The *Arabidopsis thaliana* **JASMONATE INSENSITIVE 1** Gene Is Required for Suppression of Salicylic Acid-Dependent Defenses During Infection by *Pseudomonas syringae*

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Many plant pathogens suppress antimicrobial defenses using virulence factors that modulate endogenous host defenses. The *Pseudomonas syringae* phytotoxin coronatine (COR) is believed to promote virulence by acting as a jasmonate analog, because *COR-insensitive 1 (coi1)* *Arabidopsis thaliana* and tomato mutants are impaired in jasmonate signaling and exhibit reduced susceptibility to *P. syringae*. To further investigate the role of jasmonate signaling in disease development, we analyzed several jasmonate-insensitive *A. thaliana* mutants for susceptibility to *P. syringae* pv. *tomato* strain DC3000 and sensitivity to COR. *Jasmonate-insensitive 1 (jin1)* mutants exhibit both reduced susceptibility to *P. syringae* pv. *tomato* DC3000 and reduced sensitivity to COR, whereas *jasmonate-resistant 1 (jar1)* plants exhibit wild-type responses to both COR and *P. syringae* pv. *tomato* DC3000. A *jin1 jar1* double mutant does not exhibit enhanced jasmonate insensitivity, suggesting that *JIN1* functions downstream of jasmonic acid–amino acid conjugates synthesized by *JAR1*. Reduced disease susceptibility in *jin1* mutants is correlated with elevated expression of *pathogenesis-related 1 (PR-1)* and is dependent on accumulation of salicylic acid (SA). We also show that *JIN1* is required for normal *P. syringae* pv. *tomato* DC3000 symptom development through an SA-independent mechanism. Thus, *P. syringae* pv. *tomato* DC3000 appears to utilize COR to manipulate *JIN1*-dependent jasmonate signaling both to suppress SA-mediated defenses and to promote symptom development.

Additional keywords: *axr1*, *eds8*, JA-ILE.

To successfully cause disease, a plant pathogen first must create a suitable environment for growth within the host. This process includes suppression of general plant defense responses induced upon microbial attack (Heath 2000; Ponciano et al. 2003; Thordal-Christensen 2003) and stimulation of the release of water and nutrients into the apoplast (Alfano and Collmer

1996; Ponciano et al. 2003). General antimicrobial defenses include accumulation of the signaling molecules salicylic acid (SA) and jasmonic acid (JA) and JA derivatives (collectively referred to as jasmonates), induction of pathogenesis-related genes, and production of antimicrobial compounds (Felix et al. 1999; Glazebrook et al. 1997; Hammond-Kosack and Jones 1996). Little is known about the specific mechanisms utilized by pathogens to evade or inhibit these defenses; however, several secreted pathogen virulence factors have been implicated in this process (Alfano and Collmer 1996; Kunkel and Brooks 2002; Ponciano et al. 2003).

One such virulence factor is coronatine (COR), a phytotoxin required for full virulence of several strains of the bacterial plant pathogen *Pseudomonas syringae* (Bender et al. 1999). *P. syringae* strains unable to produce COR are compromised in their ability to grow and cause disease on host plants, including *Arabidopsis thaliana* (Brooks et al. 2004; Mittal and Davis 1995). Although the exact mode of action of COR is not fully understood, several lines of evidence suggest that it may alter host physiology by mimicking one or more jasmonates (Feys et al. 1994; Staswick and Tiryaki 2004; Weiler et al. 1994). This hypothesis is suggested by the structural resemblance between the coronafacic acid moiety of COR and several jasmonates (Bender et al. 1999; Feys et al. 1994; Weiler et al. 1994) and supported by the similarities between their effects on plant tissue, including inhibition of root elongation in *A. thaliana*, production of the stress pigment anthocyanin, production of ethylene, and leaf senescence (Bender et al. 1999; Feys et al. 1994; Staswick and Tiryaki 2004; Weiler et al. 1994). There is also genetic evidence that jasmonates and COR act through the same signaling pathway in *A. thaliana* and tomato, because the jasmonate-insensitive *A. thaliana* and tomato mutants, *coronatine-insensitive 1 (coi1)* and *jasmonic acid-insensitive 1 (jai1)*, respectively, are also insensitive to COR (Feys et al. 1994; Zhao et al. 2003).

