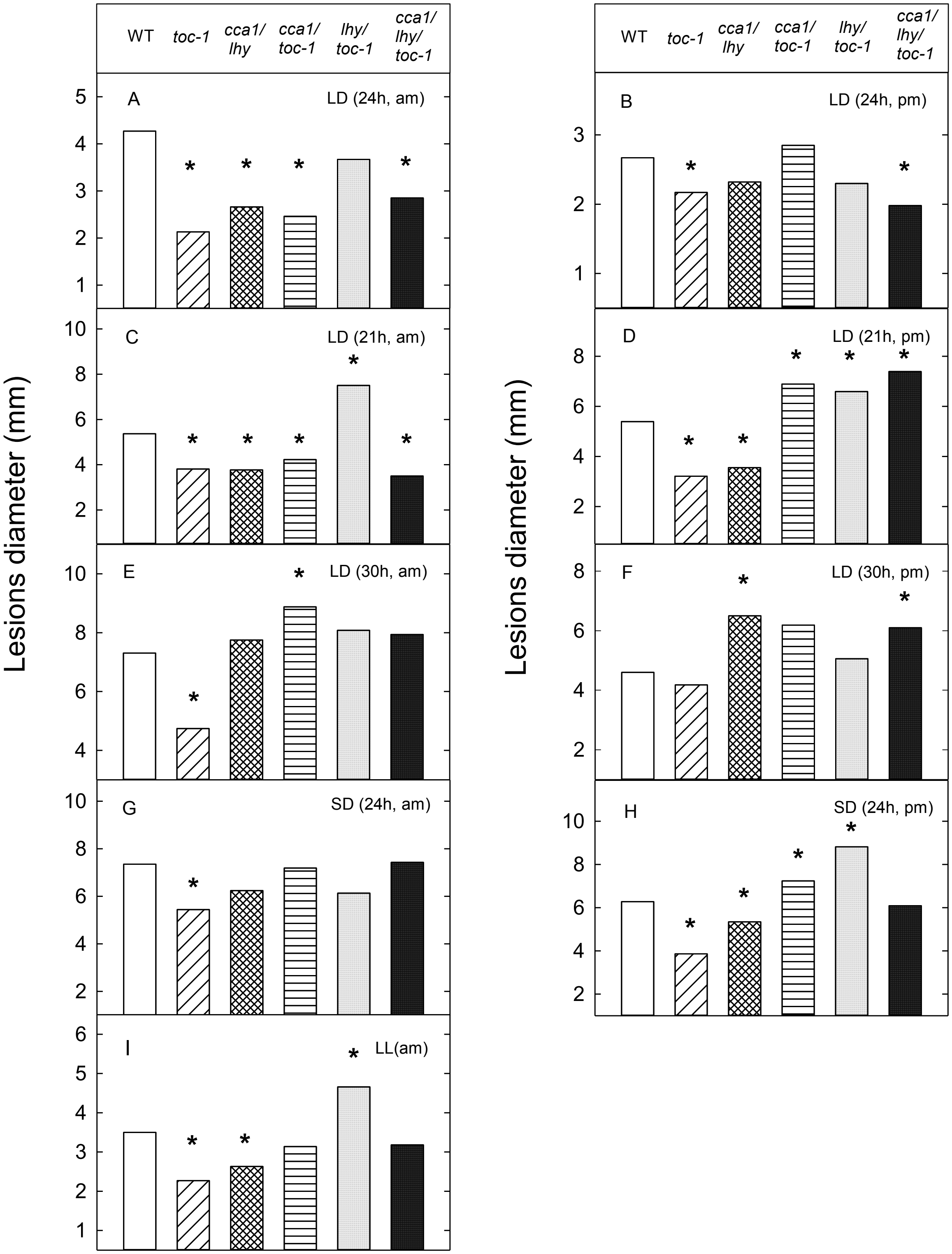
To confirm the involvement of the circadian clock in plant defense, we performed comparisons of wild type plants with all clock mutants at 52hpi and 72hpi in various photoperiods and times of infection. Previous research has shown that two days post-inoculation, small necrotic spots (primary lesions) can be seen on leaves *in vitro*. These lesions quickly spread and increase in size after four days post-inoculation (Schumacher & Tudzynski, 2012). Windram et al. (2012) have found that lesion expansion in *B. cinerea* begins between 36 and 48 hours post-inoculation. According to our observations, *B. cinerea* lesions developed at 52hpi in all mutants in different photoperiods. The data used in figure 8 was constructed from figure 7.

First, we evaluated whether clock mutants are responsible for susceptibility to *B. cinerea* infection in the leaves of *Arabidopsis* wild type plants at 52hpi in various photoperiods and times of infection under normal earth conditions. For the LD 24h morning inoculations, all mutants (*toc-1*, *cca1/toc1*, *cca1/lhy*, and *cca1/lhy/toc-1),* except *lhy/toc1* showed significantly decreased susceptibility to *B. cinerea* compared with wild type plants (**Fig. 8A; Appendix Table 3**). For the LD 24h evening inoculations, there was no difference in level of susceptibility to *B. cinerea* between wild type and all mutants except for *toc-1* and *cca1/lhy/toc-1* (**Fig. 8B; Appendix Tables 3 & 9**). In addition, for the SD 24h AM inoculations, we observed that only the *toc-1* mutant decreased the susceptibility to *B. cinerea* when compared to the wild type. No other significant differences were observed (**Fig. 8G; Appendix Table 3**). When comparing wild type and the mutants, *toc-1* and *cca1/lhy* showed decreased susceptibility to *B. cinerea*, while *cca1/toc1* and *lhy/toc1* showed increased susceptibility to *B. cinerea* for PM inoculations in the same photoperiod (**Fig. 8H; Appendix Tables 3 & 9**).

We examined susceptibility to infection by *B. cinerea* in different cycle photoperiods: LD 21h, LD 30h, and LL. For the LD 21h AM inoculations there was a difference between wild type and all clock mutants in lesion diameter (average of 4 mm). The exception is the *lhy/toc1* mutant, which obtained bigger lesions (about 8 mm) (**Fig. 8C; Appendix Table 3**). For the LD 21h PM inoculations, *toc-1* and *cca1/lhy* showed decreased susceptibility to *B. cinerea* compared to wild type plants, while *cca1/toc1*, *lhy/toc1* and *cca1/lhy/toc-1* showed increased susceptibility to *B. cinerea* (**Fig. 8D; Appendix Tables 3 & 9**).

Only the *toc-1* mutant showed less susceptibility to *B. cinerea* than the wild type in LD 30h AM inoculations, whereas *cca1/toc1* showed increases in susceptibility (**Fig. 8E; Appendix Tables 3 & 9**). For the LD 30h evening inoculations, *cca1/lhy* and *cca1/lhy/toc-1* mutants showed significantly higher susceptibility than the wild type (**Fig. 8F; Appendix Table 3**). Finally, *toc-1* and *cca1/lhy* appeared to be less susceptible to *B. cinerea* for AM inoculations in the LL condition than wild type plants, while *lhy/toc1* mutant showed increased susceptibility (**Fig. 8I; Appendix Tables 3 & 9**). Our observations demonstrate that there is a lot of variation in susceptibility to *B. cinerea* among clock mutants 52hpi in various photoperiods and times of infection (**Fig. 8A-I**). Our results suggest that the clock genes may not be solely responsible for the regulation of susceptibility to *B. cinerea*. It may be that the biological clock only plays a role in plant defense to *B. cinerea*.

Figure 8. The effects of mutations in the central oscillator of biological clocks on the susceptibility of *A. thaliana* leaves to *Botrytis cinerea* infection. The susceptibility of *A. thaliana* leaves to *B. cinerea* infection was evaluated in wild type (WT) and (circadian) biological clock mutants *(toc-1*, *cca1/lhy*, *cca1/toc-1*, *lhy/toc-1*, and *cca1/lhy/toc-1*) grown either under long day conditions LD 24h (16h: light 8h: dark) (**A,B**), LD 21h (14h: light 7h :dark) (**C,D**), and LD 30h (20h: light 7h: dark) (**E,F**) or under short day conditions SD 24h (8h: light 16h: dark) (**G,H**) or LL constant light (24:light) (**I**) The inoculations were performed on plant grown at prevailing growth conditions during different time of the day either in the morning (AM) (**A, C, E, G, I**) or in the evening (PM) (**B, D, F, H**). Detached leaves were inoculated with (6 µl) spore suspension (1 x 10^6 spores/ml). Lesion areas were measured 52 h post-infection 52 (hpi) with *B. cinerea*. The stars indicate a difference in susceptibility to *B. cinerea* in clock mutants compared with wild type for a given photoperiod and time of infection at a P-value of ≤ 0.05 (One-way ANOVA test or Student's t-test).


WT
toc-1
cca1/lhy
cca1/toc-1
lhy/toc-1
cca1/lhy/toc-1
Lesions diameter (mm)
A
LD (24h, am)
B
LD (24h, pm)
C
LD (21h, am)
D
LD (21h, pm)
E
LD (30h, am)
F
LD (30h, pm)
G
SD (24h, am)
H
SD (24h, pm)
I
LL(am)
*

First we tested susceptibility to *B. cinerea* in leaves of *Arabidopsis* among wild type and clock mutants at 72hpi in various photoperiods and times of infection in order to determine if the clock genes are responsible for controlling plant immune responses. It is useful to examine differences in susceptibility over a longer period of observation (72hpi instead of 52hpi). Lesion growth is spread over the time period and in accordance with the *B. cinerea* life cycle (Schumacher & Tudzynski, 2012). The data used in figure 9 were from the same experiments, but measurements were taken at 72h.

We compared the susceptibility to *B. cinerea* infection in the leaves of *Arabidopsis* among wild type and clock mutants at 72hpi in normal earth conditions. In LD 24h, *toc-1, cca1/lhy,* and *cca1/lhy/toc-1* showed significantly smaller lesions than the wild type for AM inoculations (**Fig. 9A; Appendix Table 4**). For the LD 24h PM inoculations, only *cca1/lhy/toc-1* showed less susceptibility to *B. cinerea* when compared to the wild type (**Fig. 9B; Appendix Table 4**).

Additionally, for AM inoculations of *B. cinerea* in SD 24h, all clock mutants except for *cca1/lhy/toc-1* showed significant decreases in susceptibility when compared to the wild type (**Fig. 9G; Appendix Table 4**). For the PM inoculations in SD 24h, only *toc-1* and *cca1/lhy* were significantly less susceptible to *B. cinerea* than the wild type (**Fig. 9H; Appendix Table 4**).

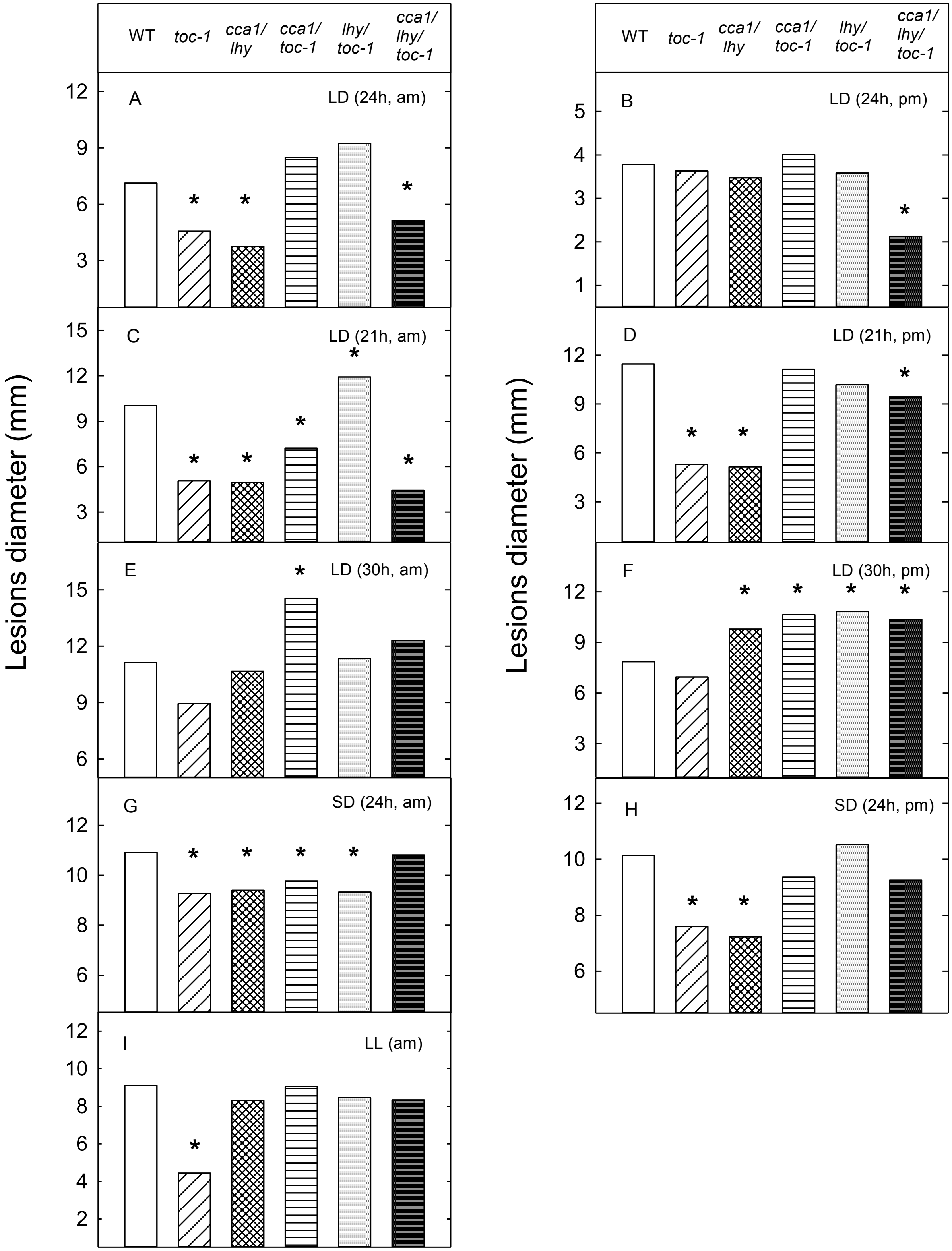
We also compared the level of susceptibility to *B. cinerea* infection in the LD 21h, LD 30h, and LL cycle photoperiods for wild type and clock mutants. For the LD21h AM inoculations, the average lesion diameter decreased (average 5.5 mm in all mutants) whereas the lesion diameter increased for *lhy/toc1* (lesions up to 12 mm) (**Fig. 9C; Appendix Table 4**). For the LD21h PM inoculations, *toc-1*, *cca1/lhy,* and *cca1/lhy/toc-1* displayed less susceptibility to *B. cinerea* when compared to the wild type (**Fig. 9D; Appendix Table 4**).

Under the LD 30h AM inoculation, only *cca1/toc1* showed an increase in susceptibility to *B. cinerea* compared to the wild type (**Fig. 9E; Appendix Table 9**). In the LD 30h PM inoculations, all mutants except for *toc-1* showed increased susceptibility to *B. cinerea* compared to the wild type (**Fig. 9F; Appendix Tables 4 & 9**). Finally, in the LL AM inoculations, *toc-1* appeared to be less susceptible to *B. cinerea* infection when compared to the wild type (**Fig. 9I; Appendix Table 4**).

