Low Red/Far-Red Ratios Reduce Arabidopsis Resistance to *Botrytis cinerea* and Jasmonate Responses via a COI1-JAZ10-Dependent, Salicylic Acid-Independent Mechanism[^1][^2][^3][^4]

Ignacio Cerrudo, Mercedes M. Keller, Miriam D. Cargnel, Patricia V. Demkura, Mieke de Wit, Micaela S. Patitucci, Ronald Pierik, Corné M.J. Pieterse, and Carlos L. Ballaré*

Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires, C1417DSE Buenos Aires, Argentina (I.C., M.M.K., M.D.C., P.V.D., M.S.P., C.L.B.); Plant Ecophysiology (M.d.W., R.P.) and Plant-Microbe Interactions (C.M.J.P.), Department of Biology, Faculty of Science, Utrecht University, 3584 CH Utrecht, The Netherlands; and Center for BioSystems Genomics, 6700 AB Wageningen, The Netherlands (C.M.J.P.)

Light is an important modulator of plant immune responses. Here, we show that inactivation of the photoreceptor phytochrome B (phyB) by a low red/far-red ratio (R:FR), which is a signal of competition in plant canopies, down-regulates the expression of defense markers induced by the necrotrophic fungus *Botrytis cinerea*, including the genes that encode the transcription factor ETHYLENE RESPONSE FACTOR1 (ERF1) and the plant defensin PLANT DEFENSIN1.2 (PDF1.2). This effect of low R:FR correlated with a reduced sensitivity to jasmonate (JA), thus resembling the antagonistic effects of salicylic acid (SA) on JA responses. Low R:FR failed to depress PDF1.2 mRNA levels in a transgenic line in which PDF1.2 transcription was up-regulated by constitutive expression of ERF1 in a *coronatine insensitive1* (coi1) mutant background (35S::ERF1/coi1). These results suggest that the low R:FR effect, in contrast to the SA effect, requires a functional SCF^COI1-JASMONATE ZIM-DOMAIN (JAZ)JA receptor module. Furthermore, the effect of low R:FR depressing the JA response was conserved in mutants impaired in SA signaling (*sid2-1* and *npr1-1*). Plant exposure to low R:FR ratios and the *phyB* mutation markedly increased plant susceptibility to *B. cinerea*; the effect of low R:FR was (1) independent of the activation of the shade-avoidance syndrome, (2) conserved in the *sid2-1* and *npr1-1* mutants, and (3) absent in two RNA interference lines disrupted for the expression of the JAZ10 gene. Collectively, our results suggest that low R:FR ratios depress Arabidopsis (*Arabidopsis thaliana*) immune responses against necrotrophic microorganisms via a SA-independent mechanism that requires the JAZ10 transcriptional repressor and that this effect may increase plant susceptibility to fungal infection in dense canopies.

The effects of canopy density on the severity of plant disease caused by microbial pathogens are well documented, both in natural and managed ecosystems (Burdon and Chilvers, 1975; Augspurger and Kelly, 1984; Bell et al., 2006; for review, see Burdon and Chilvers, 1982; Alexander and Holt, 1998; Gilbert, 2002). Fungal diseases typically show a positive relationship with plant density, and part of this density effect is caused, among other things, by changes in host resistance to fungal infection (Gilbert, 2002). The mechanisms that mediate these effects of plant density on host resistance are elusive but could reflect the influence of canopy microenvironmental factors, including light, on the plant immune system (Ballaré, 2011; Kazan and Manners, 2011; Kangasjärvi et al., 2012).

Jasmonates (JAs) are oxylipins that play a key role in the activation of plant defenses against herbivorous and pathogen organisms (Browse, 2009; Chung et al., 2009; Fonseca et al., 2009; Howe, 2010; Ballaré, 2011). In the last few years, significant progress has been made to elucidate the mechanism of JA perception by plant cells (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Melotto et al., 2008; Yan et al., 2009; Pauwels et al., 2010; Sheard et al., 2010). These studies have shown that the perception of JA-Ile, the bioactive amino acid conjugate of jasmonic acid, is achieved by a coreceptor formed by the ubiquitin ligase SCF^COI1 complex and the JASMONATE ZIM-DOMAIN (JAZ) proteins. JA-Ile stimulates the specific binding of

[^1]: This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica and the Universidad de Buenos Aires (grant nos. PICT-06, PICT-08, and UBACyT–2010, to C.L.B.), the Netherlands Organization for Scientific Research (VENI grant no. 86306001 and Toptalent grant no. 02001030 to R.P. and M.d.W.), and the European Research Council (ERC Advanced grant no. 269072 C.M.J.P.).

