Kinetics of Salicylate-Mediated Suppression of Jasmonate Signaling Reveal a Role for Redox Modulation[1][OA]

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Cross talk between salicylic acid (SA) and jasmonic acid (JA) signaling pathways plays an important role in the regulation and fine tuning of induced defenses that are activated upon pathogen or insect attack. Pharmacological experiments revealed that transcription of JA-responsive marker genes, such as *PDF1.2* and *VSP2*, is highly sensitive to suppression by SA. This antagonistic effect of SA on JA signaling was also observed when the JA pathway was biologically activated by necrotrophic pathogens or insect herbivores, and when the SA pathway was triggered by a biotrophic pathogen. Furthermore, all 18 Arabidopsis (*Arabidopsis thaliana*) accessions tested displayed SA-mediated suppression of JA-responsive gene expression, highlighting the potential significance of this phenomenon in induced plant defenses in nature. During plant-attacker interactions, the kinetics of SA and JA signaling are highly dynamic. Mimicking this dynamic response by applying SA and methyl jasmonate (MeJA) at different concentrations and time intervals revealed that *PDF1.2* transcription is readily suppressed when the SA response was activated at or after the onset of the JA response, and that this SA-JA antagonism is long lasting. However, when SA was applied more than 30 h prior to the onset of the JA response, the suppressive effect of SA was completely absent. The window of opportunity of SA to suppress MeJA-induced *PDF1.2* transcription coincided with a transient increase in glutathione levels. The glutathione biosynthesis inhibitor L-buthionine-sulfoximine strongly reduced *PDF1.2* suppression by SA, suggesting that SA-mediated redox modulation plays an important role in the SA-mediated attenuation of the JA signaling pathway.

In nature, plants interact with a wide range of microbial pathogens and herbivorous insects. During the evolutionary arms race between plants and their attackers, primary and secondary immune responses evolved to recognize common or highly specialized features of microbial pathogens (Chisholm et al., 2006; Jones and Dangl, 2006), resulting in sophisticated mechanisms of defense. Although the arms race between plants and herbivorous insects has been intensively debated (Musser et al., 2002; Schoonhoven et al., 2005), knowledge of the underlying mechanisms is relatively limited. In the past years, various genomics approaches exponentially expanded our understanding of the molecular mechanisms by which plants tailor their defense response to pathogen and insect attack (Glazebrook et al., 2003; Tao et al., 2003; Eulgem et al., 2004; Reymond et al., 2004; De Vos et al., 2005; Kempema et al., 2007; Van Oosten et al., 2008). The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) emerged as key players in the regulation of the signaling networks involved (Howe, 2004; Pozo et al., 2004; Grant and Lamb, 2006; Van Loon et al., 2006; Von Dahl and Baldwin, 2007). Other plant hormones, such as abscisic acid (Mauch-Mani and Mauch, 2005), brassinosteroids (Nakashita et al., 2003), and auxins (Navarro et al., 2006; Wang et al., 2007), have been reported to play a role in the plant immune response as well, but their significance is less well understood. SA-, JA-, and ET-dependent pathways regulate defense responses that are differentially effective against specific types of attackers. Pathogens with a biotrophic lifestyle are generally more sensitive to SA-dependent responses, whereas necrotrophic pathogens and herbivorous insects are commonly deterred by JA/ET-dependent defenses (Thomma et al., 2001; Kessler and Baldwin, 2002; Glazebrook, 2005).

There is ample evidence that SA and JA signaling pathways are mutually antagonistic (Pieterse et al., 2001; Kunkel and Brooks, 2002; Glazebrook et al., 2003; Rojo et al., 2003; Bostock, 2005; Beckers and Spoel, 2006; Koornneef and Pieterse, 2008). This pathway cross talk is thought to provide the plant with a powerful regulatory potential that helps in deciding which defensive strategy to follow, depending on the type of attacker encountered (Reymond and Farmer, 1998). Yet, it appears that attackers have also evolved ways to

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manipulate plants for their own benefit by suppressing induced defenses via modulation of the plant signaling network. A nice example is the response of Arabidopsis (*Arabidopsis thaliana*) to silverleaf whitefly (*Bemisia tabaci*) nymphs. The nymphs of this phloem-feeding insect may sabotage effectual JA-dependent host defenses by activating the antagonistic SA signaling pathway (Zarate et al., 2007). Pathogens suppress host defenses as well, by using virulence factors that antagonize the plant immune response (Nomura et al., 2005). One of these virulence factors is the *Pseudomonas syringae* phytotoxin coronatine, which functions as a jasmonate analog. During the interaction with susceptible Arabidopsis plants, coronatine suppresses SA-dependent defenses, thereby promoting susceptibility to this pathogen (Zhao et al., 2003; Brooks et al., 2005; Cui et al., 2005; Laurie-Berry et al., 2006).

Several key regulatory proteins involved in SA-JA cross talk have been identified in Arabidopsis. For instance, the transcription factor WRKY70 was shown to act as an activator of SA-responsive genes and a repressor of JA-inducible genes, thereby functioning as a molecular switch between both pathways (Li et al., 2004). Previously, we demonstrated that the defense regulatory protein NPR1 is required for SA-JA cross talk (Spoel et al., 2003). Induction of the SA response, either by pathogen infection or by exogenous application of SA, strongly suppressed JA-responsive genes, such as *PDF1.2*, *LOX2*, and *VSP2*. However, in mutant *npr1-1* plants, this SA-mediated suppression of JA-responsive gene expression was completely abolished. Nuclear localization of NPR1, which is essential for SA-mediated defense gene expression (Kinkema et al., 2000), was not required for the suppression of JA-responsive genes, indicating that the antagonistic effect of SA on JA signaling is modulated through a function of NPR1 in the cytosol (Spoel et al., 2003). Recently, overexpression of the SA-regulated glutaredoxin GRX480 was found to antagonize JA-responsive *PDF1.2* transcription (Ndamukong et al., 2007), suggesting a role for redox regulation in SA-mediated suppression of JA-responsive gene expression.

While genetic approaches are ideal for identifying key players of pathway cross talk, they do not provide full insight into the actual functioning of this regulatory mechanism in response to pathogen and insect attack. Previously, we monitored changes in the signal signature and transcriptome of Arabidopsis upon attack by various microbial pathogens and herbivorous insects (De Vos et al., 2005). Clearly, timing, magnitude, and composition of the blend of signals produced play a primary role in orchestrating the induced defense response (De Vos et al., 2005). However, additional layers of regulation, such as pathway cross talk, are needed to fine tune the final outcome of the resistance reaction (Thaler et al., 2002; De Vos et al., 2006; Mur et al., 2006; Pieterse and Dicke, 2007). Here, we demonstrate that biological or chemical induction of the SA response strongly suppresses the expression of the JA-responsive genes *PDF1.2* and *VSP2*, such as triggered upon treatment with methyl jasmonate (MeJA) or attack by the JA-inducing necrotrophs *Alternaria brassicicola* and *Botrytis cinerea*, or the insect herbivores *Frankliniella occidentalis* and *Pieris rapae*. Using a pharmacological approach to dissect the kinetics and mechanisms underlying SA-JA cross talk, we demonstrate that the SA-mediated antagonistic effect on JA-responsive gene expression is conserved among Arabidopsis accessions and that the kinetics of SA and JA signaling play an important role in the outcome of the SA-JA interaction. Furthermore, we provide evidence that the antagonistic effect of SA on JA-responsive gene transcription is linked to SA-induced changes in glutathione levels, suggesting that the antagonistic effect of SA on JA signaling is modulated by redox changes.