The observations that *coi1* and *jai1* mutants exhibit severely decreased susceptibility to *P. syringae* infection reinforce the importance of COR in *P. syringae* virulence (Feys et al. 1994; Kloeck et al. 2001; Zhao et al. 2003). These data also suggest that an intact jasmonate-signaling pathway is required for full susceptibility to infection by COR-producing strains of *P. syringae*. One hypothesis, based on mounting evidence of mutual antagonism between jasmonate and SA pathways, is that COR promotes susceptibility to *P. syringae* infection by stimulating jasmonate signaling in plants, thereby inhibiting SA-mediated defenses that normally limit growth of *P. syringae* within host tissue (Brooks et al. 2005; Kunkel and Brooks 2002). This hy-

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pothesis is supported by observations of increased expression of SA-induced defense-related genes in COR-insensitive mutants of both *A. thaliana* and tomato (Kloek et al. 2001; Zhao et al. 2003).

The *A. thaliana coil* mutants used to assess the role of jasmonate signaling in pathogenesis are pleiotropic, exhibiting defects in multiple jasmonate-dependent processes, including pollen development (Feys et al. 1994), defense against insects and necrotrophic pathogens (Penninckx et al. 1996; Thomma et al. 1998), and induction of jasmonate-responsive genes (Benedetti et al. 1995, 1998; Feys et al. 1994; Penninckx et al. 1996; Thomma et al. 1998). Thus, *COI1* appears to be a master regulator of jasmonate-dependent responses, which makes sense because it encodes an F-box protein potentially involved in targeting multiple components of jasmonate signaling for degradation (Xie et al. 1998). As a result, *coil* mutant plants may not be appropriate for examining specific aspects of jasmonate signaling required for susceptibility to *P. syringae*. Thus, it is important to assess disease susceptibility in other jasmonate-signaling mutants, especially those impaired in only a subset of jasmonate-mediated responses.

Several additional jasmonate-insensitive mutants have been identified in *A. thaliana*, including *jasmonate-resistant 1* (*jar1*)

(Staswick et al. 1992), *jasmonate-insensitive 1* (*jin1*) (Berger et al. 1996), *jasmonate-insensitive 3* and *4* (*jai13/4*) (Lorenzo et al. 2004), *auxin-resistant 1* (*axr1*) (Tiryaki and Staswick 2002), *enhanced disease susceptibility 8* (*eds8*) (Glazebrook et al. 2003; Ton et al. 2002), and several *jasmonate-under-expressing* mutants (*jue1/2/3*) (Jensen et al. 2002). However, to date, only *jar1*, *jin1*, *axr1*, and *eds8* mutants have been genetically well characterized (Berger et al. 1996; Glazebrook et al. 1996; Lorenzo et al. 2004; Staswick et al. 1992, 2002; Staswick and Tiryaki 2004). Studies using the *jar1-1* mutant demonstrated that this mutation has no detectable impact on plant susceptibility to virulent *P. syringae* (Clarke et al. 2000; Kloek et al. 2001; Nickstadt et al. 2004; Pieterse et al. 1998). This result may not be surprising because *JAR1* encodes an enzyme involved in JA modification rather than a component of the jasmonate-signaling pathway (Staswick and Tiryaki 2004; Staswick et al. 2002). *JIN1* encodes a MYC family transcription factor involved in mediating a subset of jasmonate-induced responses (Boter et al. 2004; Lorenzo et al. 2004). Like *COI1*, this gene is required for full susceptibility to *P. syringae* pv. *tomato* (Nickstadt et al. 2004), but the mechanism or mechanisms underlying this phenotype are not well understood. *AXR1* encodes an enzyme required for converting the ubiquitin-like RUB protein into an activated form necessary for proper function of the ubiquitin-ligating SCF complex and is involved in multiple hormone-signaling pathways (del Pozo et al. 2002; Tiryaki and Staswick 2002). Recently, *axr1* mutant plants have been demonstrated to have reduced disease susceptibility to *P. syringae* pv. *tomato* infection (Kunkel et al. 2004). In contrast, *eds8* mutant plants exhibit enhanced susceptibility to *P. syringae* (Glazebrook et al. 1996). Although the relevant gene has not been cloned, gene expression profiling experiments have suggested a role for *EDS8* in jasmonate or ethylene signaling (Glazebrook et al. 2003).

In this study, we further investigate the role of jasmonate signaling in *P. syringae*–*A. thaliana* interactions. We demonstrate that *JIN1* is required both for full susceptibility to *P. syringae* pv. *tomato* and full sensitivity to COR and that the decreased disease susceptibility of *jin1-1* mutant plants is dependent on accumulation of SA during infection. These results suggest that COR-mediated stimulation of a *JIN1*-dependent jasmonate-signaling pathway suppresses SA-dependent defenses, leading to susceptibility to *P. syringae* pv. *tomato* infection.

