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

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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

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CORONATINE INSENSITIVE 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.

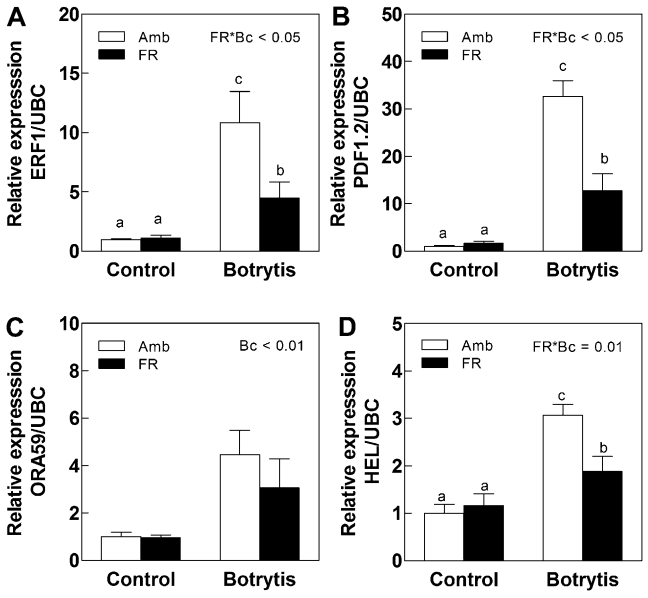

Figure 1. Supplemental FR radiation, which mimics the effects of neighbor proximity on the light environment and phyB status, down-regulates the expression of Arabidopsis defense-related genes induced by *B. cinerea*. A, Effect of FR on the response of *ERF1* to *B. cinerea* infection. B, Effect of FR on the response of *PDF1.2* to *B. cinerea* infection. C, Effect of FR on the response of *ORA59* to *B. cinerea* infection. D, Effect of FR on the response of *HEL* to *B. cinerea* infection. Expression levels were measured 24 h after inoculation of 4-week-old, soil-grown Arabidopsis plants with a 5-μL drop of *B. cinerea* spore suspension and are expressed relative to the healthy control under ambient light conditions. Amb, Ambient light; Bc, *B. cinerea*; FR, low R:FR. Error bars indicate SE ($n = 6$ replicates). The significance of the FR-*B. cinerea* interaction term (FR*Bc) is shown in each panel; different letters indicate significant differences between treatment means.

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 SYNTHASEα1* (*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 Métraux, 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. NONEXPRESOR 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).

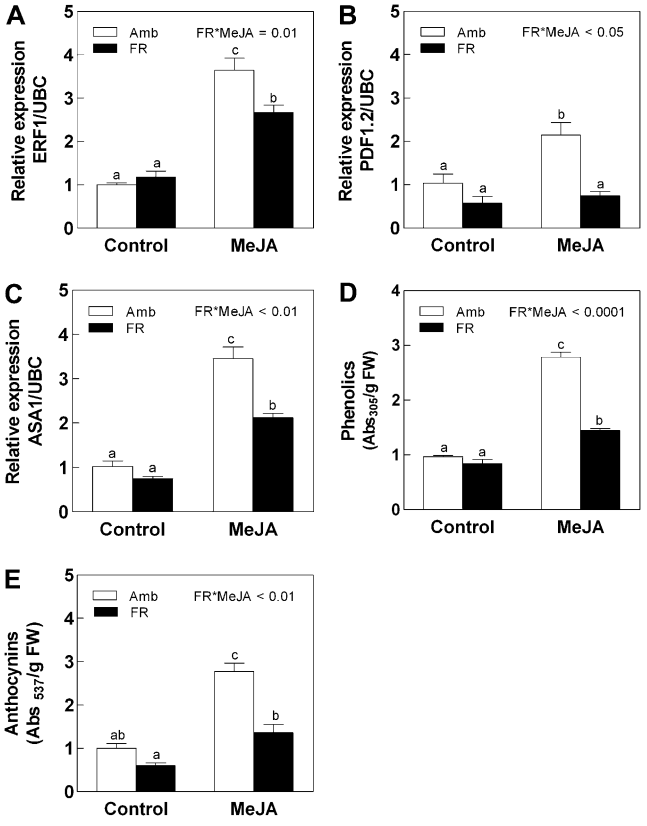

Figure 2. Phytochrome inactivation by FR radiation reduces the expression of JA response marker genes and the accumulation of leaf phenolics and anthocyanins. A, Interactive effects of MeJA and FR on the expression of *ERF1*. B, Interactive effects of MeJA and FR on the expression of *PDF1.2*. C, Interactive 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.