RESULTS

SA Suppresses JA Responses Triggered by Necrotrophic Pathogens and Herbivorous Insects

In Arabidopsis, pharmacological experiments revealed that SA can antagonize the expression of JA-responsive genes, such as *PDF1.2* and *VSP2* (Spoel et al., 2003). To investigate the potential significance of this signal interaction in the defense response of plants to multiple attackers, we tested the effect of SA on the JA response as triggered by necrotrophic pathogens and herbivorous insects. To this end, the JA response was biologically activated by inoculating wild-type Columbia (Col-0) plants with the necrotrophic fungus *A. brassicicola* or *B. cinerea*, and by infesting Col-0 plants with cell-content-feeding western flower thrips (*F. occidentalis*) or tissue-chewing caterpillars of the small cabbage white (*P. rapae*; Thomma et al., 1998; De Vos et al., 2005). After 1 d, noninduced and induced plants were treated with 1 mM SA and leaves were harvested 24 h later to analyze the expression levels of the SA-responsive marker gene *PR-1* and the JA-responsive marker gene *PDF1.2*. Because *P. rapae* specifically suppresses the expression of *PDF1.2* (De Vos, 2006), we used *VSP2* as a JA-responsive marker in the Arabidopsis-*P. rapae* interaction. Figure 1A shows that the necrotrophic pathogens and the herbivorous insects activated the JA-responsive marker genes to similar levels as did the chemical agent MeJA. In combination with SA, the JA-responsive genes were consistently suppressed, indicating that exogenously applied SA is able to antagonize the JA response as induced by a broad range of attackers.

To investigate whether biological activation of the SA pathway would similarly antagonize JA signaling, Col-0 plants were inoculated with the SA-inducing biotrophic pathogen *Hyaloperonospora parasitica*. At 3 d after inoculation, *PR-1* transcripts continuously accumulated to high levels (Fig. 1B), confirming that the SA signaling pathway was activated. Subsequently, *H. parasitica*-inoculated plants were treated with 0.1 mM MeJA. The transcription of MeJA-induced *PDF1.2* and

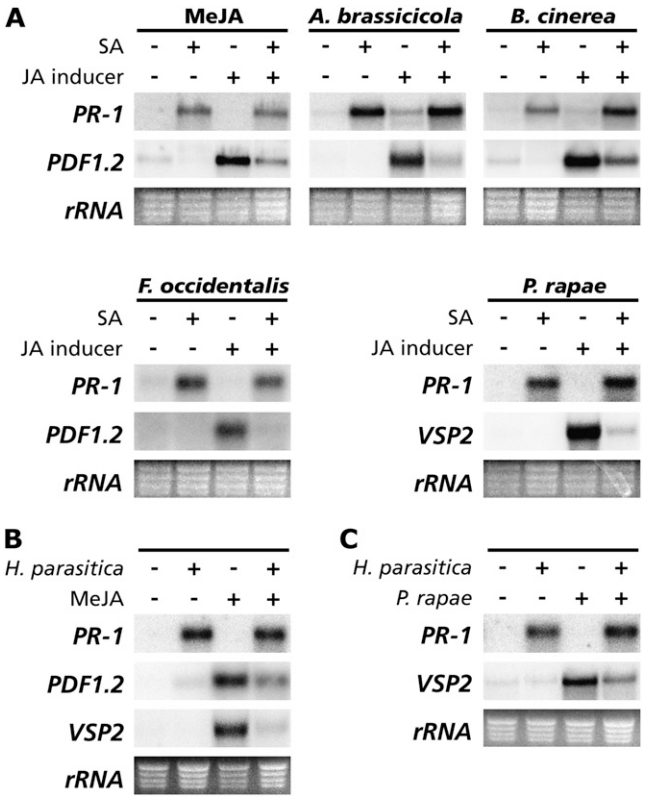

Figure 1. Biological induction of SA and JA signaling pathways results in suppression of JA-responsive gene expression. A, Exogenous application of 1 mM SA suppresses the expression of the JA-responsive marker genes *PDF1.2* and *VSP2*, triggered by MeJA, the necrotrophic pathogens *A. brassicicola* and *B. cinerea*, and the insect herbivores *F. occidentalis* and *P. rapae*. B, Infection with the SA-inducing biotrophic pathogen *H. parasitica* antagonizes MeJA-induced expression of *PDF1.2* and *VSP2*. C, *H. parasitica* suppresses *P. rapae*-induced expression of the JA-responsive gene *VSP2*. For northern-blot analysis, leaf tissue was harvested 24 h after the second treatment. Ribosomal RNA (rRNA) was used to check for equal loading of RNA samples.

VSP2 genes was found to be suppressed in *H. parasitica*-inoculated plants compared to noninoculated plants (Fig. 1B). When *P. rapae* larvae were allowed to feed on *H. parasitica*-infected Col-0 plants, the expression of *VSP2* was strongly reduced in comparison to caterpillar-infested plants that were not inoculated with the pathogen (Fig. 1C). Together, these results indicate that pathogen-induced SA negatively affects JA signaling and that, during multitrophic interactions, the SA pathway can be prioritized over the JA pathway.

SA-JA Signal Interaction Is Conserved among Arabidopsis Accessions

Naturally occurring variation in Arabidopsis accessions can be exploited to study the biological relevance and genetics of specific plant traits, such as resistance to pathogens and pests (Koornneef et al., 2004). To investigate whether Arabidopsis displays natural variation for SA-JA signal interaction, we analyzed the antagonistic effect of SA on MeJA-induced *PDF1.2* transcription in 18 Arabidopsis accessions collected from very different geographic origins. All accessions were treated with 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals. One day later, the expression of SA-responsive *PR-1* and JA-responsive *PDF1.2* was assessed (Fig. 2). The single treatments with SA or MeJA clearly activated their corresponding marker genes *PR-1* and *PDF1.2*, although the basal *PR-1* and *PDF1.2* transcript levels varied among the accessions. In the SA-MeJA combination treatments, SA-induced *PR-1* expression was not affected by MeJA in the majority of the accessions. Conversely, all accessions displayed a strong SA-mediated down-regulation of both MeJA-induced and basal levels of *PDF1.2* transcription. Hence, although several studies have reported on a differential responsiveness of Arabidopsis accessions to the plant hormones SA and (Me)JA (Rao et al., 2000; Kliebenstein et al., 2002; Van Leeuwen et al., 2007), our study demonstrates that the SA-mediated antagonism on JA-responsive gene expression is conserved among Arabidopsis accessions.

PDF1.2 Transcription Is Antagonized by Low Doses of SA

To investigate the dosage effect of SA on SA-JA cross talk, SA was applied to Col-0 plants as a foliar drench in concentrations ranging from 1,000 to 0.1 μM, either alone or in combination with 0.1 mM MeJA. After 1 d, leaf tissue was harvested and *PR-1* and *PDF1.2* expression was assessed. SA concentrations below 100 μM had no effect on *PR-1* transcription, but still antagonized MeJA-induced expression of *PDF1.2* (Fig. 3). In fact, MeJA-induced *PDF1.2* transcription was suppressed by concentrations of SA as low as 0.1 μM, although the effect was less pronounced than the suppression observed by 1,000 μM SA. A higher dose of MeJA (1 mM) could not overrule the suppressive effect of SA on *PDF1.2* expression (data not shown). These results highlight the robustness and sensitivity of the antagonistic effect of SA on JA-responsive genes, such as *PDF1.2*.

SA Triggers a Fast and Long-Lasting Antagonistic Effect on MeJA-Induced *PDF1.2* Transcription

In response to pathogen or insect attack, Arabidopsis reacts by producing an attacker-specific signal signature (De Vos et al., 2005). The kinetics of the defense signal production play an important role in shaping the final outcome of the induced defense response (Reymond and Farmer, 1998). To investigate the effectiveness of SA-JA signal interaction in view of the dynamic changes in defense signal production, we monitored the time frame during which SA is able to effectively suppress *PDF1.2* transcription. Col-0 plants were treated with SA, MeJA, or a combination of both chemicals and the expression of *PR-1* and *PDF1.2* was assessed at several time points after induction. Figure