Our observations at 72hpi differed from those at 52hpi. For AM inoculations in the LD 24h photoperiod we observed a lower susceptibility to *B. cinerea* infection in *cca1/toc1* compared to the wild type at 52hpi. There were no observed differences in susceptibility between this mutant and the wild type at 72hpi (**Fig. 8 & 9A; Appendix Tables 3 & 4**). For PM inoculations in the LD 24h photoperiod we observed significant differences in susceptibility between *toc-1* and the wild type at 52hpi. There were no observed differences in susceptibility between this mutant and the wild type at 72hpi (**Fig. 8 & 9B; Appendix Table 3 & 4**). For AM inoculations in the SD 24h photoperiod we observed significant differences in susceptibility between *cca1/lhy*, *cca1/toc1, lhy/toc1* and the wild type at72hpi but not 52hpi (**Fig. 8 & 9G; Appendix Tables 3 & 4**). For PM inoculations in the SD 24h photoperiod we observed an increase in susceptibility for *cca1/toc1*and *lhy/toc1* compared to the wild type at 52hpi but not 72hpi (**Fig. 8 & 9H; Appendix Tables 3 & 4**).

We also examined susceptibility to *B. cinerea* infection in the LD 21h, LD 30h, and LL cycle photoperiods at 52hpi and 72hpi. For PM inoculations in the LD 21h photoperiod we observed an increase in susceptibility for *cca1/toc1*and *lhy/toc1* compared to the wild type at 52hpi but not 72hpi (**Fig. 8 & 9D; Appendix Tables 3 & 4**). For the AM inoculations in the LD 30h condition we observed differences in susceptibility between *toc-1* and the wild type at 52hpi but not 72hpi. For the PM inoculations in the LD 30h condition we observed differences in susceptibility between *cca1/toc1*, *lhy/toc1* and the wild type at 72hpi but not 52hpi (**Fig. 8 & 9F; Appendix Tables 3 & 4**). Our data suggests that the clock genes are not solely responsible for plant immune responses to *B. cinerea* but rather the ways in which the biological clock system regulates the outcome pathways.

Figure 9. The effects of the mutations in the central oscillator of biological clocks on susceptibility of *A. thaliana* leaves to *B. cinerea* infection. The susceptibility of *A. thaliana* leaves to *B. cinerea* infection was evaluated in wild type (WT) and (circadian) biological clock mutants (*toc-1, cca1/lhy*, *cca1/toc-1*, *lhy/toc-1*, and *cca1/lhy/toc-1*) grown either under long day conditions LD 24h (16h: light 8h: dark) (**A,B**), LD 21h (14h: light 7h: dark) (**C,D**), and LD 30h (20h: light 7h: dark) (**E,F**) or under short day conditions SD 24h (8h : light 16h :dark) (**G,H**) or LL constant light (24:light) (**I**). The inoculations were performed on plants grown at prevailing growth conditions during the morning (AM) (**A, C, E, G, I**) or during the evening (PM) (**B, D, F, H**). Detached leaves were inoculated with (6 µl) spore suspension (1 x 10^6 spores/ml). Lesion areas were measured 72h post-infection 72 (hpi) with *B. cinerea*. The stars indicate a difference in susceptibility to *B. cinerea* of clock mutants compared to the wild type for a given photoperiod and time of infection at a P-value of ≤ 0.05 (One-way ANOVA test or Student’s t-test).


WT
toc-1
cca1/lhy
cca1/toc-1
lhy/toc-1
cca1/lhy/toc-1
Lesions diameter (mm)
A
LD (24h, am)
B
LD (24h, pm)
C
LD (21h, am)
D
LD (21h, pm)
E
LD (30h, am)
F
LD (30h, pm)
G
SD (24h, am)
H
SD (24h, pm)
I
LL (am)
*

3.2 The role of Golden 2-like (*GLK*) genes on the susceptibility to *Botrytis cinerea* in *Arabidopsis thaliana*

Studies have shown that *GLK* genes play a role in tolerance to pathogens such as *F. graminearum* and *B. cinerea* (Murmu et al., 2014; Savitch, et al., 2007). It is possible that *GLK* genes modulate the defense response through the regulation of the circadian clock. We tested this theory using the same times of infection, photoperiods, and *GLK* mutants as in the previous experiments.

We compared the level of susceptibility of the *Arabidopsis* wild type plant in normal earth conditions at 52h post-infection (hpi). Morning inoculations resulted in significantly higher susceptibility of the wild type plant in both the SD 24h and LD 24h photoperiods (**Fig. 10A; Appendix Table 5**). Wild type plants grown in the SD 24h photoperiod were more susceptible to the *B. cinerea* infection than wild type plants grown in the LD 24h photoperiod, regardless of the time of infection (**Fig. 10A; Appendix Table 6**). Wild type plants grown in the LD 24h and LL photoperiod conditions showed similar levels of susceptibility to the *B. cinerea* infection in AM inoculations (**Fig. 10A; Appendix Table 6**). Based on these results, it could suggest that the day/night length controls susceptibility to the *B. cinerea* infection.

In order to confirm that the light entrainment of the biological clock, and not the length of day/night, controls the plant immune response, we compared the level of susceptibility of the wild type plant in the LD 21h and SD 21h cycle photoperiods (**Fig. 10A; Appendix Table 5**). Wild type plants grown in LD 21h (14h: light 7h: dark) and SD 21h (7h: light 14h: dark) photoperiods showed similar level of susceptibility to *B. cinerea* for the AM and PM inoculations (**Fig. 10A; Appendix Table 5**). When compared to LD 24h grown plants, LD 21h grown plants given PM inoculations showed increased susceptibility to the *B.*

cinerea infection. In contrast, AM inoculated wild type plants displayed similar levels of susceptibility to the *B. cinerea* infection in the LD 21h and LD 24h photoperiods (**Fig. 10A; Appendix Table 6**). The data suggests that susceptibility to the *B. cinerea* infection in leaves of *A. thaliana* is controlled by the light entrainment of the biological clock and not the day/night length.

To evaluate the role of GLK genes in the control of plant immunity through the regulation of the biological clock we used the *GLK1* and *GLK2* overexpressors and *GLK1* knockout plants and compared their response to *B. cinerea* infection to wild type plants grown in LD 24h or SD 24h conditions.

We evaluated the response of *GLK1 OE* to *B. cinerea* inoculations at normal earth conditions, and compared them to wild type plants under the same conditions. For the LD 24h photoperiod, *GLK1* OE plants showed no observable differences in susceptibility between morning and evening inoculations, which is opposite to the response observed in wild type plants. In contrast, *GLK1 OE* plants grown under the SD 24h photoperiod displayed observable differences in susceptibility between morning and evening inoculations, which is similar to the response observed in wild type plants (**Fig. 10A & B; Appendix Table 5**). In addition, *GLK1 OE* plants grown in either the LD 24h or LL photoperiod conditions showed similar levels of susceptibility to the *B. cinerea* infection in AM inoculations (**Fig. 10B, Appendix Table 6**).

We evaluated the ability of light to entrain the biological clocks of *GLK* transgenic plants by growing the plants in LD 21h and SD 21h photoperiods and comparing the mutants and wild type responses. Similarly to the wild type, *GLK1* OE plants grown in LD 21h and SD 21h showed no observable differences in susceptibility between morning and evening inoculations (**Fig. 10A & B; Appendix Table 5**). *GLK1 OE* plants grown in the LD 21h and

SD 21h photoperiods displayed higher susceptibility to *B. cinerea* infection than *GLK1 OE* plants grown in the LD 24h photoperiod, regardless of the time of infection (**Fig.10B; Appendix Table 6**).

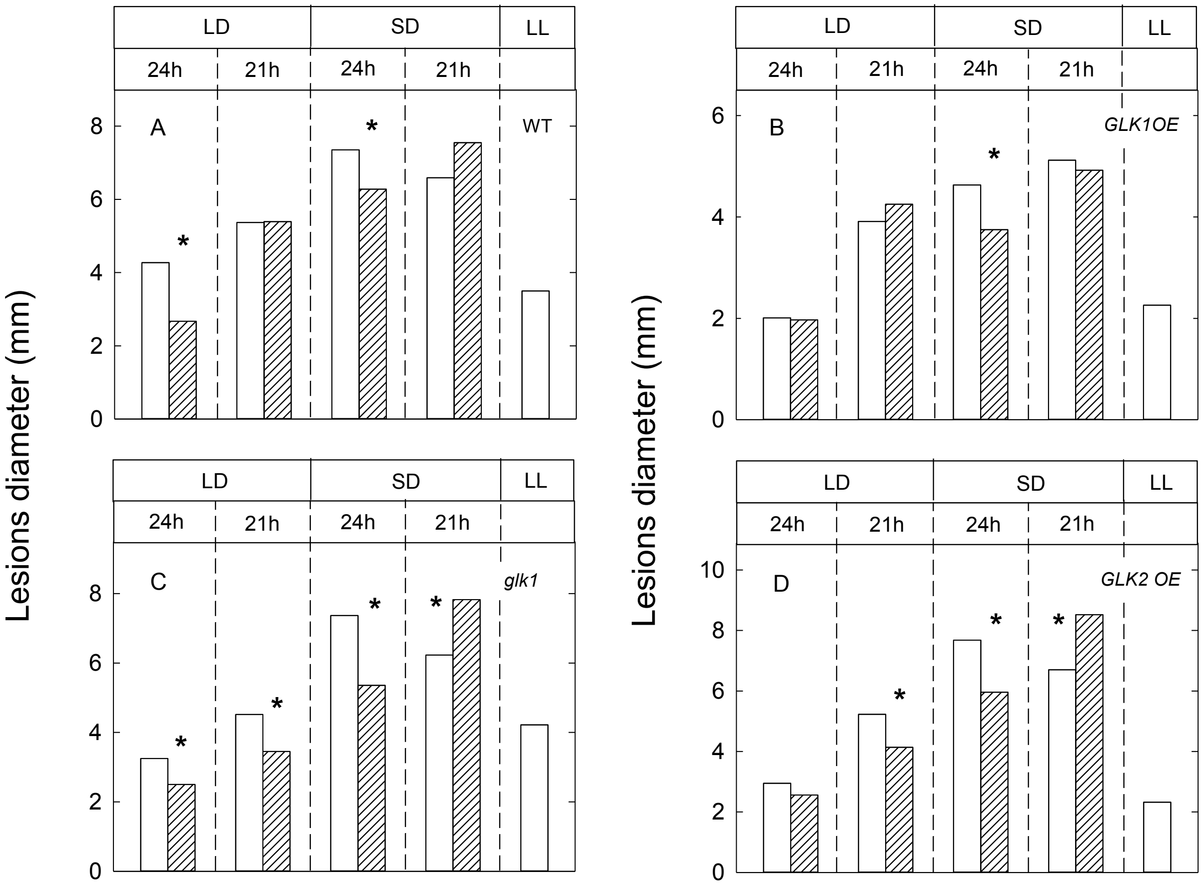
When the *GLK1* gene was knocked out there was a significant difference in susceptibility between morning and evening inoculations in both the LD 24h and SD 24h conditions which is similar to the response observed in wild type plants (**Fig. 10A & C; Appendix Table 5**). *glk1* plants grown in the SD 24h photoperiod displayed higher susceptibility to *B. cinerea* infection than *glk1* plants grown in the LD 24h photoperiod, regardless of time of infection (**Fig.10C; Appendix Table 6**). Furthermore, *glk1* plants grown in the LD 24h or LL photoperiod conditions showed similar levels of susceptibility to *B. cinerea* infection in AM inoculations (**Fig. 10C; Appendix Table 6**).

In contract to the wild type plants, there were observable differences in susceptibility to *B.cinerea* between AM and PM inoculations for *glk1* plants grown under the LD 21h and SD 21h photoperiods (**Fig. 10A & C; Appendix Table 5)**. Regardless of time of infection, *glk1* plants grown in the LD 21h photoperiod condition and *glk1* plants grown in the LD 24h photoperiod condition showed similar levels of susceptibility to *B. cinerea* infection. *glk1* plants grown in the SD 21h photoperiod condition displayed higher susceptibility to *B. cinerea* infection than *glk1* plants grown in the LD 24h photoperiod condition, regardless of time of infection (**Fig. 10C; Appendix Table 6**).

In contrast to the wild type plants, GLK2 *OE* plants displayed similar levels of susceptibility to *B. cinerea* infection in response to AM and PM inoculations when grown at LD 24h conditions. *GLK2 OE* showed significant difference in susceptibility between morning and evening inoculations under SD 24h conditions, which is similar to the response observed in wild type plants (**Fig. 10A & D; Appendix Table 5**). *GLK2 OE* plants grown in the SD 24h photoperiod displayed higher susceptibility to *B. cinerea* infection than *GLK2 OE* plants grown in the LD 24h photoperiod, regardless of the time of infection (**Fig. 10D; Appendix Table 6**). Moreover, *GLK2 OE* plants grown in either the LD 24h or LL photoperiod conditions displayed similar levels of susceptibility to *B. cinerea* infection in AM inoculations (**Fig. 10D; Appendix Table 6**).

GLK2 OE displayed a significant difference in susceptibility between AM and PM inoculations in the LD 21h and SD 21 photoperiod conditions, which is different from the response to wild type plants (**Fig. 10A & D; Appendix Table 5**). *GLK2 OE* plants grown in either the LD 21h or SD 21 photoperiods displayed higher susceptibility to *B. cinerea* infection when compared to the LD 24h photoperiod, regardless of the time of inoculation (**Fig. 10D; Appendix Table 6**).

Figure 10. The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* in leaves of *A. thaliana* wild type, *GLK1* and *GLK2* overexpressors (OE) and *GLK1* knockout (KO) plants. The susceptibility of *A. thaliana* leaves to *B. cinerea* infection was evaluated in wild type (**A**) *GLK1 OE* (**B**), *glk1* (**C**) and *GLK2 OE (***D**). WT and *GLKs* OE and KO plants were grown under different photoperiod conditions long day (LD) 24h (16 h: light 8h: dark), LD 21h (14h: light 7h: dark), short day (SD) 24h (8h: light 16h: dark), SD 21h (7h: light 14h: dark), and LL constant light (24:light). Detached leaves were inoculated with (6 µl) spore suspension (1 x 10^6 spores/ml). Inoculations were performed in AM (open bar) and PM (shaded bar) and vary on time in all photoperiod conditions. Lesion areas were measured 52h post-infection (hpi) with *B.cinerea*. The stars represent significant differences between morning and evening inoculations within different photoperiods at $P \leq 0.05$ (Student's t-test). .