[^2]: Corresponding author; e-mail ballare@ifeva.edu.ar.

[^3]: The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Carlos L. Ballaré (ballare@ifeva.edu.ar).

[^4]: Some figures in this article are displayed in color online but in black and white in the print edition.

[^5]: The online version of this article contains Web-only data.

[^6]: Open Access articles can be viewed online without a subscription.

1 This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica and the Universidad de Buenos Aires (grant nos. PICT-06, PICT-08, and UBACyT–2010, to C.L.B.), the Netherlands Organization for Scientific Research (VENI grant no. 86306001 and Toptalent grant no. 02001030 to R.P. and M.d.W.), and the European Research Council (ERC Advanced grant no. 269072 C.M.J.P.).

[^7]: Some figures in this article are displayed in color online but in black and white in the print edition.

[^8]: The online version of this article contains Web-only data.

[^9]: Open Access articles can be viewed online without a subscription.
Coronatine Inensitive 1 (COI1) and JAZ proteins, which leads to the ubiquitination of JAZs by SCF-COI1 and their subsequent degradation by the 26S proteasome. JAZ proteins are transcriptional repressors; therefore, their degradation initiates a transcriptional reprogramming of the cell and the activation of the JA response (Pauwels and Goossens, 2011; Kazan and Manners, 2012; Shyu et al., 2012). Transcription of JA-responsive genes leads to the production of plant metabolites involved in defense and the activation of JA responses in organs not directly affected by the initial event of herbivory or pathogen infection, which provides systemic protection against future attacks (Howe and Jander, 2008; Koo et al., 2009).

A growing body of evidence indicates that the JA response is modulated by the ecological context of the plant (for review, see Spoel and Dong, 2008; Pieterse et al., 2009; Verhage et al., 2010; Ballaré, 2011). In particular, the light environment, which can be strongly affected by canopy density, is emerging as a critical regulator of JA signaling (Moreno et al., 2009; Demkura et al., 2010; Radhika et al., 2010; Robson et al., 2010; Suzuki et al., 2011) and plant defense (for review, see Roberts and Paul, 2006; Ballaré, 2011; Kazan and Manners, 2011).

Plant responses to light are often mediated by informational photoreceptors, which are sensitive to specific wavelengths of the solar spectrum. The phytochromes are a family of photoreceptors that are sensitive to red (R; 660 nm) and far-red (FR; 730 nm) radiation. Plants use the phytochromes, particularly phytochrome B (phyB), to detect the proximity of other plants. Green leaves absorb R light and either reflect or transmit FR radiation. Therefore, as the density of the canopy increases, the R:FR ratio decreases (Smith, 1982; Ballaré et al., 1990). Low R:FR ratios inactivate phyB by reducing the levels of Pfr, the active (growth-repressing) form of the photoreceptor, and the depletion of Pfr unleashes the expression of many growth-related responses, collectively known as the shade-avoidance syndrome (SAS; Ballaré, 1999, 2009; Vandenbussche et al., 2005; Franklin, 2008; Kami et al., 2010; Keuskamp et al., 2010; Martínez-García et al., 2010).

Recent studies have shown that plants grown in dense canopies (low R:FR) or exposed to light treatments that mimic the proximity of other plants have reduced resistance to insect herbivory (Izaguirre et al., 2006; Moreno et al., 2009). Similarly, herbivory levels are higher on phyB mutants of several species than on the corresponding wild types (McGuire and Agrawal, 2005; Izaguirre et al., 2006; Moreno et al., 2009). This dual role of phyB Pfr (as a positive regulator of antiherbivore defense and a negative regulator of elongation and growth) is thought to be an important feature of the mechanism by which the plant incorporates information on neighbor proximity to the input signals that it uses to make adaptive decisions in the context of the “growth-versus-defense” resource allocation dilemma (Ballaré, 2009).