It has been shown that the effects of SA down-regulating *PDF1.2* induction by JA occur downstream of the SCF^{COI1}-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 *coi1-1* mutation but in which *PDF1.2* mRNA was obtained as a result of constitutive expression of the ERF1 transcription factor (*35S*::*ERF1/coi1-1*). In the ecotype Columbia (Col-0) wild type, FR down-regulated the expression of *PDF1.2*, as expected (Fig. 4A). In the *35S*::*ERF1/coi1-1* line, MeJA failed to induce *PDF1.2* expression, as expected due to the lack of COI1, and FR failed to down-regulate the constitutively high levels of *PDF1.2* mRNA (Fig. 4B). Importantly, FR radiation still depressed JA-induced *PDF1.2* expression in a *35S*::*ERF1* 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^{COI1}-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-

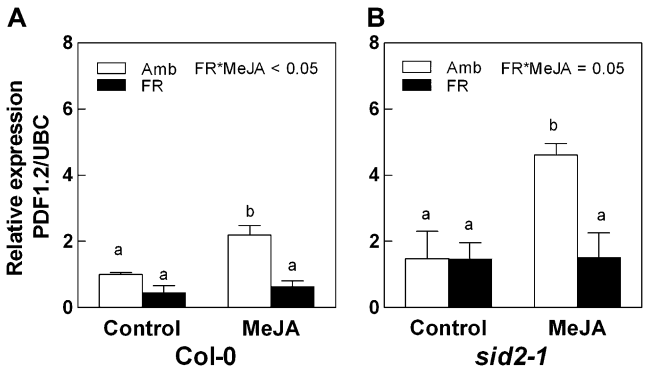

Figure 3. The effect of FR down-regulating *PDF1.2* responses to JA is conserved in the *sid2-1* mutants. A, Interactive effects of MeJA and FR on the expression of *PDF1.2* in Col-0 plants. B, Interactive effects of MeJA and FR on the expression of *PDF1.2* in the *sid2-1* mutant. Expression levels 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.

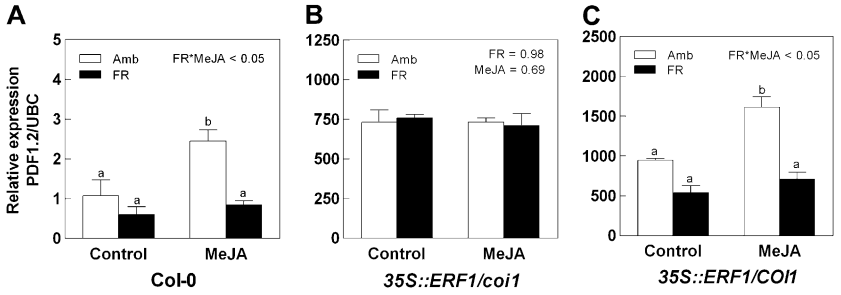

Figure 4. The effect of FR down-regulating *PDF1.2* expression requires *COI1*. A, Interactive effects of MeJA and FR on the expression of *PDF1.2* in Col-0 plants. B, Interactive effects of MeJA and FR on the expression of *PDF1.2* in the *35S::ERF1-coi1.1* line. C, Interactive effects of MeJA and FR on the expression of *PDF1.2* in the *35S::ERF1-COI1.1* line. Expression levels were measured 6 h after spraying 3-week-old, soil-grown Col-0, *35S::ERF1/coi1-1*, and *35S::ERF1/COI1-1* Arabidopsis plants with a 200 μM solution of MeJA and are expressed relative to the Col-0 control under ambient light conditions. Amb, Ambient light; FR, low R:FR. Error bars indicate SE (n = 3 replicates). When the FR-MeJA interaction term (FR*MeJA) was statistically significant, different letters indicate significant differences between treatment means.

ation (Figs. 1 and 2; Supplemental Table S1). Similarly, in the *phyB* mutant, where phyB is genetically inactivated and has very low expression of JA-induced defense markers (Moreno et al., 2009), we found a great susceptibility to *B. cinerea*, which was not further increased by FR treatment (Fig. 5B). On average, the size of the lesions caused by *B. cinerea* was two to three times larger in plants treated with FR or carrying the *phyB* mutation compared with those of Col-0 plants under the ambient light treatment. A phytochrome A null mutant (*phyA-211*) was as resistant to *B. cinerea* as the Col-0 wild type and conserved the effect of FR increasing plant susceptibility (Supplemental Fig. S3). Therefore, although phyA is also known to be a positive regulator of certain JA responses (Robson et al., 2010), our results suggest that phyA does not play an important role in mediating the effects of low R:FR on the defense phenotype of rosette-stage Arabidopsis plants. This conclusion is consistent with the classic tenets of plant photomorphogenesis that (1) phyA is not inactivated by FR supplementation of white light and (2) phyA is strongly down-regulated during deetiolation and is not an important player in the plastic responses of fully deetiolated plants to changes in the R:FR ratio (Smith, 1995).