RESULTS

The *jin1* mutants exhibit reduced susceptibility to *P. syringae*.

To further investigate the role of jasmonate signaling in susceptibility to COR-producing *P. syringae*, we infected *A. thaliana jin1-1* (Berger et al. 1996) mutant plants with the virulent *P. syringae* pv. *tomato* strain DC3000. These experiments revealed that *jin1-1* plants have reduced susceptibility to this pathogen, developing mild chlorosis and few to no water-soaked disease lesions (data not shown). To determine whether decreased symptom production in *jin1-1* plants is correlated with decreased bacterial growth in plant tissue, *P. syringae* pv. *tomato* DC3000 levels were measured over the course of infection (Fig. 1A). In wild-type Col-0 plants, bacteria multiplied more than three orders of magnitude in the first 2 days following infection and continued to increase over the next 2 days. As in wild-type plants, bacteria in *jin1-1* plants multiplied by at least three orders of magnitude during the first 2 days of infection. However, over the next 2 days, these bacterial populations failed to increase any further. Thus, *jin1-1* plants supported bacterial growth levels that were significantly lower than in wild-type plants. This differ-

Fig. 1. Growth of *Pseudomonas syringae* pv. *tomato* strain DC3000 in *Arabidopsis thaliana* jasmonate-insensitive mutants following dip inoculation. **A**, Growth in Col-0 wild-type (circle), *coi1-20* (open box), *jar1-1* (closed box), and *jin1-1 gl1* (triangle) plants. **B**, Growth in wild-type, *jin1-1 GL1*, *jar1-1* (all symbols as above), and *jin1-1 jar1-1* double mutants (open circle). Data points represent the average of three (A) or four (B) replicates $\pm$ standard error of the mean. Statistical analysis of day 4 growth data using analysis of variance followed by Tukey's method for paired comparisons indicated that *jin1 gl1*, *coi1* (A), *jin1 GL1*, and *jin1 jar1* (B) are significantly different from the wild type ($P < 0.05$). Similar results were obtained in at least three additional independent experiments.

ence in bacterial growth correlates well with the milder disease symptoms observed in *jnl1-1* plants compared with wild-type plants and is consistent with similar observations by Nickstadt and associates (2004).

For comparison, we also included two other jasmonate-insensitive mutants in this experiment, *jar1-1* and *coi1-20* (Kloek et al. 2001; Staswick et al. 1992). Consistent with previous reports, levels of bacterial growth in the *jar1-1* mutant were similar to wild-type levels at all time points examined (Fig. 1A) (Clarke et al. 2000; Kloek et al. 2001; Nickstadt et al. 2004; Pieterse et al. 1998). In contrast, bacteria multiplied only 10-fold in *coi1-20* mutant plants over the 4-day course of infection; this extremely low amount of bacterial growth is consistent with the complete absence of visible disease symptoms in these plants (Kloek et al. 2001). The reduced disease susceptibility in *jnl1-1* plants is not as pronounced as that observed in the *coi1-20* mutants. This likely can be explained by the fact that strong *coi1* alleles, such as *coi1-20* used in these studies, appear to block all jasmonate signaling, whereas *jnl1-1*, although likely to be a null allele (Lorenzo et al. 2004), affects only a subset of jasmonate responses (Berger et al. 1996). Two additional alleles, *jnl1-7* and *-8*, both of which contain early T-DNA insertions and are believed to be null alleles (Lorenzo et al. 2004), exhibited reduced disease susceptibility essentially identical to that observed in *jnl1-1* (data not shown).

The *jnl1-1* mutation originally was isolated in a *gll* (glabrous) background lacking trichomes (Berger et al. 1996). We utilized dip inoculation in our infection experiments; therefore, it is possible that the absence of trichomes may have contributed to the reduced disease susceptibility phenotype by altering epiphytic colonization or entry into the leaf by *P. syringae* pv. *tomato* DC3000 (Beattie and Lindow 1994). To determine whether the reduced disease susceptibility observed in *jnl1-1 gll* plants was enhanced by the absence of trichomes, these plants were crossed to wild-type Col-0 (discussed below). The glabrous and reduced-disease-susceptibility phenotypes segregated in the F2 progeny as single, unlinked recessive traits, whereas jasmonate-insensitivity cosegregated with reduced disease susceptibility (data not shown). Homozygous *jnl1-1 Gll* plants exhibited reduced-susceptibility phenotypes essentially identical to the original *jnl1-1 gll* lines when infected with *P. syringae* pv. *tomato* DC3000 (Fig. 1B; symptom data not shown), confirming that the decreased response to infection was not impacted significantly by the absence of trichomes. A single, homozygous *jnl1-1 Gll* line was selected for use in all further experiments to avoid possible complications from the *gll* mutation.