Low R:FR ratios, perceived by phyB, down-regulate JA responses (Moreno et al., 2009; Suzuki et al., 2011). Whether the reduction in plant resistance to fungal pathogens in high-density settings is functionally connected with the down-regulation of JA signaling by phyB-mediated neighbor detection is unknown. Double phyAphyB mutants of Arabidopsis (Arabidopsis thaliana) were found to be impaired in some of their responses to salicylic acid (SA) and more susceptible to pathogens with a biotrophic lifestyle (Genoud et al., 2002; Griebel and Zeier, 2008). Triple phyAphyBphyC mutants of rice (Oryza sativa) were also shown to be more susceptible to blast fungus (Magnaporthe grisea) than the wild type (Xie et al., 2011), and recent observations suggest that the simple phyB mutant of Arabidopsis is more susceptible to the fungal pathogen Fusarium oxysporum than wild-type plants (Kazan and Manners, 2011). However, the effects of proximity signals on pathogen resistance have not been investigated in great detail (Kazan and Manners, 2011). At the level of terminal responses (e.g., gene expression), the effect of low R:FR ratios depressing plant sensitivity to JA (Moreno et al., 2009) resembles the effects of SA (Pieterse et al., 2009; Verhage et al., 2010), but it is not known whether the low R:FR and SA effects share common mechanisms for the repression of JA responses.

In this paper, we test the effects of low R:FR treatments that mimic the proximity of neighboring plants on plant resistance to the necrotroph Botrytis cinerea and investigate the parallels between SA and low R:FR in the down-regulation of JA-mediated pathogen resistance. We found that low R:FR ratios severely down-regulate the expression of defense markers induced by B. cinerea, including the genes that encode for the transcription factor ERF1 and the plant defensin PDF1.2. The effect of phyB inactivation correlated with a reduced sensitivity to methyl jasmonate (MeJA), thereby resembling the antagonistic effects of SA on JA responses. We found that the effect of low R:FR ratios was not detectable in a transgenic line in which PDF1.2 expression was up-regulated by constitutive expression of ERF1 in a coI1 mutant background (35S::ERF1/ coI1). Therefore, the effect of phyB inactivation on the JA response, in contrast to the SA effect, requires a functional SCF-COI1-JAZ receptor module. Furthermore, the effect of low R:FR depressing the JA response was conserved in mutants impaired in SA signaling (sid2-1 and npr1-1). Inactivation of phyB (by a low R:FR treatment or the phyB mutation) markedly increased plant susceptibility to B. cinerea; this effect of low R:FR was (1) independent of the activation of the morphological components of the SAS, (2) conserved in sid2-1 and npr1-1, and (3) absent in the two RNA interference (RNAi) lines disrupted for the expression of the JAZ10 gene. Collectively, these results suggest that low R:FR ratios decrease the expression of JA-controlled immune responses via a SA-independent mechanism that involves the activity of the JAZ10 transcriptional repressor. This mechanism may be at least partially responsible for the effect of plant density.
reducing plant resistance to infection by necrotrophic microorganisms and insect herbivory.

RESULTS

Low R:FR Ratios Down-Regulate the Expression of Plant Defenses Induced by B. cinerea and Plant Sensitivity to JA

We tested the effects of low R:FR treatments on defense responses elicited by B. cinerea in fully deetiolated, soil-grown Arabidopsis rosettes. Reduction of R:FR ratio was achieved by supplementing the main light source with FR radiation, without altering the levels of photosynthetically active radiation (PAR), which produced a realistic simulation of the effect of the proximity of neighboring plants (Izaguirre et al., 2006; Moreno et al., 2009). Inoculation with B. cinerea induced the expression of several defense-related genes, including the plant defensin PDF1.2 and the transcription factor ERF1. Supplemental FR significantly reduced the defense response induced by B. cinerea (Fig. 1). A similar effect of low R:FR was found when we measured other B. cinerea-inducible genes, such as the transcription factor OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF-DOMAIN PROTEIN59 (ORA59), and HEVEIN-LIKE PROTEIN (HEL). These results demonstrate that light quality is an important modulator of pathogen-activated plant immune responses.