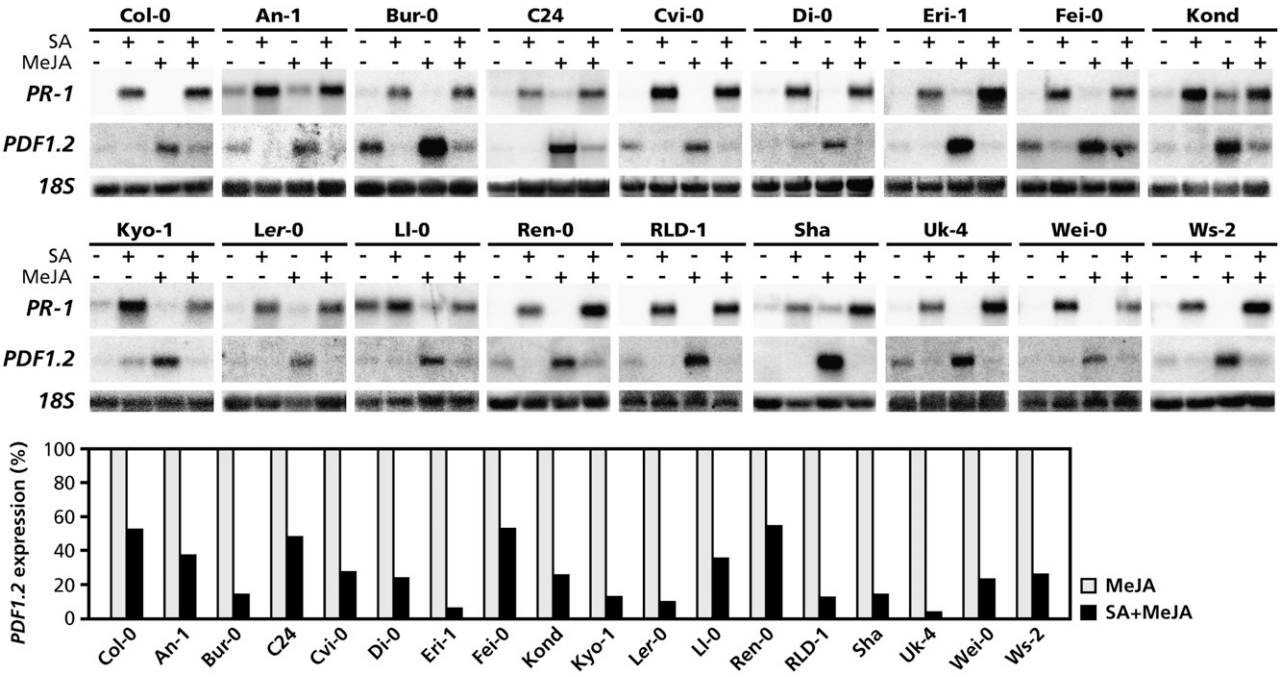

Figure 2. SA-JA signal interaction is conserved among Arabidopsis accessions. Northern-blot analysis of *PR-1* and *PDF1.2* gene expression in 18 Arabidopsis accessions after treatment with 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals. Leaf tissue was harvested 24 h after chemical treatment. Equal loading of RNA samples was checked using a probe for *18S* rRNA. Signal intensities were quantified using a phosphor imager. *PDF1.2* transcript levels in the single MeJA treatments were set to 100%.

4 shows that in the single treatments *PR-1* and *PDF1.2* transcripts were detectable 3 h after chemical application. The basal level of *PDF1.2* expression was relatively high at 6 and 12 h after treatment, which could be due to sampling at later time points during the day. In the combination treatment, again no effect of MeJA on SA-induced *PR-1* was observed. However, SA readily antagonized MeJA-induced transcription of *PDF1.2*. The suppression of *PDF1.2* by SA was clearly visible up to 4 d after chemical treatment, even though by that time SA-induced *PR-1* expression had decreased to almost undetectable levels. It can thus be concluded that the antagonistic effect of SA on JA-responsive gene expression is induced rapidly and lasts up to several days after induction of the SA signal.

Longevity of SA-JA Signal Interaction

To investigate the longevity of the SA-mediated antagonistic effect on MeJA-induced *PDF1.2* transcription, SA and MeJA were either applied simultaneously or with an interval of 3 d. Subsequently, leaf tissue was harvested 1 d after application of the last chemical for northern-blot analysis of *PR-1* and *PDF1.2* expression. Simultaneous treatment with SA and MeJA resulted in a typical suppression of MeJA-induced *PDF1.2* expression by SA (Fig. 5A, left). When SA was applied 3 d after MeJA, a similar SA-mediated suppression of *PDF1.2* was evident (Fig. 5A, middle). Note that in the middle image in Figure 5A, MeJA-induced transcript levels of *PDF1.2* are lower than in the other two images because RNA was isolated 4 d instead of 1 d

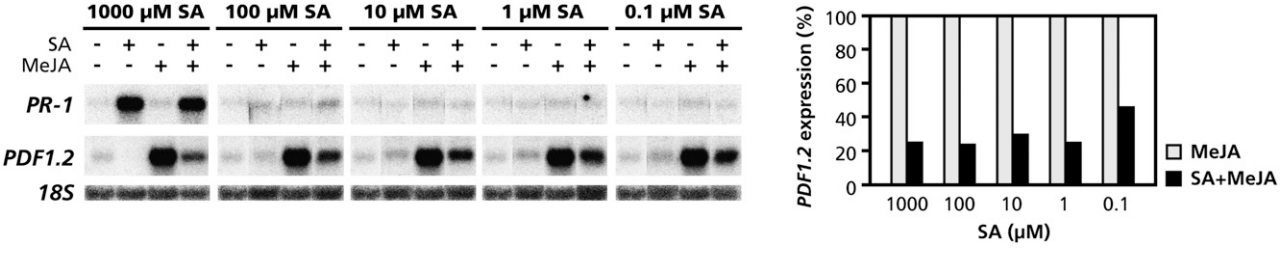

Figure 3. Very low doses of SA antagonize *PDF1.2* transcription. Northern-blot analysis of *PR-1* and *PDF1.2* gene expression in Col-0 plants treated with 1,000, 100, 10, 1, or 0.1 µM SA, with or without 0.1 mM MeJA. Leaf tissue was harvested 24 h after chemical treatment. Equal loading of RNA samples was checked using a probe for *18S* rRNA. Signal intensities were quantified using a phosphor imager. *PDF1.2* transcript levels in the single MeJA treatments were set to 100%.

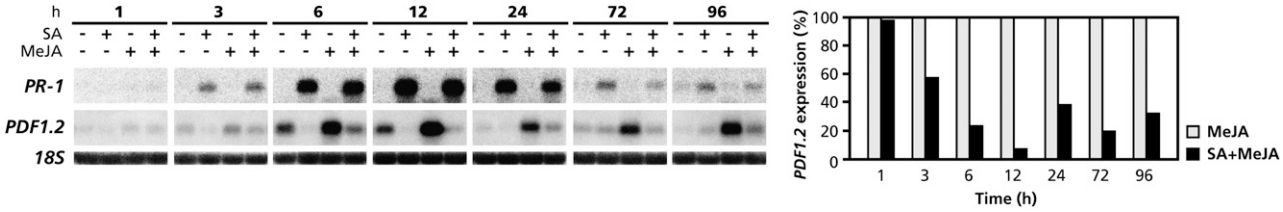

Figure 4. SA exerts a fast and long-lasting antagonistic effect on *PDF1.2* transcription. Northern-blot analysis of *PR-1* and *PDF1.2* transcript levels in Col-0 plants treated with 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals. Leaf tissue was harvested 1, 3, 6, 12, 24, 72, and 96 h after chemical treatment. To check for equal RNA loading, a probe for *18S* rRNA was used. Signal intensities were quantified using a phosphor imager. *PDF1.2* transcript levels in the single MeJA treatments were set to 100%.

after the MeJA treatment. However, when SA was applied 3 d prior to the MeJA treatment, the antagonistic effect on *PDF1.2* expression could no longer be observed (Fig. 5A, right). These results indicate that SA is capable of suppressing JA-responsive gene expression when it is produced simultaneously with or after the onset of the JA response. However, when SA is applied prior to activation of the JA pathway, the antagonistic effect of SA on JA signaling is only effective within a certain time frame after induction of the SA signal.

To investigate the window of opportunity of SA to suppress MeJA-induced expression of *PDF1.2*, we applied SA at several time points before MeJA. In all cases, Col-0 leaf tissue was harvested 1 d after the MeJA treatment for northern-blot analysis of *PDF1.2* gene expression. The antagonistic effect of SA on MeJA-induced *PDF1.2* expression was evident when SA was applied simultaneously with MeJA or up to 30 h before the MeJA treatment (Fig. 5B). However, when the time interval between the SA and MeJA treatments was extended to 48 h, the SA-mediated suppression of MeJA-induced *PDF1.2* was no longer observed. It can thus be concluded that the antagonistic effect of SA on JA signaling is transient and that the suppressive effect is lost between 30 and 48 h after induction of the SA signal.