Lesions diameter (mm)
LD
SD
LL
24h
21h
24h
21h
A
WT
B
GLK1OE
C
glk1
D
GLK2 OE
0
2
4
6
8
10
*

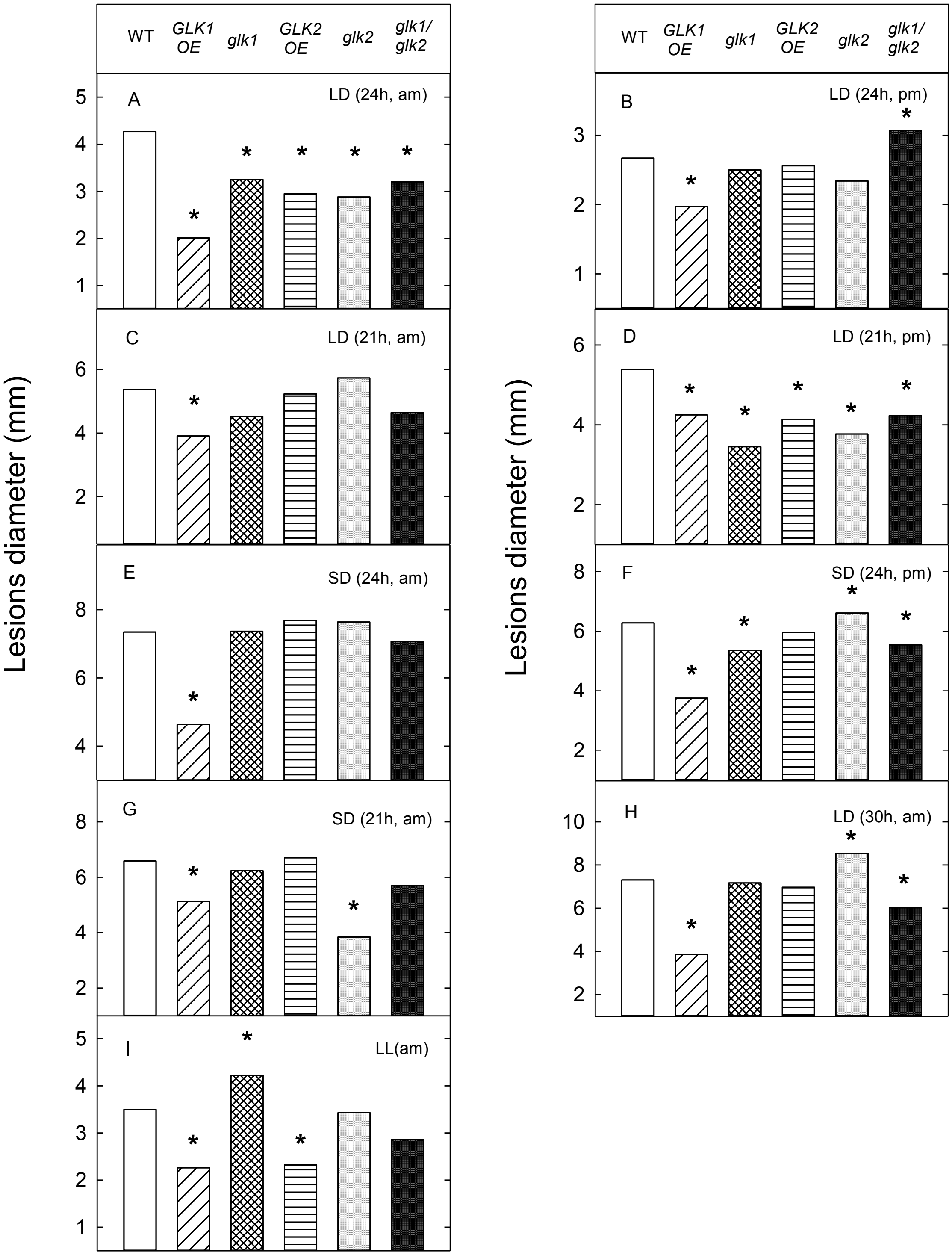
We performed comparisons of clock mutant responses at 52hpi and 72hpi at various photoperiods and times of infection. We evaluated whether or not altering the *GLK* genes affects the susceptibility of *B. cinerea* infection in leaves of *Arabidopsis* WT at 52hpi in various photoperiods and times of infection. The data used in figure 11 was constructed from figure 10.

For the LD 24h AM inoculations, all *GLKs* mutants (*GLK1 OE*, *glk1*, *GLK2 OE*, *glk2*, and *glk1/glk2*) displayed a significant decrease in susceptibility to *B. cinerea* infection when compared with wild type plants (**Fig. 11A; Appendix Table 7**). For the LD 24h PM inoculations, *GLK1 OE* displayed a significant decrease in susceptibility to *B. cinerea* infection when compared with wild type plants whereas *glk1/glk2* displayed a significant increase in susceptibility to *B. cinerea* infection when compared with wild type plants (**Fig. 11B; Appendix Tables 7 & 9**). For the SD 24h AM inoculations, *GLK1 OE* displayed a significant decrease in susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 11E; Appendix Table 7**). For the SD 24h PM inoculations, *GLK1 OE*, *glk1*, and *glk1/glk2* displayed significant decrease in the level of susceptibility to *B. cinerea* infection when compared with wild type plants, while *glk2* displayed an increase in susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 11F; Appendix Tables 7 & 9**).

For the LD 21h AM inoculation, *GLK1 OE* displayed decreased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 11C; Appendix Table 7**). For the LD 21h PM inoculations, all GLKs mutants displayed a significant decrease in susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 11D; Appendix Table 7**). For the SD 21h AM inoculations, *glk2* and *GLK1 OE* displayed decreased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 11G; Appendix Table 7**). For LD 30h AM inoculations, *GLK1 OE* and *glk1/glk2* displayed decreased susceptibility to *B.*

cinerea infection when compared to wild type plants while *glk2* displayed an increase in susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 11H; Appendix Tables 7 & 9**). For the LL AM inoculations, *GLK1 OE* and *GLK2 OE* showed significantly decreased susceptibility to *B. cinerea* infection when compared to wild type plants whereas *glk1* displayed increased susceptibility (**Fig. 11I; Appendix Tables 7 & 9**).

Figure 11. The effects of the modifications in *GLK* loci on susceptibility of *A. thaliana* leaves to *Botrytis cinerea* infection. The susceptibility of *A. thaliana* leaves to *B. cinerea* infection was evaluated in wild type (WT), *GLK1* and *GLK2* overexpressors (OE) and *glk1*, *glk2*, *glk1&glk2* knockout (KO) *A. thaliana* plants grown either under long day conditions LD 24h (16h: light 8h: dark) (**A,B**), LD 21h (14h: light 7h: dark) (**C,D**), and LD 30h (20h: light 7h: dark) (**H**) or under short day conditions SD 24h (8h: light 16h: dark) (**E,F**), SD 21h (7h: light 14h: dark) (**G**) or LL constant light (24: light) (**I**). The inoculations were performed on plants grown at prevailing growth conditions during different time of the day either in the morning (AM) (**A, C, E, G, H, I**) or in the evening (PM) (**B, D, F**). Detached leaves were inoculated with (6 µl) spore suspension (1 x 10^6 spores/ml). Lesion areas were measured 52h post- infection (hpi) with *B. cinerea*. The stars indicate a difference in susceptibility to *B. cinerea* of clock mutants compared to the wild type for a given photoperiod and time of infection at a P-value of ≤0.05 (One-way ANOVA test or Student's t-test).


WT
GLK1 OE
glk1
GLK2 OE
glk2
glk1/ glk2
Lesions diameter (mm)
A
LD (24h, am)
B
LD (24h, pm)
C
LD (21h, am)
D
LD (21h, pm)
E
SD (24h, am)
F
SD (24h, pm)
G
SD (21h, am)
H
LD (30h, am)
I
LL(am)
*

We compared the susceptibility of *B. cinerea* infection in leaves of *Arabidopsis* for wild type and *GLKs* mutants at 72hpi. The data used in figure 12 were from the same experiments, but measurements were taken at 72h. For the LD 24h AM inoculations, all GLK mutants except *glk1/glk2* displayed decreased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 12A; Appendix Tables 8 & 9**). For the LD 24h PM inoculations, *GLK1 OE* displayed decreased susceptibility to *B. cinerea* infection while *glk1/glk2* displayed increased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 12B; Appendix Table 9**). For the SD 24h AM inoculations, *GLK1 OE* and *glk1/glk2* displayed decreased susceptibility to *B.* cinerea infection when compared to wild type plants (**Fig. 12E; Appendix Tables 8 & 9**). For the SD 24h PM inoculations, *GLK1 OE* and *glk1/glk2* displayed decreased susceptibility to *B.* cinerea infection when compared to wild type plants (**Fig. 12F; Appendix Table 8**).

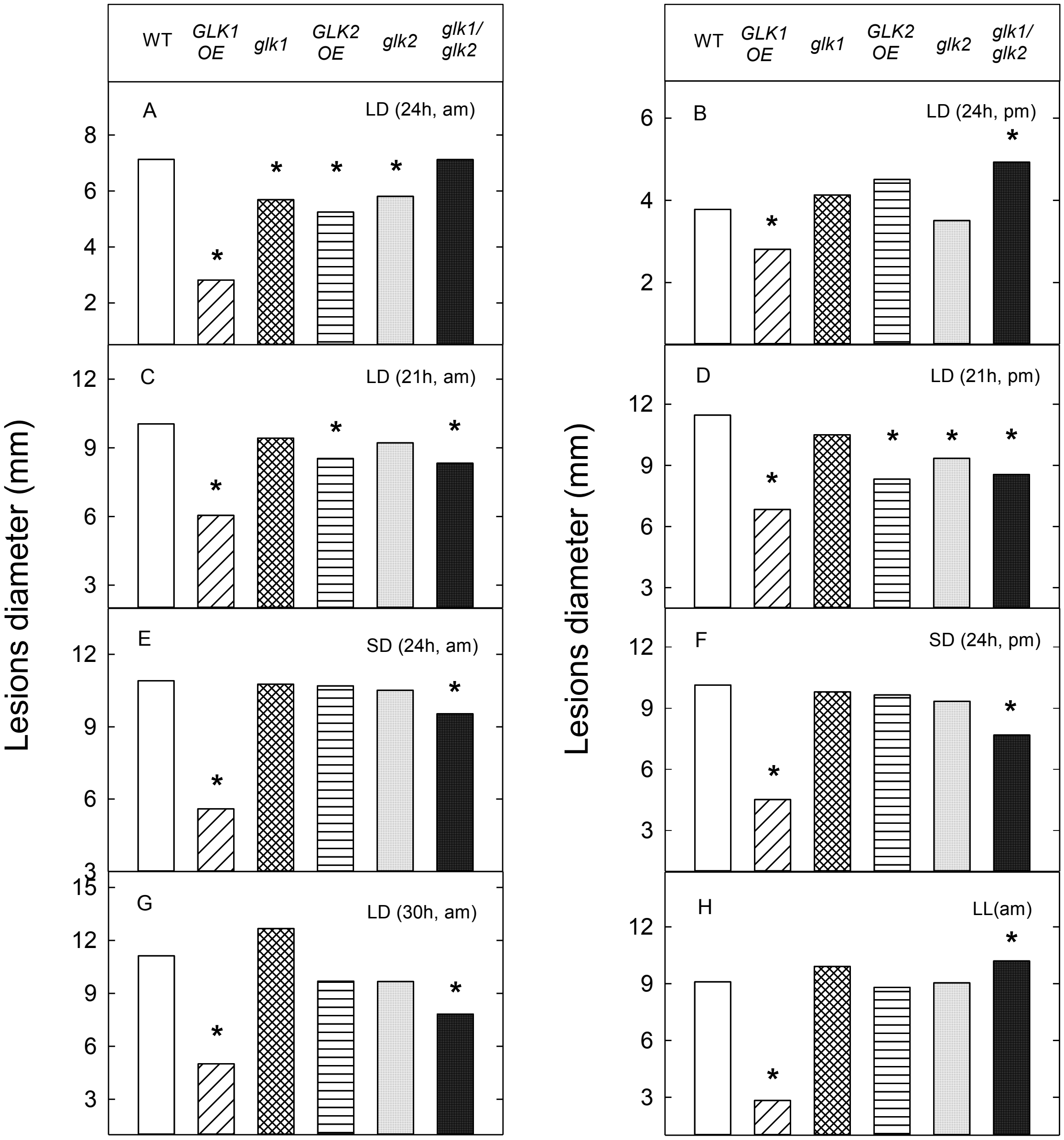
For the LD 21h AM inoculations, *GLK1 OE, GLK2 OE* and *glk1/glk2* displayed decreased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 12C; Appendix Table 8**). For the LD 21h PM inoculation, all GLKs mutants except *glk1* displayed significantly decreased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 12D; Appendix Table 8**). For the LD 30h AM inoculations, *GLK1 OE* and *glk1/glk2* displayed decreased susceptibility to *B. cinerea* infection when compared to wild type plants (**Fig. 12G; Appendix Tables 8 & 9**). For the LL AM inoculations, *GLK1 OE* displayed decreased susceptibility to *B. cinerea* infection when compared to wild type plants while *glk1/glk2* displayed increased susceptibility (**Fig. 12H; Appendix Table 8)**.

Our observations at 72hpi differed from those at 52hpi. Under normal earth conditions in the LD 24h AM inoculations we observed a decrease in susceptibility for *glk1/glk2* compared to wild type at 52hpi but no difference at 72hpi (**Fig. 11 & 12A;**

Appendix Tables 7 & 8). For the SD 24h AM inoculations there was no observable difference in susceptibility between *glk1/glk2* and the wild type at 52hpi but an observable difference at 72hpi (**Fig. 11 & 12E; Appendix Tables 7 & 8**). For the SD 24h PM inoculations, we observed differences in susceptibility between *glk1* and *glk2* compared to the wild type at 52hpi but not at 72hpi (**Fig. 11 & 12F; Appendix Tables 7 & 8**).

We also observed different susceptibility to *B. cinerea* infection in different cycle photoperiods (LD 21h, LD 30h, and LL) at 52hpi and 72hpi. For the LD 21h AM inoculations, there was no observable difference in susceptibility between *GLK2 OE* and *glk1/glk2* compared to the wild type at 52hpi but an observable difference at 72hpi (**Fig. 11 & 12C; Appendix Tables 7 & 8**). For the LD 30h PM inoculations, there was an increase in susceptibility for *glk2* when compared to the wild type at 52hpi but no difference at 72hpi (**Fig. 11 & 12H & G; Appendix Tables 7 & 8**).

Figure 12. The effects of the modifications in GLK loci on susceptibility of *A. thaliana* leaves to *Botrytis cinerea* infection. The susceptibility of *A. thaliana* leaves to *B. cinerea* infection was evaluated in wild type (WT), *GLK1* and *GLK2* overexpressors (OE) and *glk1*, *glk2*, *glk1&glk2* knockout (KO) *A. thaliana* plants grown either under long day conditions LD 24h (16h: light 8h: dark) (**A,B**), LD 21h (14h: light 7h: dark) (**C,D**), and LD 30h (20h: light 7h: dark) (**G**) or under short day conditions SD 24h (8h: light 16h: dark) (**E,F**), or LL constant light (24:light) (**H**). The inoculations were performed on plant grown at prevailing growth conditions during different time of the day either in the morning (AM) (**A, C, E, G, H, I**) or in the evening (PM) (**B, D, F**). Detached leaves were inoculated with (6 µl) spore suspension (1 x 10^6 spores/ml). Lesion areas were measured 72h post- infection (hpi) with *B. cinerea*. The stars indicate a difference in susceptibility to *B. cinerea* of clock mutants compared to the wild type for a given photoperiod and time of infection at a P-value of ≤ 0.05 (One-way ANOVA test or Student's t-test).


WT
GLK1 OE
glk1
GLK2 OE
glk2
glk1/ glk2
A
LD (24h, am)
B
LD (24h, pm)
C
LD (21h, am)
D
LD (21h, pm)
E
SD (24h, am)
F
SD (24h, pm)
G
LD (30h, am)
H
LL(am)
Lesions diameter (mm)
Lesions diameter (mm)
*
2
3
4
6
8
9
12
15

3.3 The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* under normal earth conditions in wild type and mutant plants

In order to test the theory that the biological clock controls plant immunity we compared the level of susceptibility to *B. cinerea* infection of the *Arabidopsis* wild type plant, clock and *GLKs* mutants in normal earth conditions in the LD 24h and SD 24h photoperiods at 52hpi and 72hpi. The question addressed is whether or not plants grown under normal earth conditions (LD24h and SD24h) will have similar levels of the susceptibility at 52hpi and 72hpi for the AM and PM inoculations. The data here represents a combination of all data from figures 7-9, 11-12.

In both the LD 24h and the SD 24h photoperiod conditions wild type plants have shown increased susceptibility to *B. cinerea* infection after AM inoculations when compared to PM inoculations at 52hpi and 72hpi (**Fig. 13A**). Regardless of the time of inoculations, short day plants displayed higher susceptibility to *B. cinerea* infection than long day plants at both 52hpi and 72hpi (**Fig. 13A; Appendix Table 10**).

For the LD 24h photoperiod condition, *toc-1* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at both 52hpi and 72hpi. However, there were observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations in the SD 24 at both 52hpi and 72hpi, which were similar to the response of wild type plants grown at the same conditions (**Fig. 13B)**. Similarly to the wild type plants, regardless of the time of inoculations, short day plants displayed higher susceptibility to *B. cinerea* infection than long day plants at both 52hpi and 72hpi (**Fig. 13B; Appendix Table 10**).