Since plant responses to necrotrophic pathogens are frequently orchestrated by JA (Glazebrook, 2005; Pieterse et al., 2009), we studied the effect of supplemental FR radiation on the JA response. The expression of several marker genes induced by MeJA treatment, including ERF1, PDF1.2, and ANTHRANILATE SYNTHASEa1 (ASA1), as well as the accumulation of soluble phenolic compounds were significantly down-regulated in plants exposed to supplemental FR radiation (Fig. 2). These findings were further supported by the results of a microarray exploration of the effects of supplemental FR on the JA-induced transcriptome. More than 300 genes that were significantly up-regulated by MeJA treatment under ambient light conditions were no longer up-regulated when the MeJA treatment was combined with exposure to supplemental FR. A subset of these genes (those that were induced by a factor of 2 or greater by the MeJA treatment under ambient light conditions) is presented in Supplemental Table S1. These results demonstrate that low R:FR ratios repress the JA responses of several defense-related genes and metabolic products.

The Effects of FR Down-Regulating Plant Sensitivity to JA Are Independent of the SA Pathway

In Arabidopsis, the best characterized hormonal repressor of JA sensitivity is SA (Pieterse et al., 2009), and on first examination, the effects of simulated neighbor proximity on the JA-response marker PDF1.2 are reminiscent of a SA effect (Spoel et al., 2003; Koornneef et al., 2008; Leon-Reyes, 2009). In some systems, such as sunflower (Helianthus annuus) hypocotyls (Kurepin et al., 2010), FR treatments have been shown to increase SA levels. Therefore, we tested the FR effect on JA responses in a mutant deficient in SA production (sid2-1). This mutant (Nawrath and Métrax, 1999; also known as ics1) is deficient in isochorismate synthase 1, which is essential for SA accumulation, PATHOGENESIS-RELATED PROTEIN1 (PR1) induction, and local and systemic acquired resistance responses in Arabidopsis (Wildermuth et al., 2001). We found that the effect of phytochrome inactivation on JA sensitivity was completely conserved in sid2-1 (Fig. 3). Next, we analyzed the accumulation of phenolic compounds as markers of the JA response in sid2-1 and also in npr1-1 plants. NONEXPRESSOR OF PR1 (NPR1) is a critical signaling component involved in the vast majority of SA-induced responses (Dong, 2004), including the antagonistic effect of SA on JA signaling (Spoel et al., 2003; Leon-Reyes et al., 2009). The effect of FR radiation, repressing the JA response, was clearly retained in both mutants (Supplemental Fig. S1).
It has been shown that the effects of SA down-regulating PDF1.2 induction by JA occur downstream of the SCF<sup>COI1</sup>-JAZ module of JA perception (Leon-Reyes, 2009). Therefore, we wanted to determine whether this is also the case for the low R:FR effect. To this end, we tested the effect of supplemental FR radiation on PDF1.2 expression in a transgenic line that carried the wild-type version of the COI1 gene (i.e. 35S::ERF1/COI1; Fig. 4C), indicating that the lack of effect of supplemental FR radiation in the 35S::ERF1/coi1-1 line is not simply a consequence of the strong activity of the 35S promoter. Therefore, the effect of FR down-regulating the PDF1.2 gene, in contrast to the SA effect, requires a functional SCF<sup>COI1</sup>-JAZ module of JA perception. Collectively, these results (Figs. 3 and 4; Supplemental Fig. S1) demonstrate that the effects of FR radiation and SA reducing the defense response to JA are mediated by different pathways.