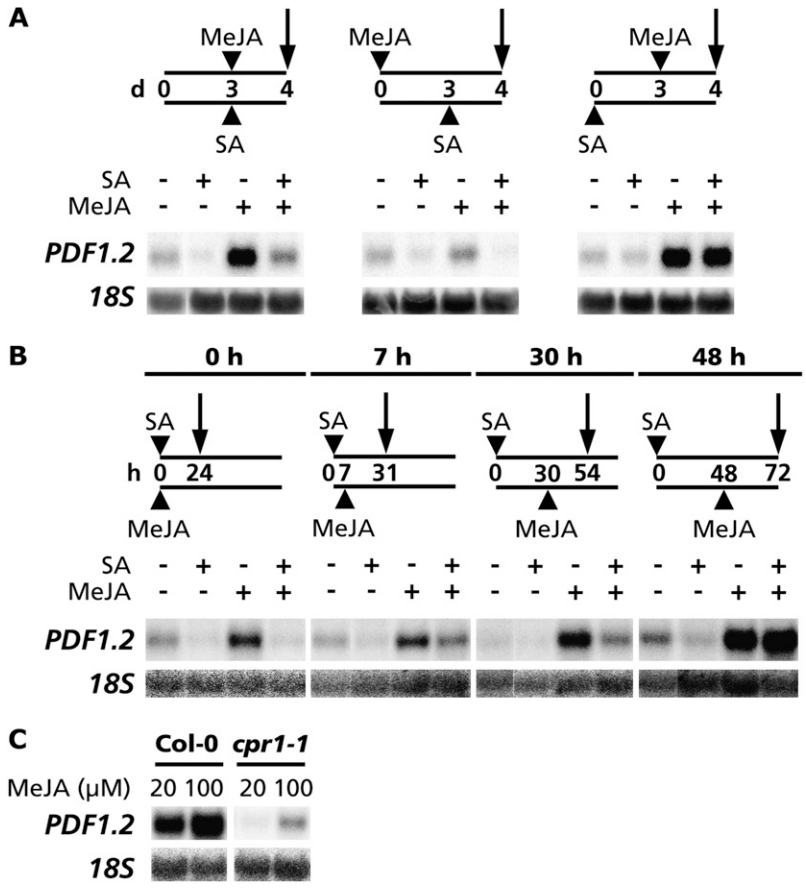

Figure 5. Longevity of the SA-mediated antagonistic effect on JA signaling. Northern-blot analysis of *PDF1.2* gene expression in Col-0 plants treated with 1 mM SA, 0.1 mM MeJA, or a combination of both chemicals. In the combination treatments, SA and MeJA were applied in different orders and with different time intervals. Leaf tissue was harvested (indicated by an arrow) 24 h after application of the last chemical. To check for equal loading, a probe for *18S* rRNA was used. A, Effect of SA on MeJA-induced *PDF1.2* transcription when SA was applied simultaneously with (left), 3 d after (middle), or 3 d before (right) MeJA. B, Effect of SA on MeJA-induced *PDF1.2* transcription when SA was supplied 0, 7, 30, or 48 h before MeJA. C, Effect of constitutive expression of the SA response on *PDF1.2* transcription. *PDF1.2* mRNA levels were determined in Col-0 and *cpr1-1* plants 24 h after treatment with 20 or 100 μM MeJA.

If the antagonistic effect of SA on JA signaling is only apparent during a certain time frame after induction of the SA signal, then constant activation of the SA-dependent signaling pathway should result in continuous down-regulation of JA-responsive genes such as *PDF1.2*. We tested this hypothesis by comparing *PDF1.2* expression in wild-type Col-0 and mutant *cpr1-1* plants after application of 20 and 100 μM MeJA. The *cpr1-1* mutant has elevated endogenous levels of SA and shows constitutive *PR-1* expression (Bowling et al., 1994). Figure 5C shows that *PDF1.2* expression was induced by both concentrations of MeJA in wild-type Col-0. However, in mutant *cpr1-1*, the effect of the MeJA treatment on the level of *PDF1.2* expression was strongly reduced. These results indicate that continuous activation of the SA response is associated with a constitutive suppression of JA-responsive gene expression.

SA-Mediated Suppression of JA Signaling Coincides with a Cellular Increase in Glutathione Levels

Changes in the cellular redox state play a major role in SA signal transduction (Després et al., 2003; Mou et al., 2003). SA-mediated redox changes activate the regulatory protein NPR1 by monomerization of inactive NPR1 oligomers, which results in the induction of SA-responsive genes such as *PR-1* (Mou et al., 2003; Dong, 2004). SA-activated NPR1 is also essential in mediating the antagonism between SA- and JA-dependent signaling (Spoel et al., 2003). Therefore, we hypothesized that the transient nature of the antagonistic effect of SA on JA signaling might be associated with changes in the cellular redox state. As a marker of the redox potential, we monitored the level of glutathione in Arabidopsis leaves upon application of SA (Fig. 6, A and B). Glutathione is a low-M_r antioxidant that functions as a major determinant of cellular redox homeostasis (Noctor and Foyer, 1998; Schafer and Buettner, 2001; Mullineaux and Rausch, 2005). Both the concentration of the total glutathione pool and the ratio between reduced (GSH) and oxidized (GSSG) glutathione can influence the redox potential of the cell (Schafer and Buettner, 2001). Basal glutathione levels fluctuated between 158 and 280 nmol g^{-1} fresh weight during the course of the experiment, which is in accordance to previously published data (Karpinski et al., 1997; Mou et al., 2003). In addition, glutathione levels were influenced diurnally, showing a general increase during daylight conditions, followed by a decrease during nighttime (Fig. 6A), as described previously (Bielawski and Joy, 1986; Koike and Patterson, 1988; Schupp and Rennenberg, 1988; Noctor et al., 1997). Pathogen attack and application of SA or one of its functional analogs have been shown to trigger an increase in total glutathione content (Fodor et al., 1997; Vanacker et al., 2001; Mou et al., 2003; Mateo et al., 2006). Similarly, SA treatment resulted in a transient increase in the level of glutathione that returned to baseline levels after 30 h (Fig. 6, A and B). A combined