For the LD 24h photoperiod condition, *cca1/lhy* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at both measurement times (52hpi and 72hpi). In the SD 24h photoperiod condition, *cca1/lhy* also displayed no observable difference in susceptibility to *B. cinerea* infection between AM and PM inoculations at 52hpi, but difference were observed at 72hpi (**Fig. 13C)**. Regardless of the time of inoculations, the growth of the *cca1/lhy* plant in the SD 24h photoperiod increased susceptibility to *B. cinerea* infection compared to LD 24h photoperiod (**Fig. 13C; Appendix Table 10**).

For the LD 24h photoperiod condition, *cca1/toc-1* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at 52 hpi, but differences in susceptibility became pounced at 72hpi. In contrast to the wild type, *cca1/toc-1* plants grown at SD 24h photoperiod condition displayed no observable differences in susceptibility to *B. cinerea* infection in response to the AM and PM inoculations (**Fig. 13D**). Regardless to the time of inoculations, the growth of the *cca1/toc-1* plants in the SD 24h photoperiod increased susceptibility to *B. cinerea* infection when compared to the LD 24h photoperiod (**Fig. 13D; Appendix Table 10**).

Similarly to wild type plants, *lhy/toc-1* plants grown at the LD 24h photoperiod condition showed increased susceptibility to *B. cinerea* infection after AM inoculations when compared to PM inoculations at both 52hpi and 72hpi. In contrast to the wild type, *lhy/ toc-1* plants grown at SD 24h photoperiod condition showed higher susceptibility to *B. cinerea* infection after evening inoculations than the morning inoculations at 52hpi. However, no differences in susceptibility to *B. cinerea* infection have been observed at 72hpi when effects of AM and PM inoculations were compared (**Fig. 13E**). Similarly to the wild type, the growth of the *lhy*/*toc-1* plants at the SD 24h photoperiod increased susceptibility to *B. cinerea* infection compared to LD 24h photoperiod independent from the time of inoculations (**Fig. 13E; Appendix Table 10**).

Interestingly, the changes in susceptibility to *B. cinerea* infection in the triple knockout *cca1/lhy/toc-1* in response to the time of inoculations and the length of the day were similar to that observed in wild type plants grown in the similar growth conditions (**Fig. 13F**).

For the LD 24h photoperiod condition, *GLK1 OE* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at both 52hpi and 72hpi. However, there were observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations in the SD 24 at both 52hpi and 72hpi, which were similar to the response of wild type plants grown at the same conditions (**Fig. 13G)**. Similarly to the wild type, the growth of the *GLK1 OE* plants in the SD 24h photoperiod increased susceptibility to *B. cinerea* infection compared to LD 24h photoperiod independent from the time of inoculations (**Fig. 13G; Appendix Table 10**).

The changes in susceptibility to *B. cinerea* infection in *glk1* plants in response to the time of inoculations and the length of the day were similar to that observed in wild type and the triple knockout *cca1/lhy/toc-1* plants grown in the similar growth conditions (**Fig. 13H**).

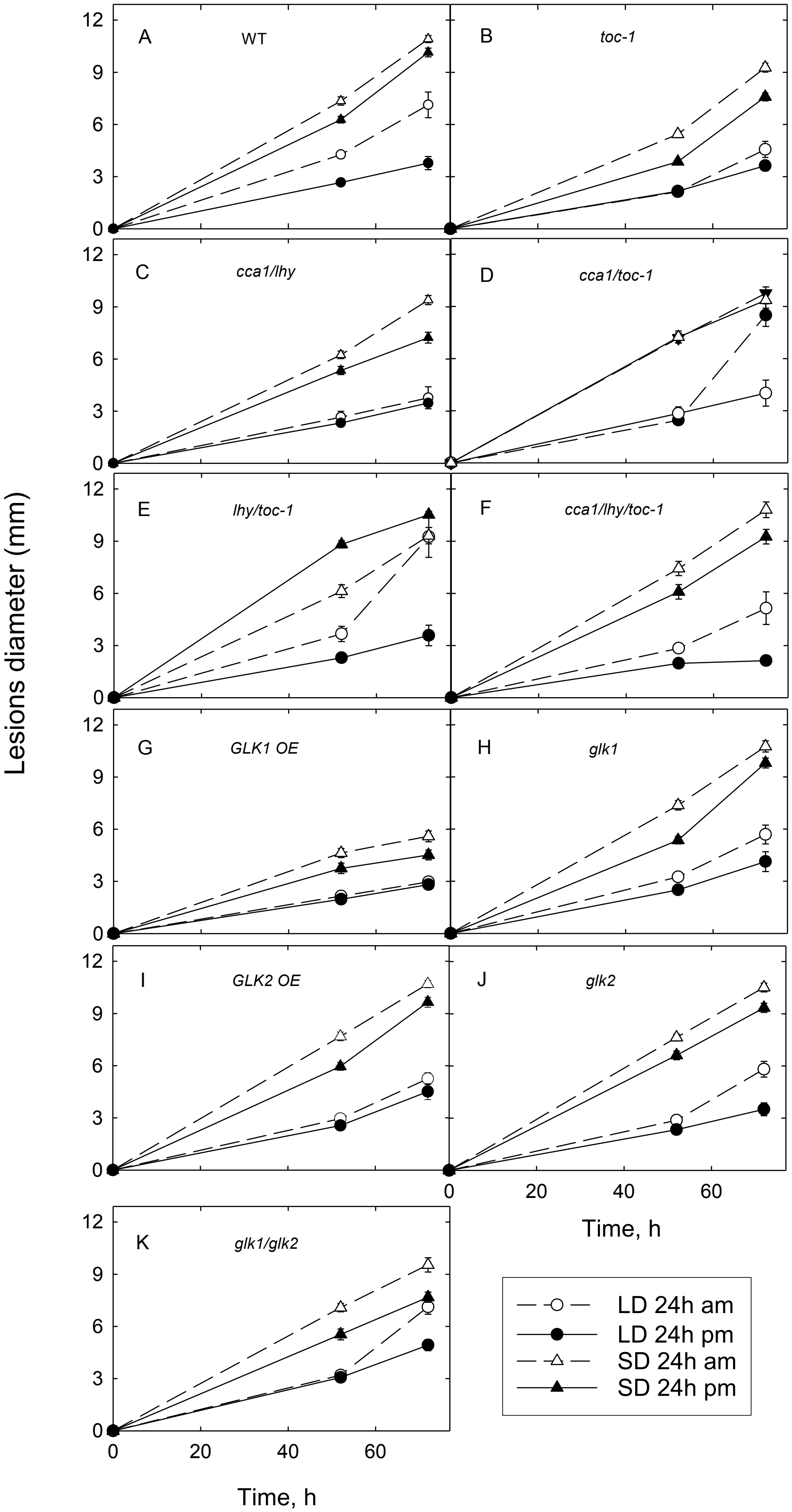
For the LD 24h photoperiod condition, *GLK2 OE* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at 52hpi and 72hpi. For the SD 24h photoperiod condition, *GLK2 OE* showed differences in in response to the time of inoculations with respect to susceptibility to *B. cinerea* (**Fig. 13I**). Independent from the time of inoculations, the growth of the *GLK2 OE* plant in the SD 24h photoperiod increased susceptibility to *B. cinerea* infection compared to LD 24h photoperiod (**Fig. 13I; Appendix Table 10**). The changes in susceptibility to *B. cinerea* infection in *GLK2 OE* plants in response to the time of inoculations and the length of the day were similar to that observed in *GLK1 OE, cca1/lhy,* and *toc-1* plants grown in the similar growth conditions (**Fig. 13B, C, G, I**).

For the LD 24h photoperiod condition, *glk2* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at 52hpi, but differences have been observed at 72hpi. For the SD 24h photoperiod condition, displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations in both 52hpi and 72hpi (**Fig. 13J**). The growth of the *glk2* plant in the SD 24h photoperiod increased susceptibility to *B. cinerea* infection compared to LD 24h photoperiod for either AM or PM inoculations (**Fig. 13J; Appendix Table 10**).

For the LD 24h photoperiod condition, *glk1/glk2* displayed no observable differences in susceptibility to *B. cinerea* infection between AM and PM inoculations at 52hpi, but differences at 72hpi. For the SD 24h photoperiod condition, *glk1/glk2* displayed increased susceptibility to *B. cinerea* infection in response to the AM inoculations compared to the PM inoculations at 52hpi and 72hpi (**Fig. 13K**). The growth of the *glk1/glk2* plant in the SD 24h photoperiod increased susceptibility to *B. cinerea* infection compared to LD 24h photoperiod for either AM or PM inoculations (**Fig. 13K; Appendix Table 10**).

We have shown that in both the LD 24h and the SD 24h photoperiod conditions wild type plants have shown increased susceptibility to *B. cinerea* infection after AM inoculations when compared to PM inoculations and regardless of the time of inoculations, short day plants displayed higher susceptibility to *B. cinerea* infection than long day plants. Modifications of the central oscillator and *GLK* loci altered this basic response of wild type such that (a) the differences in response to the time of inoculations observed in wild type plants grown in LD 24h conditions disappeared in *toc-1*, *cca1/lhy*, *glk1*, *GLK1* OE, and *GLK2 OE*, (b) the differences in response to the time of inoculations observed in wild type plants grown in SD 24h conditions disappeared in *cca1/toc-1*, and were reversed in *lhy/toc-1*. However, modifications of the central oscillator and *GLK* loci did not change the fact that growth of plants under the short day conditions promotes the susceptibility to *B. cinerea* infection.

Figure 13. The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* in leaves of *A. thaliana* wild type, circadian clock mutants and *GLK* loci mutants and overexpressors. All plants have been grown at either long day conditions (LD 24h (16 h: light 8h: dark) (circles) or short day conditions (SD 24h (8h: light 16h: dark) (triangles). Inoculations of leaves with *B. cinerea* have been performed either in the morning (AM) (solid line) or in the evening (PM) (dashed line). The susceptibility of *A. thaliana* leaves to *B. cinerea* infection have been evaluated in wild type (WT) (**A**), circadian clock mutants: *toc-1* (**B**), *cca1/lhy* (**C**), *cca1/toc-1* (**D**), *lhy/toc-1* (**E**), *cca1/lhy/toc-1* (**F**), *GLK1* (**G**), *GLK2* (**I**) overexpressors and *glk1*(**H**), *glk2* (**J**), *glk1&glk2* (**K**) knockout plants. Leaves were measured for lesion size at 52h and 72h post -infection 52 & 72(hpi). Time, h, represents hours post inoculation by *B. cinerea*. Statistical analysis has been performed using (One-way ANOVA test) at a P-value of ≤0.05 and ≤0.01. Statistical analysis is presented in Appendix Table 10 indicating significant differences in susceptibility to *B. cinerea* of wild type, circadian clock mutants and *GLK* loci mutants and overexpressors at either long day conditions or short day conditions at either in the morning or in the evening.


Lesions diameter (mm)
A WT
B toc-1
C cca1/lhy
D cca1/toc-1
E lhy/toc-1
F cca1/lhy/toc-1
G GLK1 OE
H glk1
I GLK2 OE
J glk2
K glk1/glk2
0
3
6
9
12
0
20
40
60
Time, h
LD 24h am
LD 24h pm
SD 24h am
SD 24h pm

CHAPTER 4

4. DISCUSSION

4.1 Time of infection influences susceptibility to *B. cinerea*

The circadian clock network in *Arabidopsis* regulates several biological processes. To date, very few studies have examined the role of the circadian clock in plant defense (Bhardwaj et al., 2011; Wang et al., 2011). As a result, little is known about circadian clock regulated plant immune defense in *Arabidopsis thaliana*. We used the aggressive, necrotrophic pathogen *Botrytis cinerea* to kill plant tissues in order to show that the role played by the biological clock in controlling defense to *B. cinerea* is a robust one. Overall, we provided evidence that wild type *Arabidopsis* plants infected in the morning showed the greatest susceptibility to *B. cinerea* in both normal earth conditions (LD 24h and SD 24h). In addition, infections performed on the plants grown at SD 24h led to much greater susceptibility to *B. cinerea* than infections performed on the plants grown at LD 24h photoperiod. We also used mutations in the central oscillator and modifications in GLK loci that altered the susceptibility to *B. cinerea*.

Though plants encounter different pathogens at different times in a day, *Arabidopsis* plants appear to be more susceptible in the morning than in the evening (**Fig. 7A; Appendix Table 1**). Bhardwaj et al., 2011 and Griebel & Zeier 2008 observed that wild type plants showed enhanced resistance when infiltrated with *Pseudomonas syringe* during the day than at night. Also, similar data was obtained by (Zhang et al., 2013) who observed that wild type plants infiltrated with *P. syringae* showed enhanced resistance during the day. Their data illustrated that the strong activation of defenses observed in the morning and midday inoculation compared to evening and night inoculation, result in higher salicylic acid accumulation, expression of pathogenesis-related genes, and hypersensitive response lesion development (Griebel & Zeier, 2008). Moreover, Bhardwaj et al. 2011 observed that the levels of PAMP-triggered callose deposition were higher in *Arabidopsis* plants infected with *P. syringae* in the morning than in the evening under constant light conditions. They showed that PAMP-triggered cell wall defense responses are modulated by the circadian clock, leading to the differential accumulation of pathogenesis- related genes at different times of the day. They therefore hypothesized that the *Arabidopsis* innate immune system may be primed by the circadian clock to respond most strongly to the detection of PAMPs in the morning. However, our data are in agreement with the data obtained by Zhang et al., 2013, who observed that wild type plants showed enhanced resistance at night than in the morning when spray-infected with *P. syringae*. Together, these opposite results suggest that plants use different mechanisms to defend against pathogen at different time of the day, depending on the mode of pathogen invasion.

It is a known fact that the stomata of plants are controlled by the circadian clock: opened at dawn and closed at dusk (Hotta et al., 2007; Webb, 2003). The stomata represent a natural entry site for pathogens such as *B. cinerea* into the host tissue (van Kan, 2006). Another possible explanation for differences in susceptibility during the day is the physiological state of the plant stomata. According to Zhang et al., 2013, stomatal pore size in *Arabidopsis* was smaller in the evening than in the morning. They then suggested a model for crosstalk between the circadian clock and plant innate immunity. At night, plants might depend more on closed stomata to limit pathogen invasion, however, plants have lower level of stomata-independent defense. Thus, they speculated that stomata-dependent defense could be stronger at night while stomata-independent defense could be stronger during the day. They concluded that plants depend on different mechanisms to defend against pathogen at different time of the day. Stomata-dependent and stomata-independent defenses are importation for the response to pathogen attack at different times of day (Zhang et al., 2013). We speculated that the higher susceptibility to *B. cinerea* in the morning was caused by the larger plant stomata opening, allowing *B. cinerea* to more easily gain entry into the host tissue. Taken together, these data indicate that the time of infection might play a role in controlling plant immune defense against *B. cinerea*.