**phyB Inactivation by FR Has Functional Consequences for Plant Resistance to B. cinerea**

We tested the functional consequences of phyB inactivation by low R:FR ratios on plant resistance to necrotrophic pathogens using a series of bioassays with B. cinerea. Rosette leaves of 4-week-old plants were inoculated with a spore suspension of B. cinerea; disease ratings were assessed at 2 d after inoculation based on a semiquantitative scale (Pré et al., 2008; Supplemental Fig. S2). The Col-0 wild type under the ambient light treatment (high R:FR) was relatively tolerant to B. cinerea, with a low percentage of disease symptoms. The FR treatment, which simulated the proximity of neighboring plants, had a clear effect of increasing susceptibility, with a large percentage of leaves displaying spreading necrotic lesions (Fig. 5A). This result is consistent with our observation of the down-regulation of JA response markers by FR radi-

---

**Figure 2.** Phytochrome inactivation by FR radiation reduces the expression of JA response marker genes and the accumulation of leaf phenolics and anthocyanins. A, Interactive effects of MeJA and FR on the expression of ERF1. B, Interactive effects of MeJA and FR on the expression of PDF1.2. C, Interactive effects of MeJA and FR on the expression of ASA1. D, Interactive effects of MeJA and FR on the accumulation of soluble leaf phenolics. E, Interactive effects of MeJA and FR on anthocyanin accumulation. Response levels were measured 3 h (genes) or 72 h (leaf metabolites) after spraying 3-week-old, soil-grown Col-0 Arabidopsis plants with a 200 μM solution of MeJA and are given relative to the Col-0 control under ambient light conditions. Amb, Ambient light; FR, low R:FR; FW, fresh weight. Error bars indicate SE (n = 3 replicates). Within each panel, different letters indicate significant differences between treatment means.

**Figure 3.** The effect of FR down-regulating PDF1.2 responses to JA is conserved in the sid2-1 mutants. A, Interactive effects of MeJA and FR on the expression of PDF1.2 in Col-0 plants. B, Interactive effects of MeJA and FR on the expression of PDF1.2 in the sid2-1 mutant. Expression levels were measured 6 h after spraying 3-week-old, soil-grown Arabidopsis plants with a 200 μM solution of MeJA and are given relative to the Col-0 control under ambient light conditions. Amb, Ambient light; FR, low R:FR. Error bars indicate SE (n = 4 replicates). The FR*MeJA interaction term (FR*MeJA) was statistically significant for all genotypes; within each panel, different letters indicate significant differences between treatment means.
The Effect of phyB Inactivation on Plant Resistance to B. cinerea Is Not Connected with the SAS Morphology

Besides down-regulating JA-induced defenses, phyB inactivation has a number of effects on plant morphology, including increased elongation and sometimes reduced specific leaf mass (i.e. the SAS phenotype; Franklin, 2008; Ballaré, 2009). Some of these effects on plant morphology could conceivably alter plant susceptibility to pathogen infection. For instance, the effects of low R:FR increasing the sensitivity of cucumber (Cucumis sativus) seedlings to powdery mildew fungus (Sphaerotheca cucurbitae) have been tentatively attributed to changes in leaf morphology, such as the reduced thickness of epidermal tissue (Shibuya et al., 2011). We tested this possibility using the sav3-2 mutant, which is deficient in an auxin biosynthesis pathway that is essential for the expression of the SAS morphology (Tao et al., 2008) but displays normal effects of FR radiation on defense responses (Moreno et al., 2009; M. Keller and C. Ballaré, unpublished data). Although the sav3-2 mutant fails to produce morphological responses to supplemental FR (Tao et al., 2008;...
phyB Inactivation by FR Reduces Plant Resistance to B. cinerea via a JA-Dependent, SA-Independent Mechanism

FR had few residual effects increasing the sensitivity of the jar1-1 mutant to B. cinerea (Fig. 7). The jar1 mutant is deficient in the enzyme that forms the bioactive JA-Ile conjugate (Staswick and Tiryaki, 2004), and it is known to be more susceptible to necrotrophic microorganisms (Staswick et al., 1998), including B. cinerea (Ferrari et al., 2003). These results are consistent with the idea that the effect of FR radiation increasing plant susceptibility to the fungus is functionally connected with its effects on JA synthesis or signaling.