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;

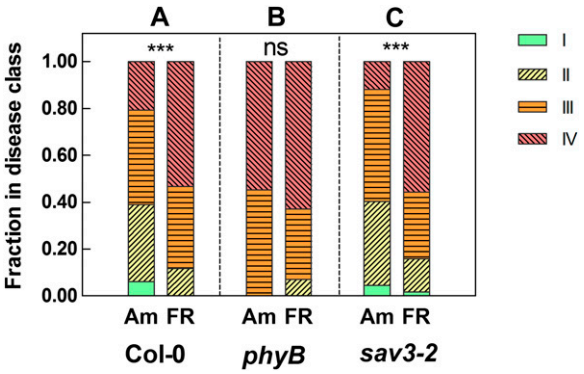

Figure 5. Phytochrome inactivation by a FR treatment that simulates the proximity of neighboring plants decreases Arabidopsis resistance to *B. cinerea*. A, FR effect in the Col-0 wild type. B, FR effect in the *phyB* mutant, which displays constitutive SAS morphology. C, FR effect in the *sav3-2* mutant, which fails to induce the SAS morphology in response to supplemental FR. The bars indicate the frequency distribution of disease symptoms 2 d after inoculation of plants of comparable leaf sizes (see "Materials and Methods"). Disease rating is expressed as the fraction of leaves falling in the following classes: I, no visible disease symptoms; II, nonspreading lesion; III, spreading lesion with less than the 30% of the leaf area; IV, spreading lesion with more than 30% of the leaf area, with additional chlorosis and leaf collapse. Statistical analysis of the disease response is based on a χ^2 comparison between the light treatments for each genotype. For each genotype, asterisks indicate significant (*** $P < 0.001$) differences between light treatments; ns = not significant. Am, Ambient; FR, Far-red. [See online article for color version of this figure.]

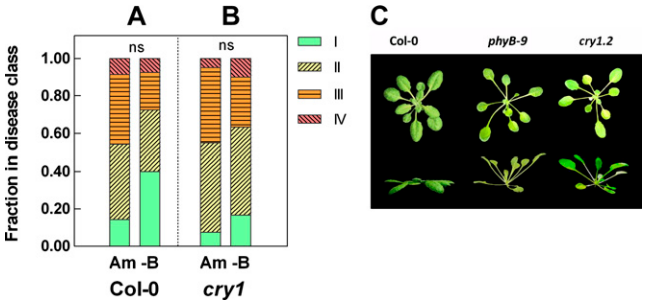

Figure 6. Inactivation of the cry1 photoreceptor (either by growing plants under PAR depleted in the blue component of the light spectrum or by the *cry1* mutation) induces a SAS phenotype that resembles the effect of phyB inactivation on plant morphology but does not reduce plant resistance to *B. cinerea*. A, Effect of blue light depletion in the Col-0 wild type. B, Effect of blue light depletion in the *cry1* mutant. Notice also that *cry1* is as resistant as Col-0 under ambient light ($P = 0.69$). C, Constitutive expression of the SAS phenotype in *phyB* and *cry1* plants. The bars indicate the frequency distribution of disease symptoms 3 d after inoculation of plants of comparable leaf sizes (for details, see Fig. 5). Am, Ambient light; -B, blue light attenuation. [See online article for color version of this figure.]

Moreno et al., 2009), it displayed a clear effect of FR radiation increasing plant susceptibility to *B. cinerea* in the infection bioassay (Fig. 5C).

As an alternative approach to determine the influence of the SAS morphology on Arabidopsis resistance to *B. cinerea*, we tested the effects of inducing SAS by manipulating cryptochrome 1 (cry1) instead of phyB. cry1 is a blue light receptor that, like phyB, plays an important role in repressing SAS responses in dense canopies (Sellaro et al., 2010; Keller et al., 2011; Keuskamp et al., 2011). Plants of the *cry1* mutant, as well as Col-0 plants exposed to light depleted in the blue region of the spectrum, displayed a strong SAS morphology, as expected (Keller et al., 2011). However, these plants were not less resistant than the corresponding controls (Col-0 plants under ambient light) in the *B. cinerea* infection bioassay (Fig. 6).