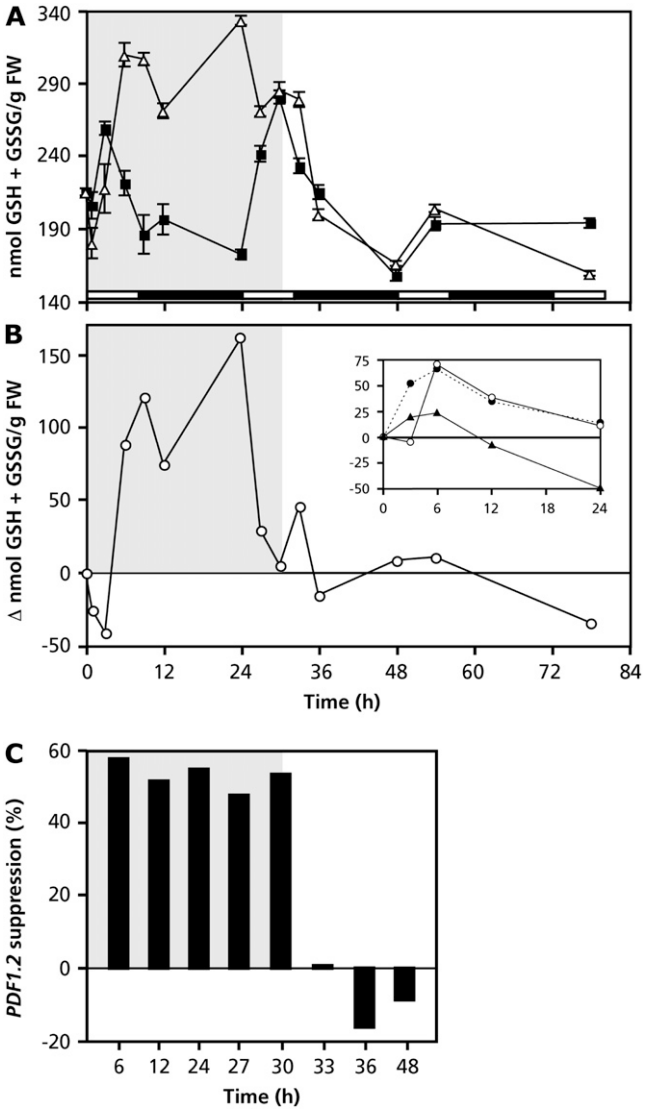

Figure 6. Suppression of *PDF1.2* by SA coincides with increased glutathione levels. A, Total glutathione levels (GSH + GSSG) in wild-type Col-0 plants, harvested 0 to 78 h after foliar drench with 1 mM SA (white triangles) or control solution (black squares). Error bars represent SE ($n = 5$). B, Glutathione levels after subtraction of control values from SA values. Inset, Increase in glutathione levels upon treatment with 1 mM (white circles), 10 μM (black circles), or 0.1 μM (black triangles) SA after subtraction of control values. C, Percentage of *PDF1.2* suppression after SA-MeJA treatment, compared to MeJA treatment alone. Signal intensities were quantified using a phosphor imager. Wild-type Col-0 plants were treated with control or 1 mM SA solutions at $t = 0$ h. Subsequently, 0.1 mM MeJA was applied at $t = 6$, 12, 24, 27, 30, 33, 36, and 48 h. Leaf tissue was harvested 24 h after application of MeJA. The shaded area represents the 30-h window of opportunity of SA to suppress *PDF1.2* expression. The white horizontal bar indicates the light period, and the black horizontal bar indicates the dark period.

treatment with SA and MeJA did not alter this pattern (data not shown). The SA-induced increase in glutathione levels was also observed when lower levels of SA (10 and 0.1 μM) were applied, albeit less pronounced in response to the lowest concentration of

0.1 μM SA (Fig. 6B, inset). Interestingly, the change in glutathione levels coincided with the window of opportunity in which SA was able to suppress MeJA-induced *PDF1.2* transcription (Fig. 6C). Hence, we postulate that the SA-mediated antagonism on JA signaling pathways is redox modulated.

Inhibition of Glutathione Biosynthesis Suppresses the Antagonistic Effect of SA on JA Signaling

To demonstrate a causal relationship between changes in glutathione levels and the down-regulation of JA-responsive gene expression by SA, we manipulated the glutathione content of the cell and monitored the effect on *PDF1.2* suppression. To deplete glutathione levels, we grew Arabidopsis seedlings on Murashige and Skoog (1962) medium, supplemented with a nontoxic and highly specific inhibitor of the first enzyme of GSH synthesis, L-buthionine-sulfoximine (BSO; Griffith and Meister, 1979; May and Leaver, 1993). Inclusion of BSO in the growth medium resulted in a strong reduction in SA-induced glutathione levels (data not shown). To assess the effect of BSO on the ability of SA to suppress JA signaling, BSO was included in the medium either during the whole growth period (2 weeks) or only during the last 48 h prior to harvest. Twelve-day-old seedlings grown on Murashige and Skoog or Murashige and Skoog supplemented with 2.5 mM BSO were transferred to Murashige and Skoog medium supplemented with 2.5 mM BSO and either 0.5 mM SA, 20 μM MeJA, or a combination of both chemicals. Leaf tissue was harvested 48 h after chemical induction and assessed for *PDF1.2* marker gene expression. Figure 7 shows normal levels of SA-JA signal interaction when the seedlings were grown on Murashige and Skoog medium without BSO (Ctrl). However, inclusion of BSO in the growth medium for 2 d clearly reduced the antagonistic effect of SA on MeJA-induced *PDF1.2* expression (Ctrl→BSO). This effect was even more pronounced when BSO was present in the medium during the whole growth period (BSO). Hence, the glutathione biosynthesis inhibitor BSO affects SA-induced suppression of JA signaling, strengthening our hypothesis that this type of SA-JA signal interaction is redox modulated.

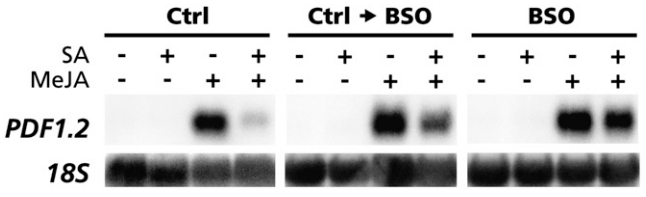

Figure 7. The glutathione biosynthesis inhibitor BSO affects the antagonistic effect of SA on JA signaling. Northern-blot analysis of *PDF1.2* expression in 14-d-old Col-0 seedlings grown on Murashige and Skoog medium with or without 2.5 mM BSO, 0.5 mM SA, 20 μM MeJA, or a combination of these chemicals. SA and MeJA treatments were performed by transferring 12-d-old seedlings to Murashige and Skoog medium with the chemicals indicated. Two days later, seedlings were harvested for northern-blot analysis. BSO was included in the medium either at day 12 (Ctrl→BSO) or during the whole growth period (BSO). Equal loading of RNA samples was checked using a probe for *18S* rRNA.

DISCUSSION

Kinetics of SA-JA Signal Interaction Demonstrate a Conserved and Robust Mechanism

Cross talk between defense signaling pathways is thought to play an important role in the regulation of induced defenses in plants. The antagonism between SA and JA signaling emerged as one of the most prominent of all signal interactions studied to date (Dong, 2004; Pieterse and Van Loon, 2004; Bostock, 2005; Nomura et al., 2005; Koornneef and Pieterse, 2008). However, the underlying molecular mechanisms of SA-JA cross talk are to a large extent unknown. In this article, we demonstrate that biological or chemical induction of the SA pathway strongly antagonizes the expression of the JA-responsive marker genes *PDF1.2* and *VSP2* as triggered by necrotrophic pathogens or insect herbivores. Moreover, we show that all 18 Arabidopsis accessions tested display SA-mediated attenuation of JA-responsive gene expression, suggesting that this trait is conserved among Arabidopsis ecotypes. Furthermore, we provide insight into how the outcome of the SA-JA signal interaction is influenced by the kinetics of the individual signaling cascades. Activation of the SA pathway resulted in an antagonistic effect on the expression of JA-responsive genes. However, when SA was applied prior to the JA trigger, SA had only a limited time frame to exert its antagonistic effect on the JA pathway. This window of opportunity of SA to down-regulate JA-responsive gene expression coincided with a transient SA-induced change in the level of the antioxidant glutathione. Moreover, inhibition of glutathione biosynthesis by BSO strongly affected SA-mediated suppression of MeJA-induced *PDF1.2* expression, suggesting a role for redox modulation in this process.

Antagonism between SA- and JA-Dependent Signaling Pathways

In this study, we predominantly observed an antagonistic effect of SA on JA-responsive gene expression, while MeJA had virtually no effect on the SA-responsive marker gene *PR-1* (Figs. 1–4). Early studies in tomato (*Solanum lycopersicum*) already revealed that SA and its acetylated form, aspirin, are potent suppressors of the JA-dependent wound response (Doherty et al., 1988; Peña-Cortés et al., 1993; Doares et al., 1995). Thus, activation of the SA pathway, such as upon infection by a biotrophic pathogen, might result in suppression of JA-dependent defenses that are triggered by necrotrophic pathogens and insect herbivores. Indeed,

we observed that inoculation with the biotrophic pathogen *H. parasitica* activated the SA pathway, resulting in down-regulation of herbivore-induced expression of the JA-responsive gene *VSP2* (Fig. 1C), indicating that during multitrophic interactions, the SA pathway can be prioritized over the JA pathway, potentially resulting in attenuation of resistance against necrotrophs and insect herbivores. Tradeoffs between SA-dependent pathogen resistance and JA-dependent defense against insect herbivory have been repeatedly reported (Thaler et al., 1999; Felton and Korth, 2000; Pieterse et al., 2001; Bostock, 2005). In Arabidopsis, the SA pathway has been shown to inhibit JA-dependent resistance against tissue-chewing herbivores, such as beet armyworm (*Spodoptera exigua*; Cipollini et al., 2004; Bodenhausen and Reymond, 2007) and cabbage looper (*Trichoplusia ni*; Cui et al., 2002, 2005), and necrotrophic pathogens, such as *A. brassicicola* (Kariola et al., 2005; Spoel et al., 2007). Intriguingly, some herbivores have been demonstrated to induce the SA pathway to actively suppress effectual JA-dependent defenses and thereby escape host defense (Zarate et al., 2007). Hence, depending on the plant attacker combination, the antagonistic effect of SA on JA-dependent defense responses may either be beneficial or deleterious.