4.2 Day length (photoperiod) influences susceptibility to *B. cinerea*

Our results showed that wild type plants as well as clock and *GLK* mutants grown in the SD 24h photoperiod showed increased susceptibility to *B. cinerea* compared to those grown in the LD 24h photoperiod as shown in (**Fig. 13; Appendix Table 11**). The LD 24h consists of 16h of light and 8h of dark, whereas SD 24h consists of 8h of light and 16h of dark, suggesting that day/night length controls susceptibility to *B. cinerea* infection. One possible explanation for these differences is that susceptibility is controlled by different output pathways that are related to the circadian clock regulatory mechanism. For example, studies have shown that *Arabidopsis* flowers earlier under long day conditions than short day conditions (Hicks et al., 2001; Strayer et al., 2000). In addition, under laboratory conditions, the germination of *B. cinerea* spores can be induced by incubation for three days in continued darkness. In our experiments, plates of *B. cinerea* were kept for 3 days in 14 h of darkness and 10 h of light (El Oirdi & Bouarab, 2007). This may explain why the SD 24h plant condition promotes susceptibility to *B. cinerea* infection since the favorable growth condition for *B. cinerea* (darkness) is longer in SD 24h condition than in the LD 24h condition. However, recent studies have suggested that light quality influences the regulation of the three MYC transcription factors (TFs) that are required for JA-mediated defenses against *B. cinerea* and are targets of JAZ repressors (Chico et al., 2014). In these studies, a high (R: red/FR: far red) ratio led to the activation of the Pfr form and increased JA-dependent defenses by mediating the MYCs stability and leading to JA-mediated degradation of JAZ repressors. However, the low (R: FR) ratio led to inactive Pr form and inhibited JA-dependent defenses by degradation of MYCs and stabilizing JAZs (Chico et al., 2014). Thus, this regulation of JA- dependent defenses by different light quality through the alteration of MYC and JAZ stability would explain at the molecular level the shade-triggered inhibition of JA sensitivity and JA- mediated defenses, resulting increasing susceptibility to *B. cinerea*. Our observation suggested that day length regulates susceptibility to *B. cinerea* infection.

Further analyses were conducted in order to determine whether day length or light entrainment of the biological clock influences plant defense to *B. cinerea*. Our observations indicated that placing clock mutants under the LD 30h (20h: light 10h: dark) photoperiod increases susceptibility to *B. cinerea* infection. A similar response was observed in the SD 24h (8h: light 16h: dark) photoperiod (**Fig. 7A-F; Appendix Tables 1 & 11**). Moreover, our results also showed consistently similar levels of susceptibility to *B. cinerea* infection when plants were exposed to the LL photoperiod (24h: light) and the LD 24h photoperiod (18h: light 6h: dark) (**Fig. 7 & 10; Appendix Tables 2 & 6**). Based on our observations, we summarized the effect of the modification of light in wild type plants as well as clock and *GLK* mutants (**Appendix Table 11**). Plants grown in the SD 24h condition showed a higher susceptibility to *B. cinerea* than plants grown in the LD 24h condition as shown in wild type, central oscillator and *GLK* mutants (**Appendix Table 11**). In addition, we compared plants grown in the LD 24h and LL and we observed similar susceptibility responses in WT and all mutants, suggesting that the length of day and night control susceptibility to *B. cinerea* (**Appendix Table 11**). Moreover, when we used different photoperiod cycles, we observed variations in response to the level of susceptibility to *B. cinerea* in wild type and all mutants which suggested that entrainment of biological clock is controlled by light rather than the length of day/night.

4.3 Genetic mutation of the clock core components influences susceptibility to *B. cinerea*

To determine whether or not the deletion of clock core genes is responsible for controlling susceptibility to *B. cinerea*, we used the single knockout of the evening gene *toc-1* and the double knockout of the morning genes *cca1/lhy* or combinations of these mutations: *cca1/toc-1, lhy/toc-1,* and *cca1/lhy/toc-1*. We observed that *toc-1* and *cca1/lhy* displayed significantly less susceptibility compared to wild type plants in some photoperiod conditions (**Fig. 8 & 9; Appendix Tables 3 & 4**). We compared the central oscillator (core loop) mutants and wild type at 52hpi, in comparison between WT and the single knockout of the evening gene toc-1, we found the level of susceptibility to *B. cinerea* decreased in all conditions were used as shown in (**Appendix Table 12**), where no change was observed in this condition LD (30h, pm). In the double knockout of the morning genes *cca1/lhy*, we found the level of susceptibility to *B. cinerea* decreased in these condition: LD (24h, am), LD (21h, am & pm), SD (24h, pm), and LL (am) and an increasing the susceptibility in one condition where no change was observed in these condition: LD (24h, pm), LD (30h, am), and SD (24h, am) (**Appendix Table 12**). Also, in the triple knockout cca1/lhy/toc-1 we observed different response in the level of susceptibility to *B. cinerea* as shown in the table (**Appendix Table 12**). Combinations of these genes (*cca1/toc-1, lhy/toc-1,* and *cca1/lhy/toc-1*) grown under different photoperiods, showed different responses to *B. cinerea* (**Appendix

Table 12). If *TOC1* or *CCA1/LHY* genes are responsible for controlling plant defense to *B. cinerea* we should see consistent susceptibility responses to *B. cinerea.*

CCA1 was the first clock component shown to influence plant defense responses to *HPA Emwal* and *Pst* DC3000 (Bhardwaj et al., 2011; Wang et al., 2011). Wang et al., 2011 suggested that the overexpression of *CCA1* leads to an increase in resistance to *HPA Emwal*, whereas the *cca1* mutant causes decreased resistance to *HPA Emwal*. However, Bhardwaj et al., 2011 found that overexpression of *CCA1* resulted in decreased resistance to *Pst* DC3000 during the day. In addition, there is evidence that overexpression of *CCA1* enhances susceptibility to *PmaDG3* compared to Co1-0 with infiltration infection in LD or LL (Zhang et al., 2013).. Also, they observed *CCA1ox* plants were also more susceptible than Col-0 to *PmaDG3* when spray-infected at ZT1 or ZT13 in LD (Zhang et al., 2013). There is also evidence that overexpression of *LHY* increases susceptibility to *Pst* DC3000 than *Landsberg erecta* (*Ler*) background with infiltration infection either at ZT1 or ZT13 in LD and also they obtained similar results with spray-infection (Zhang et al., 2013). Thus, these data strongly support a direct role of the circadian clock in plant defense. We can conclude that these variations in response to those pathogens are a function of output pathways and not the core central oscillator mechanism. It is clear from our results that the core clock genes are not solely responsible for susceptibility to *B. cinerea*.

4.4 *GLKs* genes are involved in the regulation of the susceptibility to *B. cinerea* in biological clock controlled manner

On one hand we see increasing evidence that supports the role of *GLK* genes in plant defense, as they have been shown to elicit a response against *F. graminearum*, a hemi-biotrophic pathogen (Savitch et al., 2007) and *B. cinerea*, a necrotrophic pathogen (Murmu et al., 2014). On the other hand, unpublished evidence suggests that alterations in *GLK* loci affect diurnal changes in circadian rhythmicity and the transcription of photosynthesis related genes, pathogenesis and defense related genes, cold-response related genes and major flowering locus related genes (Sprott et al. 2010, unpublished). *GLKs* play a role in plant defense and these genes alter the defense response through the regulation of the circadian clock. We observed that *GLK1 OE* was less susceptible to *B. cinerea* when compared to wild type plants at either 52hpi or 72hpi among different photoperiods (**Fig. 11 & 12; Appendix Tables 7 & 8**). Our data are in broad agreement with those previously reported in *GLK1 OE* (Murmu et al., 2014). However, we did not observe the opposite susceptibility response to *B. cinerea* with *glk1 or glk1/glk2* in some photoperiod conditions either at 52hpi or 72hpi (**Fig. 11 & 12; Appendix Tables 7 & 8**). When we compared wild type *Arabidopsis* to *GLK1* overexpressors and *glk1/glk2* mutants, we observed different responses in the level of susceptibility to *B. cinerea* in different photoperiod conditions (**Appendix Table 13**). In *GLK1 OE* plants, the level of susceptibility to *B. cinerea* decreased in all photoperiod conditions, while *GLK2 OE* plants showed a decrease in susceptibility to *B. cinerea* in certain conditions: LD (24h, am), LD (21h, pm), and LL (am) and no change in these conditions: LD (24h, pm), LD (21h, am), SD (24h, am & pm), SD (21h, am), and LD (30h, am) (**Appendix Table 13)**. In the *glk1/glk2* double knockout, the level of susceptibility to *B. cinerea* decreased in certain conditions: LD (24h, am), LD (21h, pm), SD (24h, pm), and LD (30h, am), increased in one condition, and showed no change in another condition: LD (21h, am), SD (24h, am), SD (21h, am), and LL (am) (**Appendix Table 13)**. Together these data lead us to speculate that differences in susceptibility responses to *B. cinerea* in *GLK* mutants are caused by the role of the biological clock in plant defense.

4.5 Conclusions

Our data clearly demonstrate that the circadian clock manipulates susceptibility to *B. cinerea* in clock mutants and *GLK* mutants in the leaves of *A. thaliana* in different photoperiod conditions. Overall, time of infection influences susceptibility to *B. cinerea*. Differences in plant defense responses are caused by the output pathways of the circadian system. The input pathway; light and temperature, signals entrain the plant circadian clock and the clock output pathways alter the function of the circadian oscillator. Overall, susceptibility to *B. cinerea* was higher in the morning inoculations than evening inoculations. Plants grown in the SD 24h condition showed a higher susceptibility to *B. cinerea* than plants grown in the LD 24h condition. One could suggest that day length controls plant defense, however, different responses in entrainment cycles LD 21h, LD 30h, SD21, LD21 and LL clearly demonstrate that it is not the day/night length that controls plant immunity, but rather the light entrainment of the biological clock. Our observations allowed us to determine that the deletion of core oscillator genes and *GLK* genes are probably not directly involved in the regulation of susceptibility to *B. cinerea*. Instead, we suggest that the ability of light to entrain the biological clock leads to the control of plant immunity. Our results indicate that the circadian clock is the regulator of the plant-pathogen interactions and defense responses.

4.6 Future work

It is essential to understand the link between plant defense and the circadian clock. Based on our results, the next step would consist in the assessment of gene expression profiles by identifying the cause of the differences in the susceptibility to *B. cinerea* of contrasting clock mutants and *GLKs* mutants under normal earth conditions (LD 24h and SD

24h). Two marker genes of SA and JA compound pathogen signaling (*PR1* and *PDF1)* could be used in order to assess the gene expression of plant defense genes using qPCR analysis. Moreover, it is well known that the circadian clock regulates the stomata aperture, which is opened at dawn and closed at dusk (Hotta et al., 2007; Webb, 2003). Based on our results, it would be of interest to measure stomatal aperture in wild type plant and all mutants in the morning and evening inoculations under normal earth conditions in order to test whether stomatal opening is related to susceptible in the morning. Stomatal opening can be measured by following the protocol as described by (Melotto et al., 2004). Further analysis would be essential to understand the link between the core clock genes and the *GLK* genes. Whether these genes are also on the output pathways of the circadian clock regulation remains to be answered. Our understanding would be increased by the study of plants with both clock mutations and *GLK* overexpressors or mutants. This can be done by crossing the plants as described in the protocol by (Cifuentes et al., 2013). Such information could possibly lead to a better control of plant resistance to pathogens, enhancing productivity of plants.

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Appendix

Table 1. The effect of morning and evening inoculations on susceptibility to *Botrytis cinerea* in leaves of *Arabidopsis thaliana* wild type and circadian clock mutants grown under different photoperiods conditions. Statistical analyses were performed using Student's t-test in (3-6) biological replicates with (30-60) leaves. P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01

Figure 7A. WT		
Treatment	P adj	Significance
LD 24h (AM/PM)	0.000	**
LD 21h (AM/PM)	0.829	
LD 30h (AM/PM)	0.000	**
SD 24h(AM/PM)	0.001	**
Figure 7B. ***toc-1***		
LD 24h (AM/PM)	0.815	
LD 21h (AM/PM)	0.014	*
LD 30h (AM/PM)	0.184	
SD 24h(AM/PM)	0.000	**
Figure 7C. ***cca1/lhy***		
LD 24h (AM/PM)	0.200	
LD 21h (AM/PM)	0.453	
LD 30h (AM/PM)	0.077	
SD 24h (AM/PM)	0.131	
Figure 7D. ***cca1/toc-1***		
LD 24h (AM/PM)	0.366	
LD 21h (AM/PM)	0.000	**
LD 30h (AM/PM)	0.001	**
SD 24h (AM/PM)	0.913	
Figure 7E. ***lhy/toc-1***		
LD 24h (AM/PM)	0.003	**
LD 21h (AM/PM)	0.336	
LD 30h (AM/PM)	0.000	**
SD 24h (AM/PM)	0.000	**
Figure 7F. ***lhy/toc1/cca1***		
LD 24h (AM/PM)	0.000	**
LD 21h (AM/PM)	0.000	**
LD 30h (AM/PM)	0.000	**
SD 24h (AM/PM)	0.018	**

Table 2. The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* in leaves of *Arabidopsis thaliana* wild type and circadian clock mutants. Statistical analyses were performed using a One-way ANOVA in (3-6) biological replicates with (30-60) leaves. Diff=differences, Lwr=lower, Upr=upper, P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 7A. WT AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.096	-2.849	0.657	0.409	
LD30H/LD21H	2.028	-0.545	4.601	0.188	
LL24H/LD21H	-1.598	-3.563	0.367	0.164	
SD24H/LD21H	2.001	0.479	3.523	0.004	**
LD30H/LD24H	3.1235	0.597	5.650	0.008	**
LL24H/LD24H	-0.503	-2.407	1.401	0.946	
SD24H/LD24H	3.096	1.654	4.539	0.000	**
LL24H/LD30H	-3.626	-6.304	0.948	0.003	**
SD24H/LD30H	-0.027	-2.399	2.345	1.000	
SD24H/LL24H	3.5990	1.905	5.293	0.000	**
Figure 7A. WT PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-2.708	-3.864	-1.552	0.000	**
LD30H/LD21H	-0.816	-3.022	1.391	0.764	
SD24H/LD21H	0.832	-0.190	1.853	0.149	
LD30H/LD24H	1.892	-0.379	4.164	0.135	
SD24H/LD24H	3.540	2.384	4.696	0.000	**
SD24H/LD30H	1.647	-0.559	3.853	0.210	
Figure 7B. *toc-1* AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.574	-3.098	-0.050	0.039	*
LD30H/LD21H	1.014	-0.412	2.440	0.284	
LL24H/LD21H	-1.474	-3.174	0.226	0.121	
SD24H/LD21H	1.828	0.468	3.188	0.003	**
LD30H/LD24H	2.588	1.321	3.854	0.000	**
LL24H/LD24H	0.100	-1.468	1.668	1.000	
SD24H/LD24H	3.402	2.210	4.593	0.000	**
LL24H/LD30H	-2.488	-3.962	-1.014	0.000	**
SD24H/LD30H	0.814	-0.250	1.877	0.216	
SD24H/LL24H	3.302	1.892	4.711	0.000	**
Figure 7B. *toc-1* PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-0.911	-1.674	-0.148	0.013	*
LD21H/LD30H	-0.899	-1.757	-0.041	0.036	*
SD24H/LD21H	0.696	0.076	1.316	0.021	*