Having examined the defense phenotype of light signaling mutants, we tested the expression of the SAS phenotype in JA response mutants. The JA signaling mutants *coi1-1* (Xie et al., 1998) and *jar1-1* (Staswick and Tiryaki, 2004), which are highly susceptible to necrotrophic pathogens, did not display, at the rosette stage, a SAS phenotype under ambient light (although *coi1-1* displayed slightly hyponastic leaves). Moreover, both of these mutants retained normal SAS responses to supplemental FR radiation (Supplemental Fig. S4), which agrees with the observation that the *coi1-16* mutant retains normal hypocotyl elongation responses to end-of-day R:FR manipulations (Robson et al., 2010).

Collectively, these results demonstrate that the effects of light quality on plant resistance to *B. cinerea* and JA signaling can be fully uncoupled from the effects of light on the expression of morphological components of the SAS.

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 depressing 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 upregulated 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

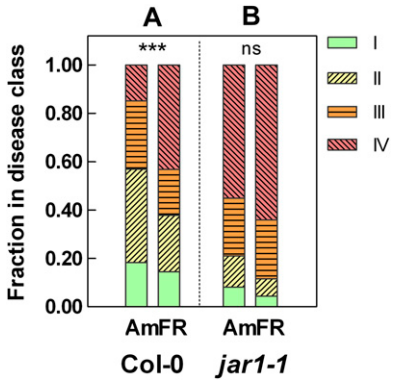

Figure 7. The effect of phyB inactivation by FR radiation reducing Arabidopsis resistance to *B. cinerea* is functionally connected with the phyB effects on JA signaling. A, FR effect in the Col-0 wild type. B, FR effect in the *jar1-1* mutant, which is deficient in the formation of the JA-Ile bioactive conjugate. The bars indicate the frequency distribution of disease symptoms 2 d after inoculation of plants of comparable leaf sizes (for details, see Fig. 5). *** $P < 0.001$; ns = not significant. Am, Ambient; FR, Far-red. [See online article for color version of this figure.]

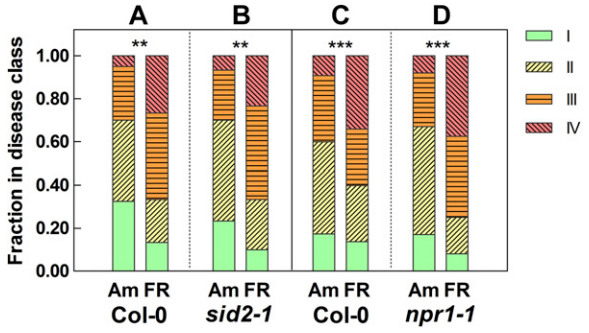

Figure 8. The effect of phyB inactivation by FR radiation on Arabidopsis resistance to *B. cinerea* is independent of SA signaling. A and C, FR effect in the Col-0 wild type. B, FR effect in the *sid2-1* mutant, which is deficient in isochorismate synthase 1. D, FR effect in the *npr1-1* mutant, which is deficient in SA signaling. The bars indicate the frequency distribution of disease symptoms 2 d after inoculation of plants of comparable leaf sizes (** $P < 0.01$, *** $P < 0.001$; for details, see Fig. 5). Am, Ambient; FR, Far-red. [See online article for color version of this figure.]

tested the effect of FR on *B. cinerea* resistance in two RNAi lines disrupted for the expression of *JAZ10*. None of the RNAi lines displayed an obvious resistance phenotype under ambient light; however, the effect of low R:FR depressing plant resistance was clearly attenuated in both of them (Fig. 9). A similar lack of FR effect was evident in a *jaz10* null mutant line (Supplemental Fig. S5). We conclude from these experiments that low R:FR ratios down-regulate plant defense against *B. cinerea* via a mechanism that requires JAZ10.

DISCUSSION

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., 2006). 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 *coi1-1* 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).

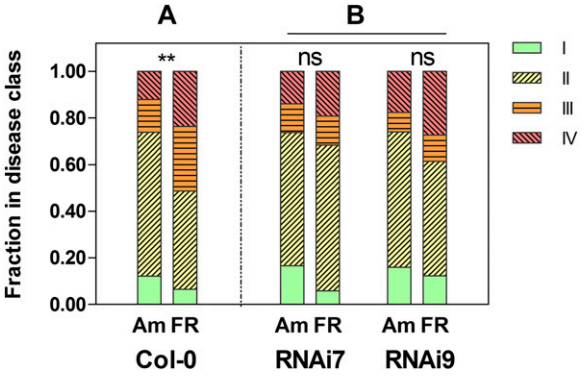

Figure 9. The effect of phyB inactivation by FR radiation decreasing Arabidopsis resistance to *B. cinerea* requires JAZ10. A, FR effect in the Col-0 wild type. B, FR effect in two independent RNAi lines silenced for the expression of the *JAZ10* gene. The bars indicate the frequency distribution of disease symptoms 2 d after inoculation of plants of comparable leaf sizes. Asterisks indicate significant (** $P < 0.01$) differences between light treatments; ns = not significant (for details, see Fig. 5). Am, Ambient; FR, Far-red. [See online article for color version of this figure.]