JIN1 and JAR1 function in the same signaling pathway mediating JA inhibition of root growth.

Although *jnl1-1* and *jar1-1* mutant plants responded differently to *P. syringae* pv. *tomato* DC3000 infection (Fig. 1), seedlings of both mutants exhibited intermediate levels of insensitivity to the inhibitory effects of methyl jasmonate (MeJA) (Berger et al. 1996; Staswick et al. 1992). These similarities and differences between *jnl1-1* and *jar1-1* mutant phenotypes suggest that jasmonate signaling may be more complex than previously described. Both *jnl1-1* and *jar1-1* mutations behave similarly to known null alleles (Lorenzo et al. 2004; Staswick et al. 2002) but lead to only partial loss of jasmonate signaling (Berger et al. 1996; Staswick et al. 2002), which might suggest functional redundancy between these two genes. However, this seems unlikely, because the two genes encode very different products (Lorenzo et al. 2004; Staswick and Tiryaki 2004; Staswick et al. 2002) that are unlikely to act at the same step in jasmonate signaling. The similarity of their MeJA insensitivity

phenotypes raises the possibility that they may function in the same pathway leading to inhibition of root growth. If JIN1 and JAR1 function in the same pathway leading to inhibition of root growth, one would predict that a *jnl1-1 jar1-1* double mutant would not exhibit enhanced MeJA insensitivity at the level of root growth, because some degree of sensitivity would be maintained via one or more additional jasmonate-signaling pathways. Alternatively, the two genes may encode components of separate jasmonate-signaling pathways, both of which are involved in physiological responses to MeJA application, such as inhibition of root elongation, but only one of which (*JIN1*) is required for *P. syringae* disease development. In this model, a *jnl1-1 jar1-1* double mutant would be predicted to exhibit increased insensitivity to MeJA treatment, perhaps similar to that observed in *coi1* mutants.

To distinguish between these two possibilities, we crossed *jnl1-1* and *jar1-1* single mutants and isolated F2 offspring homozygous for both mutations (discussed below). Because both of these alleles behave similarly to null alleles (Lorenzo et al. 2004; Staswick et al. 2002), the double mutants were presumed to lack functional JIN1 and JAR1 proteins, allowing us to examine their relative roles in jasmonate signaling. To assess jasmonate sensitivity in the resulting double mutants, we assayed root inhibition in response to exogenous application of MeJA. The *jnl1-1* and *jar1-1* single mutants each exhibited a characteristic, reproducible degree of root inhibition when grown on media containing MeJA (Fig. 2). On 10 μ M MeJA, wild-type Col-0 seedlings developed roots that were approximately 30% of their length on media lacking the hormone. In contrast, root growth in *jnl1-1* and *jar1-1* mutants was less severely inhibited by MeJA, exhibiting approximately 60 and 80%, respectively, of their untreated lengths. The *jnl1 jar1* double mutants exhibited a degree of root inhibition similar to that of *jar1-1* single mutants (approximately 75%), demonstrating that the two mutations do not produce additive effects. Thus, *JIN1* and *JAR1* appear to act in the same pathway with respect to this phenotype.

Fig. 2. Root growth inhibition of jasmonate-insensitive mutants by methyl jasmonate (MeJA) and coronatine (COR). Root lengths of Col-0 wild-type, *coi1-20*, *jar1-1*, *jnl1-1*, and *jnl1 jar1* double mutants grown on one-half strength Murashige and Skoog (0.5x MS) media (black bars), 0.5x MS containing 10 μ M MeJA (hatched bars), or 0.1 μ M COR (white bars). Roots were measured after 10 days of growth. Each value is the average of a minimum of 40 seedlings per treatment, except in the case of *coi1-20*, where approximately 10 seedlings were used. Vertical bars represent standard error of the mean. The number over each bar represents the length of seedlings on MeJA or COR as a percentage of root length when grown on MS. Similar results were obtained in a second independent experiment.

The *jin1 jar1* double mutant plants resemble *jin1-1* plants with respect to reduced disease susceptibility.