LD24H/LD30H	-1.810	-2.770	-0.850	0.000	**
SD24H/LD24H	1.607	0.853	2.362	0.000	**
SD24H/LD30H	-0.203	-1.053	0.647	0.923	
Figure 7C. ***cca1/lhy*** **AM inoculation at 52hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.150	-4.038	1.738	0.780	
LD30H/LD21H	3.958	0.349	7.568	0.026	*
LL24H/LD21H	-1.058	-3.946	1.829	0.827	
SD24H/LD21H	2.460	0.033	4.887	0.046	*
LD30H/LD24H	5.108	1.800	8.417	0.001	**
LL24H/LD24H	0.092	-2.409	2.592	1.000	
SD24H/LD24H	3.610	1.659	5.561	0.000	**
LL24H/LD30H	-5.017	-8.325	-1.708	0.001	**
SD24H/LD30H	-1.498	-4.413	1.417	0.581	
SD24H/LL24H	3.518	1.567	5.470	0.000	**
Figure 7C. ***cca1/lhy*** **PM inoculation at 72hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.208	-4.329	1.912	0.718	
LD30H/LD21H	2.986	-0.617	6.589	0.131	
SD24H/LD21H	2.103	-0.630	4.835	0.177	
LD30H/LD24H	4.194	1.074	7.315	0.005	**
SD24H/LD24H	3.311	1.257	5.365	0.001	**
SD24H/LD30H	-0.883	-3.616	1.849	0.814	
Figure 7D. ***cca1/toc-1*** **AM inoculation at 52hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.847	-3.819	0.125	0.075	
LD30H/LD21H	4.367	2.713	6.021	0.000	**
LL24H/LD21H	-1.153	-2.700	0.394	0.225	
SD24H/LD21H	2.907	1.432	4.381	0.000	**
LD30H/LD24H	6.214	4.158	8.271	0.000	**
LL24H/LD24H	0.694	-1.277	2.666	0.848	
SD24H/LD24H	4.754	2.838	6.670	0.000	**
LL24H/LD30H	-5.520	-7.174	-3.866	0.000	**
SD24H /LD30H	-1.460	-3.047	0.126	0.083	
SD24H/LL24H	4.059	2.584	5.534	0.000	**
Figure 7D. ***cca1/toc-1*** **PM inoculation at 52hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-4.053	-6.768	-1.339	0.002	**
LD30H/LD21H	-0.338	-2.761	2.085	0.980	
SD24H/LD21H	0.454	-1.451	2.358	0.912	
LD30H/LD24H	3.715	0.479	6.952	0.020	*

SD24H /LD24H	4.507	1.638	7.376	0.001	**
SD24H /LD30H	0.792	-1.803	3.387	0.834	
Figure 7E. *lhy/toc-1*AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-3.833	-6.422	1.245	0.003	**
LD30H/LD21H	0.583	-1.872	3.039	0.947	
LL24H/LD21H	-2.903	-5.218	-0.588	0.011	*
SD24H/LD21H	-1.340	-3.655	0.975	0.421	
LD30H/LD24H	4.417	2.251	6.582	0.000	**
LL24H/LD24H	0.931	-1.074	2.936	0.623	
SD24H/LD24H	2.493	0.488	4.498	0.012	*
LL24H/LD30H	-3.486	-5.316	-1.656	0.000	**
SD24H/LD30H	-1.924	-3.754	-0.093	0.037	*
SD24H/LL24H	1.563	-0.075	3.200	0.065	
Figure 7E. *lhy/toc-1*PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-4.076	-5.693	-2.459	0.000	**
LD30H/LD21H	-1.420	-3.381	0.541	0.213	
SD24H /LD21H	2.424	0.807	4.041	0.002	**
LD30H/LD24H	2.656	0.437	4.874	0.015	*
SD24H/ LD24H	6.500	4.579	8.421	0.000	**
SD24H/ LD30H	3.844	1.626	6.063	0.000	**
Figure 7F. *lhy/toc1/cca1* AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-0.708	-2.616	1.200	0.814	
LD30H/LD21H	4.436	2.609	6.263	0.000	**
LL24H/LD21H	-0.357	-2.077	1.363	0.973	
SD24H/LD21H	3.918	2.235	5.600	0.000	**
LD30H/LD24H	5.144	3.318	6.971	0.000	**
LL24H/LD24H	0.351	-1.369	2.071	0.975	
SD24H/LD24H	4.626	2.943	6.309	0.000	**
LL24H/LD30H	-4.793	-6.423	-3.164	0.000	**
SD24H/LD30H	-0.519	-2.109	1.072	0.875	
SD24H/LL24H	4.275	2.809	5.741	0.000	**
Figure 7F. *lhy/toc1/cca1* PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-5.333	-7.569	-3.098	0.000	**
LD30H/LD21H	-1.257	-4.184	1.670	0.646	
SD24H/LD21H	-1.018	-3.060	1.025	0.531	
LD30H/LD24H	4.076	1.274	6.878	0.003	**
SD24H/LD24H	4.316	2.456	6.176	0.000	**

SD24H/LD30H	0.239	-2.412	2.890	0.995	

Table 3. The effect of mutations in the central oscillator of biological clocks on the susceptibility of *Arabidopsis thaliana* leaves to *Botrytis cinerea* infection. Statistical analyses were performed using a One-way ANOVA in (3-6) biological replicates with (30-60) leaves. Diff=differences, Lwr=lower, Upr=upper P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 8A. LD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	1.979	1.307	2.651	0.000	**
WT / *cca1/lhy*	1.525	0.497	2.553	0.006	**
WT / *cca1/toc-1*	1.708	0.535	2.881	0.007	**
WT / *lhy/toc-1*	0.500	-0.919	1.919	0.462	
WT / *cca1/lhy/toc-1*	1.367	0.344	2.390	0.012	*
Figure 8B. LD (24h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT/ *toc-1*	0.454	-0.117	1.025	0.115	
WT / *cca1/lhy*	0.413	-0.257	1.083	0.213	
WT / *cca1/toc-1*	-0.212	-1.178	0.754	0.650	
WT / *lhy/toc-1*	0.319	-0.446	1.084	0.394	
WT / *cca1/lhy/toc-1*	0.740	0.141	1.338	0.018	*
Figure 8C. LD (21h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	1.596	0.684	2.508	0.002	**
WT / *cca1/lhy*	1.566	0.479	2.653	0.008	**
WT / *cca1/toc-1*	1.052	0.344	1.760	0.006	**
WT/ *lhy/toc-1*	-2.142	-3.643	-0.642	0.009	**
WT / *cca1/lhy/toc-1*	1.849	0.865	2.834	0.001	**
Figure 8D. LD (21h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT/ *toc-1*	2.251	1.540	2.961	0.000	**
WT / *cca1/lhy*	1.913	-0.194	4.020	0.073	
WT / *cca1/toc-1*	-1.557	-2.688	-0.426	0.008	**
WT / *lhy/toc-1*	-1.049	-2.210	0.112	0.075	
WT / *cca1/lhy/toc-1*	-1.886	-3.438	-0.334	0.019	*
Figure 8E. LD (30h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	2.610	0.355	4.865	0.025	*
WT / *cca1/lhy*	-0.365	-2.350	1.621	0.637	
WT / *cca1/toc-1*	-1.287	-3.088	0.514	0.140	
WT / *lhy/toc-1*	-0.698	-1.991	0.595	0.235	
WT / *cca1/lhy/toc-1*	-0.559	-1.892	0.774	0.362	
Figure 8F. LD (30h, pm)					

Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	0.536	-1.494	2.566	0.573	
WT / *cca1/lhy*	-1.889	-3.408	-0.370	0.026	*
WT / *cca1/toc-1*	-2.035	-5.936	1.867	0.238	
WT / *lhy/toc-1*	-0.444	-2.250	1.361	0.532	
WT / *cca1/lhy/toc-1*	-1.444	-2.317	-0.572	0.010	**
Figure 8G. SD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	1.807	1.030	2.583	0.000	**
WT / *cca1/lhy*	0.954	-0.066	1.973	0.066	
WT / *cca1/toc-1*	0.035	-1.270	1.340	0.957	
WT / *lhy/toc-1*	1.087	-0.550	2.724	0.186	
WT / *cca1/lhy/toc-1*	-0.179	-1.588	1.231	0.798	
Figure 8H. SD (24h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	2.386	1.788	2.984	0.000	**
WT / *cca1/lhy*	0.872	-0.031	1.776	0.058	
WT / *cca1/toc1*	-1.179	-2.273	-0.086	0.035	*
WT / *lhy/toc-1*	-2.642	-3.867	-1.416	0.000	**
WT) / *cca1/lhy/toc-1*	-0.037	-1.132	1.059	0.946	
Figure 8I. LL (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	1.472	0.618	2.325	0.002	**
WT / *cca1/lhy*	1.026	-0.243	2.295	0.104	
WT / *cca1/toc1*	0.606	-0.397	1.609	0.218	
WT / *lhy/toc-1*	-0.838	-2.077	0.401	0.168	
WT/ *cca1/lhy/toc-1*	0.608	-0.440	1.657	0.235	

Table 4. The effect of mutations in the central oscillator of biological clocks on the susceptibility of *Arabidopsis thaliana* leaves to *Botrytis cinerea* infection. Statistical analyses were performed using a One-way ANOVA in (3-6) biological replicates with (30-60) leaves. Diff=differences, Lwr=lower, Upr=upper P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 9A. LD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	3.480	1.711	5.249	0.000	**
WT / *cca1/lhy*	4.330	2.009	6.650	0.002	**
WT / *cca1/toc-1*	-0.510	-4.768	3.748	0.789	
WT / *lhy/toc-1*	-0.594	-4.309	3.121	0.726	
WT / *cca1/lhy/toc-1*	2.917	0.027	5.807	0.048	*
Figure 9B. LD (24h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT/ *toc-1*	-0.165	-1.936	1.605	0.847	
WT / *cca1/lhy*	0.307	-1.469	2.083	0.718	
WT / *cca1/toc-1*	-0.193	-2.812	2.425	0.876	
WT / *lhy/toc-1*	0.140	-2.270	2.551	0.902	
WT / *cca1/lhy/toc-1*	1.581	0.009	3.153	0.049	*
Figure 9C. LD (21h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	4.626	3.813	5.439	0.000	**
WT / *cca1/lhy*	5.065	3.632	6.498	0.000	**
WT / *cca1/toc-1*	2.834	1.495	4.173	0.000	**
WT/ *lhy/toc-1*	-1.866	-3.597	-0.134	0.036	*
WT / *cca1/lhy/toc-1*	5.659	4.464	6.854	0.000	**
Figure 9D. LD (21h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT/ *toc-1*	6.228	5.232	7.224	0.000	**
WT / *cca1/lhy*	5.989	3.777	8.200	0.000	**
WT / *cca1/toc-1*	0.539	-0.694	1.772	0.382	
WT / *lhy/toc-1*	1.311	-0.042	2.664	0.057	
WT / *cca1/lhy/toc-1*	2.121	0.418	3.824	0.016	*
Figure 9E. LD (30h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	2.231	-1.083	5.546	0.176	
WT / *cca1/lhy*	0.458	-4.020	4.936	0.703	
WT / *cca1/toc-1*	-3.225	-7.080	0.630	0.084	
WT / *lhy/toc-1*	-0.208	NaN	NaN	NaN	
WT / *cca1/lhy/toc-1*	-1.181	-3.083	0.721	0.143	
Figure 9F. LD (30h, pm)					

Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	0.866	-0.990	2.722	0.323	
WT / *cca1/lhy*	-1.958	-3.942	0.025	0.052	
WT / *cca1/toc-1*	-2.875	-7.058	1.308	0.138	
WT / *lhy/toc-1*	-2.917	-4.714	-1.120	0.011	*
WT / *cca1/lhy/toc-1*	-2.617	-5.627	0.393	0.078	
Figure 9G. SD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	1.581	0.763	2.399	0.000	**
WT / *cca1/lhy*	1.523	0.628	2.418	0.001	**
WT / *cca1/toc-1*	1.185	0.014	2.356	0.048	*
WT / *lhy/toc-1*	1.452	0.174	2.730	0.027	*
WT / *cca1/lhy/toc-1*	0.548	-0.618	1.714	0.347	
Figure 9H. SD (24h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	2.534	1.698	3.369	0.000	**
WT / *cca1/lhy*	2.465	1.351	3.579	0.000	**
WT / *cca1/toc1*	0.429	-1.011	1.870	0.548	
WT / *lhy/toc-1*	-0.379	-1.755	0.997	0.578	
WT) / *cca1/lhy/toc-1*	0.879	-0.447	2.205	0.188	
Figure 9I. LL (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *toc-1*	3.558	2.011	5.105	0.000	**
WT / *cca1/lhy*	-0.243	-2.384	1.898	0.796	
WT / *cca1/toc1*	-1.195	-4.032	1.641	0.373	
WT / *lhy/toc-1*	-0.451	-2.340	1.438	0.597	
WT/ *cca1/lhy/toc-1*	-0.511	-2.573	1.551	0.596	

Table 5. The effect of morning and evening inoculations among the photoperiods on susceptibility to *Botrytis cinerea* in leaves of *Arabidopsis thaliana* wild type, *GLK1* and *GLK2* overexpressors, and *GLK1* knockout plants. Statistical analyses were performed using Student's t-test in (3-6) biological replicates with (30-60) leaves. P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 10A. WT		
Treatment	P adj	Significance
LD 24h (AM/PM)	0.000	**
LD 21h (AM/PM)	0.829	
SD 24h (AM/PM)	0.001	**
SD 21h (AM/PM)	0.135	
Figure 10B. *GLK1 OE*		
LD 24h (AM/PM)	0.733	
LD 21h (AM/PM)	0.437	
SD 24h (AM/PM)	0.021	*
SD 21h (AM/PM)	0.621	
Figure 10C. *glk1*		
LD 24h (AM/PM)	0.000	**
LD 21h (AM/PM)	0.009	**
SD 24h (AM/PM)	0.000	**
SD 21h (AM/PM)	0.023	*
Figure 10D. *GLK2 OE*		
LD 24h (AM/PM)	0.083	
LD 21h (AM/PM)	0.004	**
SD 24h (AM/PM)	0.000	**
SD 21h (AM/PM)	0.001	**

Table 6. The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* in leaves of *Arabidopsis thaliana* wild type, *GLK1* and *GLK2* overexpressors, and *GLK1* knockout plants. Statistical analyses were performed using a One-way ANOVA in (3-6) biological replicates with (30-60) leaves. Diff=differences, Lwr=lower, Upr=upper P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 10A. WT AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD21H/LL24H	1.598	-0.148	3.345	0.089	
LD24H/LL24H	0.407	-1.310	2.125	0.963	
SD21H/LL24H	2.807	0.987	4.628	0.000	**
SD24H/LL24H	3.468	1.956	4.979	0.000	**
LD24H/LD21H	-1.191	-2.777	0.395	0.230	
SD21H/LD21H	1.209	-0.487	2.905	0.278	
SD24H/LD21H	1.869	0.510	3.229	0.002	**
SD21H/LD24H	2.400	0.734	4.066	0.001	**
SD24H/LD24H	3.060	1.738	4.383	0.000	**
SD24H/SD21H	0.660	-0.792	2.113	0.708	
Figure 10A. WT PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-2.708	-3.876	-1.540	0.000	**
SD21H/LD21H	2.123	0.336	3.910	0.013	*
SD24HLD21H	0.832	-0.200	1.863	0.156	
SD21H/LD24H	4.831	2.962	6.700	0.000	**
SD24H/LD24H	3.540	2.372	4.707	0.000	**
SD24H/SD21H	-1.292	-3.079	0.495	0.236	
Figure 10B. *GLK1 OE* AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-2.022	-3.612	-0.433	0.007	**
LL24H/LD21H	-1.779	-3.424	-0.134	0.028	*
SD21H/LD21H	1.005	-0.666	2.676	0.435	
SD24H/LD21H	0.458	-1.213	2.129	0.934	
LL24H/LD24H	0.243	-0.926	1.413	0.975	
SD21H/LD24H	3.027	1.821	4.233	0.000	**
SD24H/LD24H	2.481	1.275	3.687	0.000	**
SD21H/LL24H	2.784	1.506	4.061	0.000	**
SD24H/LL24H	2.238	0.960	3.515	0.000	**
SD24H/SD21H	-0.546	-1.857	0.764	0.757	
Figure 10B. *GLK1 OE* PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-2.252	-3.054	-1.451	0.000	**