While in our study SA-mediated inhibition of JA signaling seems to dominate over the reciprocal effect, several studies have demonstrated that JA-mediated suppression of SA signaling plays an important role in specific plant-pathogen interactions as well. A well-studied example is the suppression of SA-dependent host defenses by the jasmonate-mimicking virulence factor coronatine of the bacterial pathogen *P. syringae* (Zhao et al., 2003; Brooks et al., 2005; Cui et al., 2005; Laurie-Berry et al., 2006). Coronatine produced by *P. syringae* in a susceptible host actively inhibits the SA signaling pathway, thereby promoting susceptibility to this pathogen. Whole-genome expression profiling of Arabidopsis plants treated with either SA, MeJA, or a combination of both chemicals revealed that a substantial part of the genes that are sensitive to the SA-JA antagonism are JA-responsive genes that are suppressed by SA. Nevertheless, a significant portion of the genes that are antagonistically affected by the combination treatment with SA and MeJA consists of SA-responsive genes that are suppressed by MeJA (A. Koornneef and C.M.J. Pieterse, unpublished data). Thus, while SA and JA signaling pathways can be mutually antagonistic, the differences observed in the outcome of this signaling interaction between studies are likely to be related to the plant-attacker combination and marker genes tested.

Onset of SA-Mediated Suppression of JA Signaling Requires a Transient Change in Glutathione Levels

Our studies on the kinetics of SA and JA signaling in relation to the outcome of the SA-JA signal interaction revealed that low doses of SA are able to suppress JA-responsive *PDF1.2* transcription, suggesting that this down-regulation is highly sensitive (Fig. 3). However, the antagonistic effect was only apparent when the SA pathway was activated after the onset of the JA response, or within a time frame of about 30 h prior to the activation of the JA response, indicating that the ability of SA to suppress JA-responsive gene expression is transient (Fig. 5, A and B). These experiments were carried out with a single application of SA. Thus, when SA production is triggered upon pathogen attack, the time frame during which SA is effective may be different (Fig. 1, B and C). Although our results are to a large extent consistent with previous findings in tomato, tobacco (*Nicotiana tabacum*), and Arabidopsis (Thaler et al., 2002; Mur et al., 2006), Mur et al. (2006) demonstrated that transient synergistic effects between SA and JA signaling may occur during early stages of the SA-JA signal interaction. However, this synergism was observed only when the chemicals were applied at low doses for short durations, which may account for the differences observed. Also, these experiments were performed with Arabidopsis explants, making it difficult to directly compare the outcome of both studies (Mur et al., 2006). So how does SA manipulate JA-dependent defenses? In this article, we demonstrated that SA-mediated antagonism coincides with a transient increase in the level of glutathione, and that an inhibitor of glutathione synthesis, BSO, reduced the suppressive effect of SA on MeJA-induced *PDF1.2* expression (Figs. 6 and 7). Glutathione is a major cellular antioxidant and an important determinant of the redox state in eukaryotes (Schafer and Buettner, 2001). Previously, Mou et al. (2003) determined both total glutathione levels and the ratio of reduced (GSH) and oxidized (GSSG) glutathione in Arabidopsis upon application of the SA analog 2,6-dichloroisonicotinic acid, and observed comparable changes in kinetics in both glutathione pool size and redox status. In addition, SA-accumulating mutants with constitutive *PR-1* expression were shown to have an increased glutathione pool size (Mateo et al., 2006). Our data indicate that the SA-induced change in glutathione levels plays an important role in initiating the antagonistic effect on JA-responsive gene transcription. This finding demonstrates that redox modulation is not only important in the activation of SA-dependent genes (Mou et al., 2003), but also in the suppression of JA-responsive gene expression. The involvement of redox modulation is supported by the observation that overexpression of the SA-regulated glutaredoxin GRX480 antagonizes JA-responsive transcription of *PDF1.2* (Ndamukong et al., 2007). In addition, EDS1 and PAD4 have been implicated in transduction of redox signals in response to biotic and abiotic stresses (Wiermer et al., 2005), as well as in the regulation of cross talk as activators and repressors of SA and JA defenses, respectively (Brodersen et al., 2006).

Previously, it was demonstrated that SA-activated NPR1 is required for the suppression of JA-responsive gene expression by SA (Spoel et al., 2003) and that

activation of NPR1 is redox regulated (Mou et al., 2003). In uninduced cells, NPR1 is present as an oligomer formed through intermolecular disulfide bonds. SA mediates a change in the cellular redox potential, resulting in the reduction of the NPR1 oligomer to its active monomeric form. Monomeric NPR1 is then translocated into the nucleus where it functions as a coactivator of SA-responsive genes, such as *PR-1* (Dong, 2004). For the suppression of JA-responsive gene expression, translocation of SA-activated NPR1 into the nucleus is not required, as has been demonstrated in both Arabidopsis and rice (*Oryza sativa*; Spoel et al., 2003; Yuan et al., 2007), suggesting an important role for cytosolic NPR1 in SA-JA signal interaction. Thus, although the role of NPR1 in SA-JA cross talk and SA-induced *PR-1* gene expression seems to be dissimilar, it is plausible that both defense responses are controlled by active NPR1 monomers that are produced upon changes in the redox state. However, additional SA-dependent signaling components are required for the suppression of JA signaling because Arabidopsis transgenic plants with constitutively monomerized NPR1 did not affect JA-responsive marker gene expression in the absence of SA (Beckers and Spoel, 2006). Uncovering these players in pathway cross talk will be the focus of future research.

MATERIALS AND METHODS

Cultivation of Plants

Seeds of Arabidopsis (*Arabidopsis thaliana*) accessions Col-0 (N1092; Poland), An-1 (N944; Belgium), Bur-0 (CS6643; Ireland), C24 (N906; Portugal), Cvi-0 (N8580; Cape Verde Islands), Di-0 (N1106; France), Eri-1 (CS22548; Sweden), Fei-0 (CS22645; Portugal), Kond (CS6175; Tajikistan), Kyo-1 (W10372; Japan), L*er*-0 (NW20; Poland), Ll-0 (N1338; Spain), Ren-0 (CS22535; Netherlands), RLD-1 (N913; Russia), Sha (CS929; Tajikistan), Uk-4 (N1580; Germany), Wei-0 (N3110; Germany), Ws-2 (CS2360; Belarus), and mutants *npr1-1* and *cpr1-1* (Col-0 background) were kindly provided by M. Koornneef (Wageningen University) and X. Dong (Duke University). Seeds were sown in quartz sand. Two weeks later seedlings were transferred to 60-mL pots containing a sand-and-potting soil mixture (5:12 [v/v]) that was autoclaved twice for 20 min. Plants were cultivated in a growth chamber with an 8-h day (200 μE m^{-2} s^{-1} at 24°C) and 16-h night (20°C) cycle at 70% relative humidity for another 3 weeks. Plants were watered every other day and received one-half-strength Hoagland solution (Hoagland and Arnon, 1938) containing 10 μM Sequestreen (CIBA-Geigy) once a week.

Chemical Induction

Induction treatments were performed by dipping the leaves of 5-week-old plants in an aqueous solution containing 0.015% (v/v) Silwet L-77 (Van Meeuwen Chemicals BV), supplemented with 0.1, 1, 10, 100, or 1,000 μM SA (Mallinckrodt Baker), or 20 or 100 μM MeJA (Serva, Brunschwig Chemie), or a combination of both chemicals. Control plants were treated with 0.015% Silwet L-77 only. MeJA was added to the medium from a 1,000-fold stock solution in 96% ethanol. Solutions without MeJA were supplemented with equal amounts of ethanol. Plants were harvested between 1 and 96 h after induction treatment and immediately frozen in liquid nitrogen.