The above data suggest that *JIN1* and *JAR1* act in a single signaling pathway affecting root sensitivity to MeJA. However, the different responses of *jar1-1* and *jin1-1* mutants to *P. syringae* pv. *tomato* DC3000 suggest that *JIN1* mediates disease susceptibility via a mechanism that does not require *JAR1*. To confirm that loss of *JAR1* does not affect *P. syringae* pv. *tomato* DC3000 susceptibility in the context of the *jin1-1* mutation, we examined the disease response of the *jin1 jar1* double mutant. The double mutant plants responded similarly to *jin1-1* single mutants when infected with *P. syringae* pv. *tomato* DC3000, both in terms of symptom development (data not shown) and levels of bacterial growth (Fig. 1B). This demonstrates that the *jar1-1* mutation does not alter the plant's response to infection with virulent *P. syringae* pv. *tomato* DC3000, even in the context of a *jin1-1* mutation, confirming that *JAR1* does not play a significant role in the process of *P. syringae* pv. *tomato* DC3000 infection.

The *jin1* mutants exhibit reduced sensitivity to COR.

The reduced disease susceptibility of *jin1* and *coi1* mutants to infection with COR-producing *P. syringae* led us to hypothesize that *jin1* mutants also would be less sensitive to COR than wild-type plants, whereas *jar1-1* plants, which exhibit wild-type disease susceptibility, would respond normally to COR treatment. This is supported by the strong correlation in *coi1* mutants between COR insensitivity and reduced disease susceptibility to COR-producing bacterial strains (Feys et al. 1994; Kloeck et al. 2001). Previous studies have shown that *jin1-1* plants have reduced sensitivity to coronalon, a COR analog; however, this study was not quantitative, nor was it confirmed with authentic COR (Schuler et al. 2004). To examine the COR sensitivity of these jasmonate-signaling mutants, *jin1-1*, *jar1-1*, and *jin1 jar1* double mutant seedlings were grown on media containing 0.1 μ M COR, and root inhibition was assayed (Fig. 2). As expected, root growth of wild-type plants on COR was severely inhibited to approximately 30% of their untreated length. Conversely, roots of *coi1-20* seedlings were completely uninhibited by COR, also as expected,

Fig. 3. Coronatine (COR)-induced anthocyanin accumulation in jasmonate-insensitive mutants. Anthocyanin levels in leaves from Col-0 wild-type, *coi1-20*, *jin1-1*, and *jar1-1* plants following infiltration with a 20% (vol/vol) methanol solution (open bars) or infiltration with 5 nM COR in 20% (vol/vol) methanol (black bars). Samples with absorption values at or below background are represented as 0. Values represent an average of absorbance at 530 nm readings of six replicates corrected for chlorophyll absorption (see methods). Vertical bars represent standard error of the mean. Similar results were obtained in three independent experiments.

and, in two of three experiments, they exhibited enhanced elongation in the presence of 0.1 μ M COR. The *jin1-1*, *jin1-7*, and *jin1-8* seedlings exhibited an intermediate level of root growth on this media (approximately 55% of the untreated length), demonstrating that *jin1* mutants are partially insensitive to COR (Fig. 2 and data not shown). On the other hand, root inhibition of *jar1-1* plants appeared almost identical to that of wild-type plants (approximately 30%), a phenotype which correlates with their fully susceptible response to *P. syringae* pv. *tomato* DC3000 (Fig. 1). The *jin1 jar1* double mutants also exhibited intermediate levels of sensitivity to COR (approximately 65%), similar to the *jin1-1* single mutant. These results demonstrate a clear correlation between a plant's level of sensitivity to COR and its susceptibility to *P. syringae* pv. *tomato* DC3000 infection.

The inhibition assays described above monitored COR sensitivity in seedling roots. However, this may not adequately reflect COR sensitivity in adult leaf tissue where *P. syringae* infection occurs. To examine COR sensitivity in mature plants, a dilute solution of 5 nM COR was infiltrated into the abaxial side of leaves. Seven days later, we measured accumulation of the stress pigment anthocyanin in the infiltrated leaves, a typical response of Col-0 plants to exogenous application of JA or COR (Bent et al. 1992; Feys et al. 1994; Greenberg and Ausubel 1993). The resulting data indicated levels of COR sensitivity similar to those observed in roots (Fig. 3). Mock-treated wild-type Col-0 plants produced a relatively small amount of pigment, potentially due to environmental stress combined with the wounding response to infiltration. This response to mock treatment was not observed in the mutant lines examined. Following COR treatment, anthocyanin accumulated to significantly higher levels in wild-type plants, primarily in the petiole and midvein of the infiltrated leaf, as well as the abaxial leaf surface. Occasional pigmentation also was observed on the adaxial side of the leaf, concentrated most strongly around the site of infiltration. Similar levels and patterns of anthocyanin production were observed in *jar1-1* mutants following COR application. As expected for a fully jasmonate-insensitive mutant that does not respond to wounding (Titarenko et al. 1997) or COR application (Feys et al. 1994), *coi1-20* plants did not produce detectable levels of anthocyanin in response to either mock or COR treatment. The *jin1-1* mutant plants treated with COR accumulated small amounts of anthocyanin (Fig. 3), localized to the petiole and leaf midvein. These observations are consistent with those from seedling assays (Fig. 2), indicating that *jin1-1* mutants exhibit intermediate sensitivity to COR. Further, these results indicate that COR sensitivity as monitored in seedlings by root inhibition assays accurately reflects sensitivity in adult leaf tissue.

axr1 mutants exhibit decreased COR sensitivity, whereas *eds8* mutants do not.