SD21H/LD21H	0.708	-0.566	1.983	0.455	
SD24H/LD21H	-0.527	-1.432	0.379	0.416	
SD21H/LD24H	2.961	1.718	4.203	0.000	**
SD24H/LD24H	1.726	0.865	2.586	0.000	**
SD24H/SD21H	-1.235	-2.548	0.078	0.072	
Figure 10C. *glk1* AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.202	-2.899	0.495	0.285	
LL24H/LD21H	0.114	-1.762	1.990	1.000	
SD21H/LD21H	1.750	-0.210	3.710	0.102	
SD24H/LD21H	2.850	1.190	4.510	0.000	**
LL24H/LD24H	1.316	-0.284	2.916	0.156	
SD21H/LD24H	2.952	1.255	4.649	0.000	**
SD24H/LD24H	4.052	2.712	5.392	0.000	**
SD21H/LL24H	1.636	-0.240	3.512	0.116	
SD24H/LL24H	2.736	1.176	4.296	0.000	**
SD24H/SD21H	1.100	-0.560	2.760	0.351	
Figure 10C. *glk1* PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-0.930	-2.052	0.192	0.139	
SD21H/LD21H	4.369	2.922	5.816	0.000	**
SD24H/LD21H	1.941	0.979	2.902	0.000	**
SD21H/LD24H	5.299	3.839	6.759	0.000	**
SD24H/LD24H	2.870	1.889	3.852	0.000	**
SD24H/SD21H	-2.428	-3.769	-1.088	0.000	**
Figure 10D. *GLK2 OE* AM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-2.341	-3.990	-0.692	0.002	**
LL24H/LD21H	-2.944	-4.828	-1.060	0.000	**
SD21H/LD21H	1.420	-0.415	3.255	0.200	
SD24H/LD21H	2.406	0.780	4.033	0.001	**
LL24H/LD24H	-0.603	-2.062	0.855	0.767	
SD21H/LD24H	3.760	2.367	5.154	0.000	**
SD24H/LD24H	4.747	3.641	5.853	0.000	**
SD21H/LL24H	4.364	2.698	6.029	0.000	**
SD24H/LL24H	5.351	3.917	6.784	0.000	**
SD24H/SD21H	0.987	-0.381	2.354	0.261	
Figure 10D. *GLK2 OE* PM inoculation at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD24H/LD21H	-1.575	-2.710	-0.440	0.003	**
Figure 10D. *GLK2 OE* PM inoculation at 52hpi					

Treatment	Diff	Lwr	Upr	P adj	Significance
SD21H/LD21H	4.335	2.856	5.814	0.000	**
SD24H/LD21H	1.823	0.744	2.901	0.000	**
SD21H/LD24H	5.910	4.515	7.304	0.000	**
SD24H/LD24H	3.398	2.438	4.358	0.000	**
SD24H/D21H	-2.512	-3.861	-1.163	0.000	**

Table 7. The effects of the modifications in GLK loci on the susceptibility of *Arabidopsis thaliana* leaves to *Botrytis cinerea* infection. Statistical analyses were performed using a one-way ANOVA in (3-6) biological replicates with (30-80) leaves. Diff=differences, Lwr=lower, Upr=upper P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 11A. LD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	2.139	1.548	2.730	0.000	**
WT / *glk1*	0.894	0.183	1.604	0.016	*
WT / *GLK2 OE*	1.217	0.382	2.051	0.006	**
WT / *glk2*	1.365	0.588	2.143	0.001	**
WT / *glk1/glk2*	0.964	0.245	1.682	0.010	**
Figure 11B. LD (24h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	0.758	0.351	1.165	0.001	**
WT / *glk1*	0.253	-0.218	0.723	0.282	
WT / *GLK2 OE*	0.171	-0.376	0.717	0.530	
WT / *glk2*	0.404	-0.131	0.939	0.134	
WT / *glk1/glk2*	-0.312	-0.866	0.242	0.262	
Figure 11C. LD (21h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	1.420	0.271	2.569	0.019	*
WT / *glk1*	0.826	-0.185	1.837	0.103	
WT / *GLK2 OE*	0.141	-0.707	0.989	0.728	
WT / *glk2*	-0.426	-1.362	0.511	0.348	
WT / *glk1/glk2*	0.681	-0.377	1.738	0.193	
Figure 11D. LD (21h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	1.198	0.121	2.274	0.030	*
WT / *glk1*	2.020	1.099	2.941	0.000	**
WT / *GLK2 OE*	1.343	0.292	2.395	0.014	*
WT / *glk2*	1.633	0.770	2.495	0.000	**
WT / *glk1/glk2*	1.018	0.196	1.839	0.016	*
Figure 11E. SD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	2.677	1.404	3.950	0.000	**
WT / *glk1*	-0.217	-1.343	0.909	0.700	
WT / *GLK2 OE*	-0.423	-1.440	0.594	0.407	
WT / *glk2*	-0.363	-1.348	0.623	0.463	
WT / *glk1/glk2*	0.144	-0.996	1.284	0.800	
Figure 11F. SD (24h, pm)					

Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	2.496	1.505	3.487	0.000	**
WT / *glk1*	0.910	0.132	1.688	0.023	*
WT / *GLK2 OE*	0.173	-0.606	0.953	0.657	
WT / *glk2*	-0.320	-1.179	0.539	0.456	
WT / *glk1/glk2*	0.800	-0.225	1.825	0.122	
Figure 11G. SD (21h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	1.401	0.209	2.592	0.024	*
WT / *glk1*	0.109	-0.789	1.008	0.801	
WT / *GLK2 OE*	-0.313	-1.176	0.549	0.454	
WT / *glk2*	2.704	1.613	3.795	0.000	**
WT / *glk1/glk2*	0.961	-0.284	2.206	0.121	
Figure 11H. LD (30h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	3.427	1.144	5.710	0.014	*
WT / *glk1*	0.219	-1.899	2.336	0.788	
WT / *GLK2 OE*	0.202	-1.484	1.889	0.785	
WT / *glk2*	-1.156	-2.405	0.092	0.064	
WT / *glk1/glk2*	1.362	-0.207	2.930	0.081	
Figure 11I. LL (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	1.497	0.677	2.316	0.001	**
WT / *glk1*	-0.796	-2.176	0.584	0.242	
WT / *GLK2 OE*	1.420	0.429	2.411	0.008	**
WT / *glk2*	0.179	-0.983	1.341	0.751	
WT / *glk1/glk2*	0.926	-0.066	1.918	0.066	

Table 8. The effects of the modifications in *GLK* loci on the susceptibility of *Arabidopsis thaliana* leaves to *Botrytis cinerea* infection. Statistical analyses were performed using a One-way ANOVA in (3-6) biological replicates with (30-80) leaves. Diff=differences, Lwr=lower, Upr=upper P adj= p value adjust. Different stars indicates significance *P≤ 0.05, ** P≤ 0.01.

Figure 12A. LD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	5.219	3.409	7.029	0.000	**
WT / *glk1*	1.885	-0.466	4.237	0.109	
WT / *GLK2 OE*	2.253	-0.213	4.720	0.070	
WT / *glk2*	2.509	0.735	4.283	0.008	**
WT / *glk1/glk2*	0.735	-1.591	3.061	0.521	
Figure 12B. LD (24h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	0.971	-0.240	2.183	0.112	
WT / *glk1*	-0.471	-2.128	1.187	0.561	
WT / *GLK2 OE*	-0.643	-2.458	1.172	0.472	
WT / *glk2*	0.179	-1.198	1.557	0.791	
WT / *glk1/glk2*	-1.295	-2.760	0.169	0.081	
Figure 12C. LD (21h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	3.947	2.638	5.256	0.000	**
WT / *glk1*	0.612	-0.504	1.727	0.270	
WT / *GLK2 OE*	1.701	0.506	2.896	0.007	**
WT / *glk2*	0.759	-0.249	1.768	0.133	
WT / *glk1/glk2*	1.670	0.494	2.846	0.007	**
Figure 12D. LD (21h, pm)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	4.600	3.223	5.978	0.000	**
WT / *glk1*	0.566	-1.174	2.306	0.514	
WT / *GLK2 OE*	3.138	1.834	4.442	0.000	**
WT / *glk2*	2.040	0.607	3.474	0.006	**
WT / *glk1/glk2*	2.919	1.865	3.973	0.000	**
Figure 12E. SD (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	5.428	4.332	6.523	0.000	**
WT / *glk1*	0.000	-1.201	1.200	0.999	
WT / *GLK2 OE*	0.224	-0.600	1.047	0.586	
WT / *glk2*	0.378	-0.540	1.296	0.410	
WT / *glk1/glk2*	1.070	-0.024	2.165	0.055	
Figure 12F. SD (24h, pm)					

Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	5.449	4.153	6.744	0.000	**
WT / *glk1*	0.178	-0.869	1.225	0.735	
WT / *GLK2 OE*	0.664	-0.236	1.565	0.145	
WT / *glk2*	0.883	-0.132	1.899	0.086	
WT / *glk1/glk2*	2.573	1.391	3.755	0.000	**
Figure 12G. LD (30h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	5.917	-0.904	12.737	0.065	
WT / *glk1*	-1.542	-5.470	2.387	0.234	
WT / *GLK2 OE*	1.283	-2.020	4.587	0.364	
WT / *glk2*	1.458	-0.016	2.932	0.052	
WT / *glk1/glk2*	3.292	1.223	5.361	0.007	**
Figure 12H. LL (24h, am)					
Treatment	Diff	Lwr	Upr	P adj	Significance
WT / *GLK1 OE*	5.070	3.874	6.267	0.000	**
WT / *glk1*	-2.164	-4.452	0.123	0.062	
WT / *GLK2 OE*	-0.707	-2.343	0.929	0.362	
WT / *glk2*	-1.333	-3.214	0.548	0.152	
WT / *glk1/glk2*	-2.281	-4.314	-0.248	0.030	*

Table 9. The effect of modifications in the central oscillator of the biological clock and GLK loci on the susceptibility of *Arabidopsis thaliana* leaves to *Botrytis cinerea* infection. Statistical analyses were performed using Student's t-test with selected variance, in addition to a One-way ANOVA statistical analysis presented in table (3-4 & 7-8) in (3-6) biological replicates with (30-80) Different stars indicate significance *P≤ 0.05, ** P≤ 0.01.

<table>
<tr><th colspan="3">Figure 8B. LD (24h, pm)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>toc-1</i></td><td>0.001</td><td>**</td></tr>
<tr><td>WT / <i>cca1/lhy</i></td><td>0.089</td><td></td></tr>
<tr><th colspan="3">Figure 8D. LD (21h, pm)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/lhy</i></td><td>0.000</td><td>**</td></tr>
<tr><td>WT / <i>lhy/toc-1</i></td><td>0.004</td><td>**</td></tr>
<tr><th colspan="3">Figure 8E. LD (30h, am)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/toc-1</i></td><td>0.003</td><td>**</td></tr>
<tr><td>WT / <i>lhy/toc-1</i></td><td>0.112</td><td></td></tr>
<tr><td>WT / <i>cca1/lhy/toc-1</i></td><td>0.113</td><td></td></tr>
<tr><th colspan="3">Figure 8F. LD (30h, pm)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/toc-1</i></td><td>0.053</td><td></td></tr>
<tr><th colspan="3">Figure 8H. SD (24h, pm)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/lhy</i></td><td>0.003</td><td>**</td></tr>
<tr><th colspan="3">Figure 8I. LL (24h, am)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/lhy</i></td><td>0.022</td><td>*</td></tr>
<tr><td>WT / <i>lhy/toc-1</i></td><td>0.016</td><td>*</td></tr>
<tr><th colspan="3">Figure 9A. LD (24h, am)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/toc-1</i></td><td>0.556</td><td></td></tr>
<tr><td>WT / <i>lhy/toc-1</i></td><td>0.201</td><td></td></tr>
<tr><th colspan="3">Figure 9E. LD (30h, am)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>toc-1</i></td><td>0.129</td><td></td></tr>
<tr><td>WT / <i>cca1/toc-1</i></td><td>0.008</td><td>**</td></tr>
<tr><th colspan="3">Figure 9F. LD (30h, pm)</th></tr>
<tr><td>Treatment</td><td>P value</td><td>Significance</td></tr>
<tr><td>WT / <i>cca1/lhy</i></td><td>0.019</td><td>*</td></tr>
<tr><td>WT / <i>cca1/toc-1</i></td><td>0.007</td><td>**</td></tr>
</table>

WT / *cca1/lhy/toc-1*	0.002	**
Figure 11B. LD (24h, pm)		
Treatment	P value	Significance
WT / *glk1/glk2*	0.048	*
Figure 11C. LD (21h, am)		
Treatment	P value	Significance
WT / *glk1/glk2*	0.070	
Figure 11E. SD (24h, am)		
Treatment	P value	Significance
WT / *glk1/glk2*	0.455	
Figure 11F. SD (24h, pm)		
Treatment	P value	Significance
WT / *glk1/glk2*	0.040	*
Figure 11G. SD (21h, am)		
Treatment	P value	Significance
WT / *glk1/glk2*	0.133	
Figure 11H. LD (30h, am)		
Treatment	P value	Significance
WT / *glk2*	0.001	**
WT / *glk1/glk2*	0.011	*
Figure 11I. LL (24h, am)		
Treatment	P value	Significance
WT / *glk1*	0.016	*
WT / *glk1/glk2*	0.065	
Figure 12A. LD (24h, am)		
Treatment	P value	Significance
WT / *glk1*	0.029	*
WT / *GLK2 OE*	0.016	*
Figure 12B. LD (24h, pm)		
Treatment	P value	Significance
WT / *GLK1 OE*	0.024	*
WT / *GLK2 OE*	0.233	
WT / *glk1/glk2*	0.033	*
Figure 12E. SD (24h, am)		
Treatment	P value	Significance
WT / *glk1/glk2*	0.005	**
Figure 12G. LD (30h, am)		
Treatment	P value	Significance
WT / *GLK1 OE*	0.000	**
WT / *glk1*	0.071	
WT / *GLK2 OE*	0.217	

Table 10. The effect of time of infection and photoperiod on susceptibility to *Botrytis cinerea* in leaves of *Arabidopsis thaliana* wild type, circadian clock mutants and GLK loci mutants and overexpressors. Statistical analyses were performed using a One-way ANOVA in (3-6) biological replicates with (30-80) leaves. Diff=differences, Lwr=lower, Upr=upper P adj= p value adjust. Stars indicate significance *P≤ 0.05, ** P≤ 0.01.