Pathogen and Insect Bioassays

Alternaria brassicicola strain MUCL 20297 and *Botrytis cinerea* strain B0510 were grown on potato dextrose agar (Difco Laboratories) plates for 2 weeks at 22°C. Subsequently, conidia were collected as described previously (Broekaert et al., 1990). Five-week-old Col-0 plants were inoculated by applying 5-μL drops of one-half-strength potato dextrose broth containing 5×10^5 spores mL^{-1}. *Pieris rapae* and *Frankliniella occidentalis* were reared as described previously (De Vos et al., 2005) and transferred to 5-week-old Col-0 plants. Infestation was carried out by transferring five first-instar larvae of *P. rapae* or 20 larvae of *F. occidentalis* to each plant using a fine paintbrush. SA (1 mM) was applied as a foliar drench 24 h after pathogen inoculation or herbivore infestation and leaf tissue was harvested another 24 h later. Sporangia from *Hyaloperonospora parasitica* strain WACO9 were collected by rinsing sporulating Col-0 leaves in 10 mM $MgSO_4$ as described previously (Van der Ent et al., 2008). Next, 5-week-old Col-0 plants were inoculated by spraying the leaves with the spore suspension containing 5×10^4 sporangia mL^{-1}. To ensure infection, plants were placed at 17°C and kept at 100% relative humidity for 24 h. After this period, plants were kept at 70% to 80% relative humidity to facilitate growth of the pathogen. MeJA (0.1 mM) was applied as a foliar drench 3 d after *H. parasitica* inoculation. In the case of two biological inducers, *P. rapae* larvae were applied 3 d after *H. parasitica* inoculation. Leaf material was harvested 24 h after MeJA or *P. rapae* treatment.

RNA Extraction and Northern-Blot Analysis

Total RNA was extracted from five to 10 plants as described previously (De Vos et al., 2005). For northern-blot analysis, 15 μg RNA were denatured using glyoxal and dimethyl sulfoxide (Sambrook et al., 1989), electrophoretically separated on a 1.5% agarose gel, and blotted onto Hybond-N^+ membrane (Amersham) by capillary transfer. The electrophoresis and blotting buffer consisted of 10 and 25 mM sodium phosphate (pH 7.0), respectively. Northern blots were hybridized with gene-specific probes for *PR-1*, *PDF1.2*, and *VSP2* as described previously (Pieterse et al., 1998). After hybridization with α-[^{32}P]dCTP-labeled probes, blots were exposed for autoradiography and signals quantified using a Bio-Rad molecular imager FX (Bio-Rad) with Quantity One software (Bio-Rad). To check for equal loading, the blots were stripped and hybridized with a probe for *18S* rRNA. The AGI numbers for the genes studied are At2g14610 (*PR-1*), At5g44420 (*PDF1.2*), and At5g24770 (*VSP2*). The probe for *18S* rRNA was derived from an Arabidopsis cDNA clone (Pruitt and Meyerowitz, 1986). All gene expression analyses have been repeated with similar results.

Glutathione Assay

Total levels of glutathione (GSH + GSSG) were measured using a glutathione assay kit (Sigma) according to the manufacturer's protocol. Leaf tissue was frozen in liquid nitrogen and ground to a fine powder. Subsequently, 500 μL of 5% 5-sulfosalicylic acid were added to 0.1 g of pulverized leaf tissue to deproteinize the sample. Glutathione was then determined in a kinetic assay in which the reduction of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) to yellow TNB was spectrophotometrically measured at 415 nm. The amount of total glutathione was calculated using a standard curve of reduced glutathione. Five plants per treatment were harvested at each time point, and each sample was measured six times.

BSO Assay

Col-0 seedlings were grown for 12 d on Murashige and Skoog (1962) medium with or without 2.5 mM BSO (Sigma) and with 10 g L^{-1} Suc and 6 g L^{-1} plant agar, pH 5.7. Seedlings were then transferred to Murashige and Skoog plates containing 2.5 mM BSO, 0.5 mM SA, 20 μM MeJA, or a combination of these chemicals. Leaf tissue was harvested 48 h later. BSO was included in the Murashige and Skoog medium either continuously or only during the last 48 h, together with the SA and MeJA treatments.

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LITERATURE CITED

Beckers GJM, Spoel SH (2006) Fine-tuning plant defence signalling: salicylate versus jasmonate. Plant Biol **8:** 1–10

Bielawski W, Joy KW (1986) Reduced and oxidised glutathione and glutathione-reductase activity in tissues of Pisum sativum. Planta **169:** 267–272

Bodenhausen N, Reymond P (2007) Signaling pathways controlling induced resistance to insect herbivores in Arabidopsis. Mol Plant Microbe Interact **20:** 1406–1420

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol **43:** 545–580

Bowling SA, Guo A, Cao H, Gordon AS, Klessig DF, Dong X (1994) A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. Plant Cell **6:** 1845–1857

Brodersen P, Petersen M, Bjorn Nielsen H, Zhu S, Newman MA, Shokat KM, Rietz S, Parker J, Mundy J (2006) Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J **47:** 532–546

Broekaert WF, Terras FRG, Cammue BPA, Vanderleyden J (1990) An automated quantitative assay for fungal growth. FEMS Microbiol Lett **69:** 55–60

Brooks DM, Bender CL, Kunkel BN (2005) The *Pseudomonas syringae* phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in *Arabidopsis thaliana*. Mol Plant Pathol **6:** 629–639

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell **124:** 803–814

Cipollini D, Enright S, Traw MB, Bergelson J (2004) Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. Mol Ecol **13:** 1643–1653

Cui J, Bahrami AK, Pringle EG, Hernandez-Guzman G, Bender CL, Pierce NE, Ausubel FM (2005) *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores. Proc Natl Acad Sci USA **102:** 1791–1796

Cui J, Jander G, Racki LR, Kim PD, Pierce NE, Ausubel FM (2002) Signals involved in Arabidopsis resistance to *Trichoplusia ni* caterpillars induced by virulent and avirulent strains of the phytopathogen *Pseudomonas syringae*. Plant Physiol **129:** 551–564

De Vos M (2006) Signal signature, transcriptomics and effectiveness of induced pathogen and insect resistance in Arabidopsis. PhD thesis. Utrecht University, Utrecht, The Netherlands

De Vos M, Van Oosten VR, Van Poecke RMP, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Métraux JP, Van Loon LC, Dicke M, et al (2005) Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. Mol Plant Microbe Interact **18:** 923–937

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Dicke M, Van Loon LC, Pieterse CMJ (2006) Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol **142:** 352–363

Després C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert PR (2003) The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell **15:** 2181–2191

Doares SH, Narváez-Vásquez J, Conconi A, Ryan CA (1995) Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol **108:** 1741–1746

Doherty HM, Selvendran RR, Bowles DJ (1988) The wound response of tomato plants can be inhibited by aspirin and related hydroxy-benzoic acids. Physiol Mol Plant Pathol **33:** 377–384

Dong X (2004) NPR1, all things considered. Curr Opin Plant Biol **7:** 547–552

Eulgem T, Weigman VJ, Chang HS, McDowell JM, Holub EB, Glazebrook J, Zhu T, Dangl JL (2004) Gene expression signatures from three genetically separable resistance gene signaling pathways for downy mildew resistance. Plant Physiol **135:** 1129–1144

Felton GW, Korth KL (2000) Trade-offs between pathogen and herbivore resistance. Curr Opin Plant Biol **3:** 309–314

Fodor J, Gullner G, Adam AL, Barna B, Komives T, Kiraly Z (1997) Local and systemic responses of antioxidants to tobacco mosaic virus infection and to salicylic acid in tobacco (role in systemic acquired resistance). Plant Physiol **114:** 1443–1451

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol **43:** 205–227

Glazebrook J, Chen W, Estes B, Chang HS, Nawrath C, Métraux JP, Zhu T, Katagiri F (2003) Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J **34:** 217–228

Grant MR, Lamb C (2006) Systemic immunity. Curr Opin Plant Biol **9:** 414–420

Griffith OW, Meister A (1979) Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl homocysteine sulfoximine). J Biol Chem **254:** 7558–7560