Two additional jasmonate-insensitive mutants also have been shown to have altered responses to *P. syringae* pv. *tomato* DC3000 infection: *eds8* and *axr1*. The *eds8* plants had reduced sensitivity to MeJA (Glazebrook et al. 2003); however, unlike *coi1* and *jin1*, they exhibited enhanced susceptibility to *P. syringae* infection (Glazebrook et al. 1996). To investigate this apparent discrepancy, we examined the response of *eds8* mutants to 0.1 μ M COR and determined that they have wild-type sensitivity to the phytotoxin (data not shown). Thus, the reduced jasmonate sensitivity of *eds8* mutant plants does not correlate with altered sensitivity to COR.

The recent findings that *AXR1* plays a role in both disease responses to *P. syringae* pv. *tomato* DC3000 (Kunkel et al. 2004) and jasmonate signaling (Tiryaki and Staswick 2002) suggested that this mutant also may exhibit reduced COR sen-

sitivity, similar to that observed in *coi1* and *jin1* plants. To examine this hypothesis, *axr1-12* (Lincoln et al. 1990) plants were tested for COR sensitivity in root inhibition and anthocyanin accumulation assays. Results from the root inhibition assays are presented in Figure 4. Compared with wild-type Col-0, *axr1-12* plants showed significantly less inhibition of root growth when treated with COR (approximately 60% of untreated length) (Fig. 4). This intermediate level of sensitivity to COR is similar to that observed in *jin1-1* plants (Figs. 2 and 4). Similar results were obtained when COR sensitivity was assayed by monitoring anthocyanin accumulation in mature leaves (data not shown). These data suggest that *AXR1* is required for normal COR-induced responses, which is likely to account, at least in part, for the reduced disease susceptibility of *axr1* mutant plants to *P. syringae* pv. *tomato* DC3000 infection (Kunkel et al. 2004). It is important to note that, despite the role of *AXR1* in both jasmonate and auxin signaling, *JIN1* appears to be specific to jasmonate because *jin1* mutants exhibit normal sensitivity to auxin (J. Agnew and N. Laurie-Berry, unpublished data), ethylene, and ABA (Lorenzo et al. 2004).

***jin1-1* mutants exhibit decreased induction of jasmonate-responsive genes following *P. syringae* pv. *tomato* DC3000 infection.**

To further assess the jasmonate-signaling defect in the *jin1-1* mutant, we examined expression of two jasmonate-responsive genes, *lipoxygenase 2* (*LOX2*) and *coronatine-induced 1* (*COR11*), by RNA blot analysis. *LOX2* is involved in jasmonate biosynthesis and also is regulated by jasmonates (Bell and Mullet 1993). Expression of *COR11*, which encodes a predicted chlorophyllase, is stimulated by jasmonate or COR treatment and is induced during infection with *P. syringae* (Benedetti et al. 1998; Brooks et al. 2005; Tsuchiya et al. 1999). Expression of these two genes in wild-type Col-0 and *jin1-1* plants over the course of infection with *P. syringae* pv. *tomato* DC3000 is shown in Figure 5A. In wild-type plants, transcripts of both *LOX2* and *COR11* were induced within 24 h after infection, reaching their highest levels 1 to 2 days after infection. Although these genes also were induced upon infection in *jin1-1* plants, levels of both transcripts were markedly decreased rela-

Fig. 4. Inhibition of root growth by coronatine (COR) on *axr1-12* mutants. Root lengths of Col-0 wild-type, *coi1-20*, *jin1-1*, and *axr1-12* mutants grown on one-half strength Murashige and Skoog (0.5x MS) plates (black bars) or 0.5x MS plates containing 0.1 μ M COR (white bars). Roots were measured after 10 days of growth. Each value is the average of at least 17 seedlings, with the exception of *coi1-20*, where 8 seedlings were used. Vertical bars represent standard error of the mean. The number over each bar represents the percentage of untreated root length. Similar results were obtained in two independent experiments.

tive to wild-type levels at all time points examined (Fig. 5A). These data are consistent with the identification of *JIN1* as a transcription factor mediating expression of a subset of jasmonate-responsive genes (Lorenzo et al. 2004) and also with previous observations that *jin1* plants do not exhibit wild-type induction of several jasmonate-responsive genes following MeJA treatment (Berger et al. 1996; Lorenzo et al. 2004; Nickstadt et al. 2004).