<table>
<tr><th colspan="6">Figure 13A.WT in normal earth conditions (LD &SD 24H) at 52hpi</th></tr>
<tr><td>Treatment</td><td>Diff</td><td>Lwr</td><td>Upr</td><td>P adj</td><td>Significance</td></tr>
<tr><td>LD 24h pm /LD 24h am</td><td>-1.448</td><td>-2.844</td><td>-0.051</td><td>0.039</td><td>*</td></tr>
<tr><td>SD 24h am /LD 24h am</td><td>3.060</td><td>1.812</td><td>4.309</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm /LD 24h am</td><td>2.092</td><td>0.813</td><td>3.371</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h am /LD 24h pm</td><td>4.508</td><td>3.344</td><td>5.673</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm /LD 24h pm</td><td>3.540</td><td>2.342</td><td>4.737</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm /SD 24h am</td><td>-0.969</td><td>-1.989</td><td>0.052</td><td>0.069</td><td></td></tr>
<tr><th colspan="6">Figure 13A.WT in normal earth conditions (LD &SD 24H) at 72hpi</th></tr>
<tr><td>Treatment</td><td>Diff</td><td>Lwr</td><td>Upr</td><td>P adj</td><td>Significance</td></tr>
<tr><td>LD 24h pm / LD 24h am</td><td>-4.308</td><td>-6.412</td><td>-2.204</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h am / LD 24h am</td><td>2.869</td><td>1.029</td><td>4.708</td><td>0.001</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h am</td><td>2.083</td><td>0.252</td><td>3.913</td><td>0.020</td><td>*</td></tr>
<tr><td>SD 24h am / LD 24h pm</td><td>7.177</td><td>5.538</td><td>8.815</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h pm</td><td>6.390</td><td>4.762</td><td>8.019</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / SD 24h am</td><td>-0.786</td><td>-2.055</td><td>0.482</td><td>0.367</td><td></td></tr>
<tr><th colspan="6">Figure 13B. <i>toc-1</i> in normal earth conditions (LD &SD 24H) at 52hpi</th></tr>
<tr><td>Treatment</td><td>Diff</td><td>Lwr</td><td>Upr</td><td>P adj</td><td>Significance</td></tr>
<tr><td>LD 24h pm / LD 24h am</td><td>0.077</td><td>-0.885</td><td>1.040</td><td>0.997</td><td></td></tr>
<tr><td>SD 24h am / LD 24h am</td><td>3.253</td><td>2.466</td><td>4.040</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h am</td><td>1.685</td><td>0.866</td><td>2.503</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h am / LD 24h pm</td><td>3.175</td><td>2.350</td><td>4.000</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h pm</td><td>1.607</td><td>0.752</td><td>2.463</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / SD 24h am</td><td>-1.568</td><td>-2.220</td><td>-0.916</td><td>0.000</td><td>**</td></tr>
<tr><th colspan="6">Figure 13B. <i>toc-1</i> in normal earth conditions (LD &SD 24H) at 72hpi</th></tr>
<tr><td>Treatment</td><td>Diff</td><td>Lwr</td><td>Upr</td><td>P adj</td><td>Significance</td></tr>
<tr><td>LD 24h pm / LD 24h am</td><td>-0.285</td><td>-3.045</td><td>2.476</td><td>0.993</td><td></td></tr>
<tr><td>SD 24h am / LD 24h am</td><td>5.676</td><td>3.473</td><td>7.878</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h am</td><td>3.947</td><td>1.755</td><td>6.139</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h am / LD 24h pm</td><td>5.960</td><td>3.758</td><td>8.162</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h pm</td><td>4.232</td><td>2.040</td><td>6.424</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / SD 24h am</td><td>-1.728</td><td>-3.154</td><td>-0.303</td><td>0.012</td><td>*</td></tr>
<tr><th colspan="6">Figure 13C. <i>cca1/lhy</i> in normal earth conditions (LD &SD 24H) at 52hpi</th></tr>
<tr><td>Treatment</td><td>Diff</td><td>Lwr</td><td>Upr</td><td>P adj</td><td>Significance</td></tr>
<tr><td>LD 24h pm / LD 24h am</td><td>-0.336</td><td>-2.941</td><td>2.268</td><td>0.986</td><td></td></tr>
<tr><td>SD 24h am / LD 24h am</td><td>3.610</td><td>1.488</td><td>5.733</td><td>0.000</td><td>**</td></tr>
<tr><td>SD 24h pm / LD 24h am</td><td>2.975</td><td>0.824</td><td>5.126</td><td>0.003</td><td>**</td></tr>
</table>

SD 24h am / LD 24h pm	3.946	1.974	5.918	0.000	**
SD 24h pm / LD 24h pm	3.311	1.309	5.313	0.000	**
SD 24h pm / SD 24h am	-0.635	-1.950	0.680	0.578	
Figure 13C. *cca1/lhy* in normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.285	-3.045	2.476	0.993	
SD 24h am / LD 24h am	5.676	3.473	7.878	0.000	**
SD 24h pm / LD 24h am	3.947	1.755	6.139	0.000	**
SD 24h am / LD 24h pm	5.960	3.758	8.162	0.000	**
SD 24h pm / LD 24h pm	4.232	2.040	6.424	0.000	**
SD 24h pm / SD 24h am	-1.728	-3.154	-0.303	0.012	*
Figure 13D. *cca1/toc-1* in normal earth conditions (LD &SD 24H) at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	0.472	-2.366	3.310	0.967	
SD 24h am / LD 24h am	4.754	2.584	6.923	0.000	**
SD 24h pm / LD 24h am	4.979	2.704	7.255	0.000	**
SD 24h am / LD 24h pm	4.282	1.861	6.702	0.000	**
SD 24h pm / LD 24h pm	4.507	1.991	7.023	0.000	**
SD 24h pm / SD 24h am	0.225	-1.501	1.952	0.983	
Figure 13D. *cca1/toc-1* in normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-4.625	-9.469	0.219	0.065	
SD 24h am / LD 24h am	1.285	-2.963	5.534	0.835	
SD 24h pm / LD 24h am	1.143	-3.342	5.627	0.893	
SD 24h am / LD 24h pm	5.910	2.712	9.108	0.000	**
SD 24h pm / LD 24h pm	5.768	2.262	9.274	0.001	**
SD 24h pm / SD 24h am	-0.142	-2.765	2.480	0.999	
Figure 13E. *lhy/toc-1* in normal earth conditions (LD &SD 24H) at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-1.267	-2.917	0.384	0.165	
SD 24h am / LD 24h am	2.493	0.895	4.091	0.002	**
SD 24h pm / LD 24h am	5.233	3.583	6.884	0.000	**
SD 24h am / LD 24h pm	3.760	2.391	5.128	0.000	**
SD 24h pm / LD 24h pm	6.500	5.071	7.929	0.000	**
SD 24h pm / SD 24h am	2.740	1.372	4.109	0.000	**
Figure 13E. *lhy/toc-1* in normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-5.042	-8.602	-1.481	0.004	**
SD 24h am / LD 24h am	0.823	-2.333	3.979	0.879	
SD 24h pm / LD 24h am	1.868	-1.428	5.165	0.399	
SD 24h am / LD 24h pm	5.865	3.010	8.719	0.000	**

SD 24h pm / LD 24h pm	6.910	3.900	9.919	0.000	**
SD 24h pm / SD 24h am	1.045	-1.473	3.563	0.647	
Figure 13F. ***cca1/lhy/toc-1*** **in normal earth conditions (LD &SD 24H) at 52hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.821	-3.133	1.491	0.771	
SD 24h am / LD 24h am	4.626	2.364	6.888	0.000	**
SD 24h pm / LD 24h am	3.495	1.361	5.629	0.001	**
SD 24h am / LD 24h pm	5.447	3.476	7.417	0.000	**
SD 24h pm / LD 24h pm	4.316	2.493	6.138	0.000	**
SD 24h pm / SD 24h am	-1.131	-2.890	0.628	0.318	
Figure 13F. ***cca1/lhy/toc-1*** **in normal earth conditions (LD &SD 24H) at 72hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-2.972	-6.282	0.338	0.091	
SD 24h am / LD 24h am	5.238	2.154	8.321	0.000	**
SD 24h pm / LD 24h am	4.121	1.127	7.114	0.004	**
SD 24h am / LD 24h pm	8.209	5.656	0.763	0.000	**
SD 24h pm / LD 24h pm	7.092	4.648	9.537	0.000	**
SD 24h pm / SD 24h am	-1.117	-3.245	1.010	0.495	
Figure 13G. ***GLK1 OE*** **in normal earth conditions (LD &SD 24H) at 52hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.063	-0.856	0.729	0.997	
SD 24h am / LD 24h am	2.581	1.665	3.496	0.000	**
SD 24h pm / LD 24h am	1.663	0.771	2.554	0.000	**
SD 24h am / LD 24h pm	2.644	1.785	3.503	0.000	**
SD 24h pm / LD 24h pm	1.726	0.893	2.559	0.000	**
SD 24h pm / SD 24h am	-0.918	-1.869	0.033	0.062	
Figure 13G. ***GLK1 OE*** **in normal earth conditions (LD &SD 24H) at 72hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.080	-1.299	1.139	0.998	
SD 24h am / LD 24h am	2.640	1.220	4.060	0.000	**
SD 24h pm / LD 24h am	1.781	0.258	3.303	0.016	*
SD 24h am / LD 24h pm	2.720	1.458	3.981	0.000	**
SD 24h pm / LD 24h pm	1.861	0.484	3.237	0.004	**
SD 24h pm / SD 24h am	-0.859	-2.416	0.698	0.459	
Figure 7H. ***glk1*** **in normal earth conditions (LD &SD 24H) at 52hpi**					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.753	-2.043	0.538	0.426	
SD 24h am / LD 24h am	4.052	2.876	5.228	0.000	**
SD 24h pm / LD 24h am	2.124	1.034	3.214	0.000	**

SD 24h am / LD 24h pm	4.805	3.552	6.057	0.000	**
SD 24h pm / LD 24h pm	2.877	1.705	4.048	0.000	**
SD 24h pm / SD 24h am	-1.928	-2.973	-0.883	0.000	**
Figure 13H. *glk1* in normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-1.951	-4.446	0.543	0.176	
SD 24h am / LD 24h am	4.762	2.464	7.059	0.000	**
SD 24h pm / LD 24h am	3.697	1.540	5.853	0.000	**
SD 24h am / LD 24h pm	6.713	4.542	8.884	0.000	**
SD 24h pm / LD 24h pm	5.648	3.627	7.669	0.000	**
SD 24h pm / SD 24h am	-1.065	-2.837	0.708	0.394	
Figure 13I. *GLK2 OE in* normal earth conditions (LD &SD 24H) at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.329	-1.486	0.829	0.878	
SD 24h am / LD 24h am	4.747	3.575	5.919	0.000	**
SD 24h pm / LD 24h am	3.069	1.977	4.161	0.000	**
SD 24h am / LD 24h pm	5.076	3.954	6.198	0.000	**
SD 24h pm / LD 24h pm	3.398	2.359	4.436	0.000	**
SD 24h pm / SD 24h am	-1.678	-2.733	-0.624	0.000	**
Figure 13I. *GLK2 OE in* normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-1.411	-3.567	0.745	0.317	
SD 24h am / LD 24h am	4.899	2.779	7.018	0.000	**
SD 24h pm / LD 24h am	3.620	1.591	5.648	0.000	**
SD 24h am / LD 24h pm	6.310	4.729	7.891	0.000	**
SD 24h pm / LD 24h pm	5.031	3.573	6.488	0.000	**
SD 24h pm / SD 24h am	-1.279	-2.682	0.124	0.086	
Figure 13J. *glk2 in* normal earth conditions (LD &SD 24H) at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.486	-1.457	0.485	0.556	
SD 24h am / LD 24h am	4.788	3.846	5.730	0.000	**
SD 24h pm / LD 24h am	3.777	2.789	4.765	0.000	**
SD 24h am / LD 24h pm	5.275	4.246	6.304	0.000	**
SD 24h pm / LD 24h pm	4.264	3.193	5.335	0.000	**
SD 24h pm / SD 24h am	-1.011	-2.056	0.033	0.061	
Figure 13J. *glk2 in* normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-2.017	-3.549	-0.484	0.005	**
SD 24h am / LD 24h am	4.961	3.511	6.410	0.000	**
SD 24h pm / LD 24h am	3.617	2.150	5.084	0.000	**
SD 24h am / LD 24h pm	6.977	5.528	8.427	0.000	**

SD 24h pm / LD 24h pm	5.634	4.167	7.101	0.000	**
SD 24h pm / SD 24h am	-1.344	-2.724	0.037	0.059	
Figure 13K. *glk1/glk2 in* normal earth conditions (LD &SD 24H) at 52hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-0.172	-1.058	0.714	0.956	
SD 24h am / LD 24h am	3.880	2.828	4.933	0.000	**
SD 24h pm / LD 24h am	2.255	1.149	3.362	0.000	**
SD 24h am / LD 24h pm	4.053	3.015	5.090	0.000	**
SD 24h pm / LD 24h pm	2.428	1.335	3.520	0.000	**
SD 24h pm / SD 24h am	-1.625	-2.856	-0.394	0.005	**
Figure 13K. *glk1/glk2 in* normal earth conditions (LD &SD 24H) at 72hpi					
Treatment	Diff	Lwr	Upr	P adj	Significance
LD 24h pm / LD 24h am	-2.277	-4.055	-0.500	0.007	**
SD 24h am / LD 24h am	2.533	0.393	4.674	0.014	*
SD 24h pm / LD 24h am	0.192	-1.948	2.333	0.995	
SD 24h am / LD 24h pm	4.811	2.745	6.876	0.000	**
SD 24h pm / LD 24h pm	2.470	0.404	4.535	0.013	*
SD 24h pm / SD 24h am	-2.341	-4.726	0.044	0.056	

Table 11. Summary table of list of different photoperiods used in wild type, central oscillator and *GLKs* mutants.

Modification of light					
Treatment	**Results**	**Wild type plant**	**Central oscillator mutants**	***GLKs* mutants**	**Suggestions**
SD 24h / LD 24h "Either AM or PM inoculations"	SD 24h condition enhanced the susceptibility	√	√	√	Length of day and night control susceptibility to *B. cinerea* infection
LD 24h / LL "AM inoculations"	Either the LD 24h or LL conditions showed similar levels of susceptibility	√	√	√	
LD 21h,LD 30h, and SD 21h "AM and PM inoculations"	Variation in susceptibility	√	√	√	Entrainment of biological clock by light rather than the length of day/night

Table 12. Summary table of list of modification of core clock mutants.

Treatment	**↓ susceptibility**	**↑ susceptibility**	**No change**	**Suggestions**

WT / *toc-1*	LD (24h, am)
	LD (24h, pm)
	LD (21h, am)
	LD (21h, pm)
	LD (30h, am)
	SD (24h, am)
	SD (24h, pm)
	LL (am)
			LD (30h, pm)	Core clock genes are not solely responsible for susceptibility to *B. cinerea.*

WT / *cca1/lhy*	LD (24h, am)
	LD (21h, am)
	LD (21h, pm)
	SD (24h, pm)
	LL (am)
		LD (30h, pm)	LD (24h, pm)
			LD (30h, am)
			SD (24h, am)	

WT/ *cca1/toc-1*	LD (24h, am)
	LD (21h, am)
		LD (21h, pm)
		LD (30h, am)
		SD (24h, pm)
			LD (24h, pm)
			LD (30h, pm)
			SD (24h, am)
			LL (am)	

WT / *lhy/toc-1*		LD (21h, am)
		LD (21h, pm)
		SD (24h, pm)
		LL (am)
			LD (24h, am)
			LD (24h, pm)
			LD (30h, am)
			LD (30h, pm)
			SD (24h, am)	

WT/ *cca1/lhy/toc-1*	LD (24h, am)
	LD (24h, pm)
	LD (21h, am)
		LD (21h, pm)
		LD (30h, pm)
			LD (30h, am)
			SD (24h, am)
			SD (24h, pm)
			LL (am)	

Table 13. Summary table of list of modification of GLK loci.

Treatment	↓ susceptibility	↑ susceptibility	No change	Suggestions

WT / *GLK1 OE*	LD (24h, am)
	LD (24h, pm)
	LD (21h, am)
	LD (21h, pm)
	SD (24h, am)
	SD (24h, pm)
	SD (21h, am)
	LD (30h, am)
	LL (am)
				We speculated that differences in susceptibility responses to *B. cinerea* in *GLK* genes are caused by the biological clock regulation in plant defense

WT / *glk1*	LD (24h, am)
	LD (21h, pm)
	SD (24h, pm)
		LL (am)	LD (24h, pm)
			LD (21h, am)
			SD (24h, am)
			SD (21h, am)
			LD (30h, am)	

WT / *GLK2 OE*	LD (24h, am)
	LD (21h, pm)
	LL (am)
			LD (24h, pm)
			LD (21h, am)
			SD (24h, am)
			SD (24h, pm)
			SD (21h, am)
			LD (30h, am)	

WT / *glk2*	LD (24h, am)
	LD (21h, pm)
	SD (21h, am)
		SD (24h, pm)
		LD (30h, am)
			LD (24h, pm)
			LD (21h, am)
			SD (24h, am)
			LL (am)	

WT / *glk1/glk2*	LD (24h, am)
	LD (21h, pm)
	SD (24h, pm)
	LD (30h, am)
		LD (24h, pm)	LD (21h, am)
			SD (24h, am)
			SD (21h, am)
			LL (am)	