Next, we tested the influence of FR radiation on resistance to B. cinerea in SA synthesis and signaling mutants. In our bioassays, the sid2-1 and npr1-1 defense phenotypes were very similar to that of Col-0 plants under ambient light, and the effect of FR radiation, increasing plant susceptibility to B. cinerea, was clearly conserved in both mutants (Fig. 8). These results indicate that the effect of phyB inactivation depresses plant resistance to B. cinerea is independent of the well-known effects of SA repressing JA-mediated defenses.

The Effect of phyB Inactivation Reducing Plant Resistance to B. cinerea Requires JAZ10

JAZ10 is one of the members of the JAZ family in Arabidopsis and is known to repress JA signaling (Yan et al., 2007; Chung and Howe, 2009). Previous work has shown that the expression of JAZ10 can be up-regulated by FR treatment (Moreno et al., 2009) and that jaz10 mutants are more sensitive than the wild type to the biotrophic bacterial pathogen Pseudomonas syringae strain DC3000 (Demianski et al., 2012). We
Our results demonstrate that the low R:FR ratio of canopy light, which is a signal of neighbor proximity, has a major effect down-regulating Arabidopsis resistance to the necrotrophic pathogen *B. cinerea*. This effect is mediated, at least in part, by a reduced sensitivity to JA, and our study sheds light on the mechanisms recruited by phyB to regulate JA signaling and plant immunity.

Effects of plant density on vulnerability to disease have important agronomic implications (Burdon and Chilvers, 1982) and are thought to play a central role in theories that explain species diversity in mixed stands (Augspurger and Kelly, 1984; Gilbert, 2002; Bell et al., 2009). However, the mechanisms that mediate these effects of plant proximity on disease severity are not completely clear. We show that phyB inactivation reduces plant defense responses and resistance to *B. cinerea* (Figs. 5 and 7–9). In this regard, our results resemble recent findings indicating that FR and the phyB mutation reduce plant resistance to herbivorous insects (McGuire and Agrawal, 2005; Izaguirre et al., 2006; Moreno et al., 2009) and, collectively, are consistent with a major negative effect of phyB-perceived neighbor proximity signals on JA-mediated defenses (Ballaré, 2009, 2011; Kazan and Manners, 2011). These effects of phyB inactivation have been interpreted on the basis of the paradigm of opportunity costs associated with the allocation of resources to growth or defense (i.e. "the dilemma of plants: to grow or defend"; Herms and Mattson, 1992). Down-regulation of the JA response under conditions of high risk of competition would appear to be an adaptive strategy, as JAs are positive regulators of costly defenses (Baldwin, 1998) and also strong inhibitors of elongation (Yan et al., 2007).

The effect of low R:FR ratios on plant resistance to *B. cinerea* is independent of the SAS morphology and most likely is connected with the phytochrome modulation of defense signaling. This is demonstrated by the results of our experiments showing a lack of correspondence between light effects on morphology and susceptibility to disease. First, the results with the *sav3-2* mutant (Fig. 5C) indicate that induction of the SAS morphology is not a requirement for the FR effects on Arabidopsis susceptibility to *B. cinerea*, because this mutant does not produce a SAS morphology when exposed to low R:FR ratios (Tao et al., 2008; Moreno et al., 2009) and yet conserves a FR-induced disease phenotype. Second, even though growth under attenuated blue light levels, or mutation of the blue light receptor cry1, induces drastic SAS phenotypes in Arabidopsis (Fig. 6; Keller et al., 2011), none of these conditions increased plant sensitivity to *B. cinerea* (Fig. 6). Finally, the high susceptibility to necrotrophic pathogens of the JA signaling mutants *jar1-1* and *col1-1* under ambient light, which at least in the case of *jar1-1* could not be further increased by FR supplementation (Fig. 7), was not accompanied by the constitutive expression of a low R:FR morphological phenotype (Supplemental Fig. S4).
Attenuation of the JA response is a typical effect of SA, and this antagonism is one of the best studied cases of hormone cross talk in plant defense (Kunkel and Brooks, 2002; Bostock, 2005; Lorenzo and Solano, 2005; Koornneef and Pieterse, 2008; Pieterse et al., 2009). In fact, it has been shown that many herbivorous insects (Stotz et al., 2002; Cipollini et al., 2004; Zarate et al., 2007; Rayapuram and Baldwin, 2007; Weech et al., 2008; Diezel et al., 2009) and some pathogens (Preston et al., 1999), including some strains of B. cinerea (El Oirdi et al., 2011), can activate the SA pathway to repress the JA response mounted by the host plant. Previous studies have shown that phyA phyB double mutants are impaired in some SA responses (Genoud et al., 2002; Griebel and Zeier, 2008); however, increased SA accumulation in response to low R:FR ratios also has been observed in some systems (Kurepin et al., 2010). Our experiments provide compelling evidence that SA and phyB inactivation by low R:FR repress the JA response using different mechanisms. This evidence is based on the observation of a COI1 requirement for the FR effect on PDF1.2 expression (Fig. 4) and the demonstration that the effect of FR radiation depressing the JA response (Fig. 3; Supplemental Fig. S1) and plant resistance to B. cinerea are fully conserved in the npr1-1 and sid2-1 SA signaling mutants (Fig. 8).