We also examined expression of the jasmonate- and ethylene-inducible defense gene *plant defensin 1* (*PDF1.2*) (Penninckx et al. 1996). This gene was induced weakly upon *P. syringae* pv. *tomato* DC3000 infection, reaching detectable levels 1 day after infection (Fig. 5B). Expression of *PDF1.2* was much more strongly induced in *jin1-1* plants throughout the infection process. Although this result seems surprising at first, it is consistent with earlier observations that *JIN1* negatively regulates expression of this gene in response to MeJA treatment (Boter et al. 2004; Lorenzo et al. 2004).

Reduced susceptibility to *P. syringae* pv. *tomato* DC3000 in *jin1-1* plants correlates with elevated *PR-1* expression and is dependent on SA accumulation.

The reduced susceptibility of *coi1* mutants to *P. syringae* appears to result from hyperactivation of the SA-responsive defense pathway (Kloek et al. 2001). To determine whether *jin1-1* also exhibits enhanced SA signaling, we examined expression of the SA-responsive gene *pathogenesis-related 1* (*PR-1*) during the course of infection with *P. syringae* pv. *tomato* DC3000 (Fig. 5). In wild-type plants, this defense-related marker typically was induced within 48 h after dip inoculation with *P. syringae* pv. *tomato* DC3000 (Chen et al. 2004). In *jin1-1* plants, *PR-1* was more strongly induced than in wild-type plants and, in one of three Northern blot experiments, was

Fig. 5. Expression of jasmonic acid-dependent (*LOX2*, *COR11*, and *PDF1.2*) and salicylic acid-dependent (*PR-1*) defense response genes in wild-type Col-0 and *jin1-1* plants after dip inoculation with *Pseudomonas syringae* pv. *tomato* strain DC3000. **A** and **B**, Data shown are from two independent experiments. Total RNA was prepared from tissue harvested on the indicated days post inoculation (dpi). Approximately 7 μ g of total RNA was loaded for each sample in **A** and 10 μ g in **B**. Ethidium bromide staining of rRNA is included as a control for equal loading. Similar results were obtained for *PR-1* in a third independent experiment and in a second independent experiment for *PDF1.2*, *LOX2* and *COR11*.

observed as early as 24 h following infection (Fig. 5A). These results are consistent with observations by Nickstadt and associates (2004) that *jin1-1* mutants accumulated elevated levels of SA 24 h after infection with *P. syringae* pv. *tomato* DC3000. These data support the hypothesis that the reduced susceptibility observed in *jin1-1* mutants results from increased expression of SA-dependent defenses.

To directly test this hypothesis, we examined the effect of the *jin1-1* mutation on disease susceptibility in the context of plants impaired in their ability to accumulate SA. If the reduced disease susceptibility of *jin1-1* plants results from hyperactivation of SA-responsive defenses, overall reduction of SA levels in the plant should result in wild-type susceptibility in *jin1-1* plants. A transgenic construct containing the *P. putida* salicylate hydroxylase *nahG* gene, which encodes an enzyme that degrades SA, was introduced into the *jin1-1* line (discussed below). Disease responses were examined in the resulting *jin1-1 nahG* double homozygous lines. As expected, wild-type Col-0 plants carrying the *nahG* transgene supported higher levels of bacterial growth (Fig. 6A) and exhibited more severe disease symptoms than wild-type plants, including an increase in chlorosis and a greater number of individual water-soaked disease lesions that coalesced into patches (Fig. 6B). These results are consistent with the role of SA in limiting growth and spread of virulent *P. syringae* in *A. thaliana* plants (Delaney et al. 1994; Dewdney et al. 2000; Nawrath and Metraux 1999). The pres-

ence of the *nahG* transgene also led to significantly more severe disease symptoms in the *jin1-1* background; *jin1 nahG* plants developed chlorosis and disease lesions, neither of which was observed in the *jin1-1* parental line (Fig. 6B). The observed increase in disease symptom severity in *jin1 nahG* plants correlated with increased levels of bacterial growth (Fig. 6A). Because *nahG* suppresses the reduced disease susceptibility of *jin1-1* mutants, this phenotype appears to be dependent on accumulation of SA. However, *nahG* plants have been shown to accumulate high levels of catechol upon SA degradation, a phenomenon that promotes bacterial growth (van Wees and Glazebrook 2003), making it potentially difficult to interpret results using these plants.