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 photomorphogenesis. 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), *phyA-211* (Reed et al., 1994), *sav3-2* (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 Métraux, 1999), and *npr1-1* (Cao et al., 1994) mutants, the *35S*::*ERF1* and *35S*::*ERF1/coi1-1* 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 chamico (*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 legends. 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

legends, and immediately frozen in liquid nitrogen for analysis of gene expression or extracted for metabolite determinations.

Bioassays with *Botrytis cinerea*

Droplets of 5 μL of spore suspension of *B. cinerea* (5×10^5 spores mL^{-1}) were placed on the adaxial surface of five mature leaves (one droplet per leaf) of 4-week-old plants (Muckenschnabel et al., 2002). Pots were enclosed in individual clear polyester chambers to prevent desiccation of the inoculation droplets. The progress of fungal infection was rated 2 or 3 d after inoculation using a qualitative scale (Pré et al., 2008), with some modifications. The following disease classes were recognized in this study: I, no visible disease symptoms; II, nonspreading lesion; III, spreading lesion with less than 30% necrotic leaves; IV, spreading lesion with more than 30% extensive tissue maceration (Supplemental Fig. S2). A χ^2 test was used to compare the distribution of disease categories between treatments in each genotype or between genotypes. For the analysis of changes in gene expression induced by fungal infection, plants were harvested 24 h after infection and immediately frozen in liquid nitrogen.

Gene Expression and Metabolite Accumulation

Arabidopsis rosettes were harvested at the indicated times after infection or MeJA treatment, and total RNA from the aerial plant 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 *UBIQUITIN* (*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 the sixth youngest leaf of the rosette. Normalized gene expression and metabolite levels were expressed as fold change relative to the control genotype under ambient light conditions. The results are based on three to six independent biological replicates. In the case of gene expression analyses, each replicate consisted of a pool of three individual plants.

Genome-wide transcriptome analyses were performed on 5-mm-long third youngest petioles (for detailed growth conditions, see Pierik et al., 2009) of plants exposed for 2 h to control, low R:FR (R:FR = 0.2; Philips Green Power FR light-emitting diodes), or low R:FR + 100 μM MeJA. To this end, total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) with on-column DNA digestion following the company's instructions. These materials were then processed (cDNA and copy RNA synthesis, hybridization to ATH1 chip) by Service XS (authorized service provider for Affymetrix). Three biological replicates, each consisting of three petioles from three different plants, were used. The microarray data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE35700 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35700).

Statistics and Analysis

Statistical analyses were carried out using INFOSTAT software (InfoStat/Professional version 1.1). Data on gene expression were analyzed using factorial ANOVA, with light treatment, MeJA (or *B. cinerea*) elicitation, and genotype as factors. Differences between means were tested when interaction terms were significant in the factorial ANOVA. Appropriate transformations of the primary data were used when needed to meet the assumptions of the analysis.

Microarray data were analyzed using the Bioconductor package in the R statistical package (www.bioconductor.org). Data normalization was done using the robust multiarray average algorithm, and differential expression was calculated using the empirical Bayes method in the R limma package (Smyth, 2004) and the multiple testing correction of Benjamini and Hochberg (1995). Genes were considered differentially expressed at adjusted $P < 0.01$ and fold change greater than 2.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. The effect of FR depressing the accumulation of soluble leaf phenolics induced by MeJA is conserved in *sid2-1* and *npr1-1* mutants.

Supplemental Figure S2. Disease severity classes identified in this study.

Supplemental Figure S3. *phyA-1* was as resistant to *B. cinerea* as the Col-0 wild type and conserved the effect of FR increasing plant susceptibility.

Supplemental Figure S4. SAS responses in JA signaling mutants *coi1-1* and *jar1-1*.

Supplemental Figure S5. FR effect on plant resistance to *B. cinerea* in a *jaz10* null mutant.

Supplemental Table S1. Genes induced 2 h after MeJA (100 μM) spray (adjusted $P < 0.01$, $\log_2$ fold change [FC] greater than 1) that were not significantly induced when the MeJA treatment was combined with exposure to low R:FR

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

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