Hoagland DR, Arnon DI (1938) The water culture method for growing plants without soil. Calif Agric Exp Stn Bull **347:** 36–39

Howe GA (2004) Jasmonates as signals in the wound response. J Plant Growth Regul **23:** 223–237

Jones JDG, Dangl JL (2006) The plant immune system. Nature **444:** 323–329

Kariola T, Brader G, Li J, Palva ET (2005) Chlorophyllase 1, a damage control enzyme, affects the balance between defense pathways in plants. Plant Cell **17:** 282–294

Karpinski S, Escobar C, Karpinska B, Creissen G, Mullineaux PM (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. Plant Cell **9:** 627–640

Kempema LA, Cui X, Holzer FM, Walling LL (2007) Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. Plant Physiol **143:** 849–865

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol **53:** 299–328

Kinkema M, Fan W, Dong X (2000) Nuclear localization of NPR1 is required for activation of *PR* gene expression. Plant Cell **12:** 2339–2350

Kliebenstein DJ, Figuth A, Mitchell-Olds T (2002) Genetic architecture of plastic methyl jasmonate responses in *Arabidopsis thaliana*. Genetics **161:** 1685–1696

Koike S, Patterson BD (1988) Diurnal variation of glutathione levels in tomato seedlings. HortScience **23:** 713–714

Koornneef A, Pieterse CMJ (2008) Cross talk in defense signaling. Plant Physiol **146:** 839–844

Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu Rev Plant Biol **55:** 141–172

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol **5:** 325–331

Laurie-Berry N, Joardar V, Street IH, Kunkel BN (2006) The Arabidopsis thaliana *JASMONATE INSENSITIVE 1* gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. Mol Plant Microbe Interact **19:** 789–800

Li J, Brader G, Palva ET (2004) The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell **16:** 319–331

Mateo A, Funck D, Muhlenbock P, Kular B, Mullineaux PM, Karpinski S (2006) Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. J Exp Bot **57:** 1795–1807

Mauch-Mani B, Mauch F (2005) The role of abscisic acid in plant-pathogen interactions. Curr Opin Plant Biol **8:** 409–414

May MJ, Leaver CJ (1993) Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension cultures. Plant Physiol **103:** 621–627

Mou Z, Fan WH, Dong XN (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell **113:** 935–944

Mullineaux P, Rausch T (2005) Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. Photosynth Res **86:** 459–474

Mur LAJ, Kenton P, Atzorn R, Miersch O, Wasternack C (2006) The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol **140:** 249–262

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15:** 473–497

Musser RO, Hum-Musser SM, Eichenseer H, Peiffer M, Ervin G, Murphy JB, Felton GW (2002) Herbivory: caterpillar saliva beats plant defences—a new weapon emerges in the evolutionary arms race between plants and herbivores. Nature **416:** 599–600

Nakashita H, Yasuda M, Nitta T, Asami T, Fujioka S, Arai Y, Sekimata K, Takatsuto S, Yamaguchi I, Yoshida S (2003) Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. Plant J **33:** 887–898

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science **312:** 436–439

Ndamukong I, Abdallat AA, Thurow C, Fode B, Zander M, Weigel R, Gatz C (2007) SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive *PDF1.2* transcription. Plant J **50:** 128–139

Noctor G, Arisi ACM, Jouanin L, Valadier M-H, Roux Y, Foyer CH (1997) Light-dependent modulation of foliar glutathione synthesis and associated amino acid metabolism in poplar overexpressing γ-glutamylcysteine synthetase. Planta **202:** 357–369

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol **49:** 249–279

Nomura K, Melotto M, He SY (2005) Suppression of host defense in compatible plant-*Pseudomonas syringae* interactions. Curr Opin Plant Biol **8:** 361–368

Peña-Cortés H, Albrecht T, Prat S, Weiler EW, Willmitzer L (1993) Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. Planta **191:** 123–128

Pieterse CMJ, Dicke M (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. Trends Plant Sci **12:** 564–569

Pieterse CMJ, Ton J, Van Loon LC (2001) Cross-talk between plant defence signalling pathways: boost or burden? AgBiotechNet **3:** ABN 068

Pieterse CMJ, Van Loon LC (2004) NPR1: the spider in the web of induced resistance signaling pathways. Curr Opin Plant Biol **7:** 456–464

Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, Van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis.* Plant Cell **10:** 1571–1580

Pozo MJ, Van Loon LC, Pieterse CMJ (2004) Jasmonates—signals in plant-microbe interactions. J Plant Growth Regul **23:** 211–222

Pruitt RE, Meyerowitz EM (1986) Characterization of the genome of *Arabidopsis thaliana.* J Mol Biol **187:** 169–183

Rao MV, Lee HI, Creelman RA, Mullet JE, Davis KR (2000) Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. Plant Cell **12:** 1633–1646

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcriptional pattern in response to a specialist and a generalist herbivore. Plant Cell **16:** 3132–3147

Reymond P, Farmer EE (1998) Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol **1:** 404–411

Rojo E, Solano R, Sanchez-Serrano JJ (2003) Interactions between signaling compounds involved in plant defense. J Plant Growth Regul **22:** 82–98

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med **30:** 1191–1212

Schoonhoven LM, Van Loon JJA, Dicke M (2005) Insect-Plant Biology. Oxford University Press, Oxford

Schupp R, Rennenberg H (1988) Diurnal changes in the glutathione content of spruce needles (*Picea Abies* L.). Plant Sci **57:** 113–117

Spoel SH, Johnson JS, Dong X (2007) Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc Natl Acad Sci USA **104:** 18842–18847

Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux JP, Brown R, Kazan K, et al (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell **15:** 760–770

Tao Y, Xie Z, Chen W, Glazebrook J, Chang HS, Han B, Zhu T, Zou GZ, Katagiri F (2003) Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae.* Plant Cell **15:** 317–330

Thaler JS, Fidantsef AL, Bostock RM (2002) Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. J Chem Ecol **28:** 1131–1159

Thaler JS, Fidantsef AL, Duffey SS, Bostock RM (1999) Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. J Chem Ecol **25:** 1597–1609

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci USA **95:** 15107–15111

Thomma BPHJ, Penninckx IAMA, Broekaert WF, Cammue BPA (2001) The complexity of disease signaling in *Arabidopsis.* Curr Opin Immunol **13:** 63–68

Van der Ent S, Verhagen BWM, Van Doorn R, Bakker D, Verlaan MG, Pel MJC, Joosten RG, Proveniers MCG, Van Loon LC, Ton J, et al (2008) MYB72 is required in early signaling steps of rhizobacteria-induced systemic resistance in Arabidopsis. Plant Physiol **146:** 1293–1304

Van Leeuwen H, Kliebenstein DJ, West MAL, Kim K, Van Poecke R, Katagiri F, Michelmore RW, Doerge RW, St.Clair DA (2007) Natural variation among *Arabidopsis thaliana* accessions for transcriptome response to exogenous salicylic acid. Plant Cell **19:** 2099–2110

Van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci **11:** 184–191

Van Oosten VR, Bodenhausen N, Reymond P, Van Pelt JA, Van Loon LC, Dicke M, Pieterse CMJ (2008) Differential effectiveness of microbially induced resistance against herbivorous insects in Arabidopsis. Mol Plant Microbe Interact (in press)

Vanacker H, Lu H, Rate DN, Greenberg JT (2001) A role for salicylic acid and NPR1 in regulating cell growth in Arabidopsis. Plant J **28:** 209–216

Von Dahl CC, Baldwin IT (2007) Deciphering the role of ethylene in plant–herbivore interactions. J Plant Growth Regul **26:** 201–209

Wang D, Pajerowska-Mukhtar K, Hendrickson Culler A, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr Biol **17:** 1784–1790

Wiermer M, Feys BJ, Parker JE (2005) Plant immunity: the EDS1 regulatory node. Curr Opin Plant Biol **8:** 383–389

Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Li Q, Yang D, He Z (2007) Functional analysis of rice *NPR1*-like genes reveals that *OsNPR1/NH1* is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. Plant Biotechnol J **5:** 313–324

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol **143:** 866–875

Zhao Y, Thilmony R, Bender CL, Schaller A, He SY, Howe GA (2003) Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. Plant J **36:** 485–499