Our results suggest that the phyB effect on plant defense involves the regulation of some of the core elements of the JA-Ile coreceptor module (Figs. 4 and 9). A possible mechanism may be based on phytochrome-mediated changes in JAZ gene expression or JAZ protein stability. Increased expression of certain JAZ genes has been observed in response to FR supplementation (Moreno et al., 2009), including JAZ10, which can give rise to splicing products that are strong suppressors of the JA response because they are resistant to JA-induced degradation (Chung and Howe, 2009; Chung et al., 2010; Howe, 2010). A phytochrome effect on JAZ stability has been demonstrated under light conditions in which phyA is the predominant player controlling seedling phenotypic responses. Thus, COI1-mediated degradation of JAZ1-GUS in response to JA treatment was shown to require active phyA (Robson et al., 2010). This effect of phyA Pfr, promoting JAZ degradation, could explain the reduced JA sensitivity observed in phyA mutants in growth inhibition bioassays (Robson et al., 2010). However, in fully deetiolated plants at the rosette stage, where responses to low R:FR are controlled predominantly by phyB (Smith, 1995; Ballaré, 1999), effects of low R:FR ratio (mediated by phyB) on JAZ function have yet to be demonstrated. Light effects on JAZ function could occur in response to the degradation of DELLA proteins triggered by phyB inactivation (Djakovic-Petrovic et al., 2007), as DELLA proteins directly interact with JAZs and can prevent their function as transcriptional repressors (Hou et al., 2010). Our experiments with the JAZ10 RNAi lines provide functional evidence that JAZ10 is required for the effect of low R:FR ratios dampening plant resistance to B. cinerea. Preliminary evidence suggests that JAZ10 is also required for the effects of low R:FR ratios on other JA responses, including growth inhibition (M. Leone and C.L. Ballaré, unpublished data). The mechanism that connects phyB with JAZ10 remains to be elucidated.

In conclusion, our results establish that the inactivation of phyB by low R:FR ratios reduces plant resistance to a necrotrophic pathogen, which along with light responses mediated by other photoreceptors (Demkura and Ballaré, 2012) could help explain the effects of plant density on disease severity that have been observed in many agronomic and ecological studies. Our experiments suggest that the effect of phyB inactivation is mediated at least in part by decreased JA sensitivity. Furthermore, the effect of low R:FR desensitizing the JA response is not dependent on the classic JA-SA antagonism and most likely involves interactions of the phyB signal with components of the JA-Ile perception module.

**MATERIALS AND METHODS**

**Plant Cultivation**

Surface-sterilized seeds of Arabidopsis (Arabidopsis thaliana) were germinated on 0.8% agar plates at 22°C. Seven days after sowing, the seedlings were transferred to soil in individual pots as described previously (Moreno et al., 2009). Seedlings were grown in a growth chamber (10 h of light/14 h of dark, temperature of 22°C, PAR of 150 μmol m⁻² s⁻¹ provided by fluorescent bulbs, R:FR ratio of 4.5). The phyb-9 (Reed et al., 1993), phyb-211 (Reed et al., 1994), smr3-2 (Tao et al., 2008; Moreno et al., 2009), cry1-301 (Mockler et al., 1999), jar1-1 (Staswick and Tiryaki, 2004), coi1-1 (Xie et al., 1998), sid2-1 (Nawrath and Metraux, 1999), and npr1-1 (Cao et al., 1994) mutants, the 35S:ERF1 and 35S:ERF1::ERF1 lines (Solano et al., 1998; Lorenzo et al., 2003; Leon-Reyes, 2009), and the JAZ10 RNAi7 and JAZ10 RNAi9 lines (Yan et al., 2007) were all in the Col-0 background. In all experiments, 3- to 4-week-old plants were used for gene expression, metabolite analysis, and infection bioassays.

**Light Treatments**

Arabidopsis plants receiving 150 μmol m⁻² s⁻¹ PAR from fluorescent bulbs were placed in front of banks of incandescent lamps covered with either opaque screens (“ambient” light treatment) or FR-transmitting filters (“FR” treatment; Moreno et al., 2009). The FR treatment reduced the R:FR ratio of the integrated horizontal radiation to approximately 0.55. Previous studies in canopies of mustard (Sinapis alba) and chamoico (Datura ferox) seedlings indicated that this R:FR ratio in the horizontal light flux corresponds to a leaf area index of approximately 0.5, in which mutual shading among neighboring plants is negligible (Ballaré et al., 1991). Neither air temperature nor the level of PAR received by the plants was affected by the FR treatment. Blue light attenuation was achieved by interposing a yellow film between the PAR source and the plants, essentially as described previously (Keller et al., 2011). Plants were exposed to the relevant light treatments for 5 d prior to the elicitation experiments and B. cinerea infection bioassay, and the light treatments were maintained until harvest for gene expression or metabolite analysis or until the completion of the infection bioassay.

**JA Treatments**

Chemical induction was performed by spraying an aqueous solution of MeJA (Sigma) at the concentrations indicated in the relevant figure or table legend. Although the MeJA treatments were effective in inducing typical phenolic and gene expression responses, they did not cause visible growth inhibition in these soil-grown plants at the rosette stage. Plants were harvested 3, 6, or 72 h after the elicitation treatment, as indicated in the relevant figure.
Gene Expression and Metabolite Accumulation

Arabidopsis rosettes were harvested at the indicated times after infection or MeJA treatment, and total RNA from the aerial parts was extracted using the LiCl-phenol/chloroform method (Izaguirre et al., 2003). Quantitative real-time PCR analysis was performed as described previously (Moreno et al., 2009). PCR was carried out in the 7500 PCR Real System (Applied Biosystems) with FastStart Universal SYBR Green Master (RoX; Roche). The UBQUITIN (UBC) gene was used as an internal standard; the primers for the genes of interest are listed in Supplemental Table S2. The accumulation of soluble UV-absorbing phenolic compounds and anthocyanins was measured spectrophotometrically (Singh et al., 1999), 72 h after elicitation, in the petioles of six independent biological replicates. In the case of gene expression analyses, each replicate consisted of a pool of three individual plants.

Supplemental Table S2. Primer sequences used for quantitative PCR assays.

ACKNOWLEDGMENTS

We thank Carlos Mazza, Miriam Izaguirre, Javier Moreno, and Amy Austin for many helpful discussions and Edward Farmer for the JAZ10 RNAi lines.

LITERATURE CITED

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-
Pierik R, Dijkova-Petrovick T, Keuskamp DH, de Wit M, Voesenek LACJ

Demkura PV, Ballaré CL (March 23, 2012) UVR8 mediates UV-B-induced Arabidopsis defense responses against Botrytis cinerea by controlling sinapate accumulation. Mol Plant (http://dx.doi.org/10.1093/mp/ss2025)
Djakovic-Petrovic T, de W it M, Voesenek LA, Pierik R, Djakovic-Petrovic T, de W it M, Voesenek LA, Pierik R, Djakovic-Petrovic T, de W it M, Voesenek LA, Pierik R


Downloaded from on October 30, 2017 - Published by www.plantphysiol.org
Copyright © 2012 American Society of Plant Biologists. All rights reserved.
Cerrudo et al.