To verify the apparent SA-dependence of reduced susceptibility in *jin1-1* plants, we investigated the disease susceptibility of *jin1-1* mutants carrying the *SA-induction deficient 2* (*sid2-2*) mutation. The *sid2-2* mutation, which results in disruption of the SA biosynthetic gene isochorismate synthase (*ICS1*), significantly reduces the plant's ability to synthesize SA in response to infection (Wildermuth et al. 2001). As previously reported (Dewdney et al. 2000; Nawrath and Metraux 1999), *sid2-2* mutant plants exhibited more severe disease symptoms than wild-type plants following infection with virulent *P. syringae* strains (Fig. 6B). The *jin1 sid2* double mutants also had visibly increased symptoms, compared with the *jin1-1* parental line, with the double mutants developing extensive chlo-

Fig. 6. Growth and symptom production in salicylic acid-deficient *Arabidopsis thaliana* plants. **A**, Growth of *Pseudomonas syringae* pv. *tomato* strain DC3000 in Col-0 wild-type (circle), *nahG* (square), *jin1-1* (upright triangle), and *jin1 nahG* (upside-down triangle). **B**, Disease symptoms exhibited by Col-0 wild-type, *nahG*, *jin1-1*, *jin1 nahG*, *sid2-2*, and *jin1 sid2* plants 4 days after dip inoculation with *P. syringae* pv. *tomato* strain DC3000. **C**, Growth in Col-0 wild-type (circle), *sid2-2* (square), *jin1-1* (triangle), and *jin1sid2* (open diamond). Data points in A and C represent the average of three replicates $\pm$ standard error of the mean. Asterisks indicate that day 4 growth is significantly different from the wild type ($P < 0.05$) as determined using analysis of variance followed by Tukey's method for paired comparisons. In each case, similar results were obtained in an additional independent experiment.

rosis and some disease lesions (Fig. 6B). Bacterial growth levels correlated well with the increased disease symptoms observed in these plants; *jin1 sid2* double mutants supported bacterial levels nearly identical to those seen in *sid2-2* mutants and well in excess of those observed in *jin1-1* plants (Fig. 6C). These data are consistent with the results obtained from *jin1 nahG* lines and demonstrate that the reduced disease susceptibility of *jin1-1* plants depends on the presence of SA.

Further, although the *jin1 sid2* and *JIN1 sid2* plants supported equivalent levels of bacterial growth, we reproducibly observed that the *jin1 sid2* plants developed fewer disease lesions and less chlorosis than the *sid2-2* parent (Fig. 6B and C; Table 1). These results suggest that, although *sid2-2* suppresses the inability of *jin1-1* plants to support high levels of pathogen growth, it does not fully restore disease susceptibility. Thus, *JIN1* also may be required for wild-type disease symptom development via an SA-independent mechanism.

DISCUSSION

Our results provide important experimental data in support of the hypothesis that *P. syringae* pv. *tomato* DC3000 utilizes the phytotoxin COR to manipulate jasmonate signaling within the host in a manner that promotes both pathogen growth and disease development. Our data are consistent with earlier observations (Kloek et al. 2001; Nickstadt et al. 2004; Zhao et al. 2003) that disease susceptibility requires an intact jasmonate-signaling pathway. This requires the activity of the *JIN1* transcription factor but is independent of synthesis of the JA-amino acid conjugates produced through the activity of JAR1 (Fig. 1). Reduced susceptibility to *P. syringae* pv. *tomato* DC3000 is observed in several jasmonate-signaling mutants, such as *jin1*, *coi1*, and *jai1* (Fig. 1) (Kloek et al. 2001; Zhao et al. 2003); therefore, it seems likely that manipulation of jasmonate signaling is an important virulence strategy for *P. syringae* on both *A. thaliana* and tomato.

A unified model for jasmonate signaling.

Based on several recent studies, it is becoming clear that the jasmonate-signaling pathway is more complex than previously described, and we have made an effort to incorporate these findings into a new, more comprehensive model (Fig. 7). The recent discovery that *JAR1* encodes an active JA-amino acid conjugase (Staswick and Tiryaki 2004) reinforces the idea that there are multiple forms of jasmonate acting within the plant, including MeJA and JA-amino acid conjugates, most notably JA-Ile. The main feature of this model is that jasmonate signaling occurs through a branched pathway with different jasmonates controlling distinct processes. For example, *jar1* mutants are fully fertile and only partially impaired in their responses to exogenous JA treatment (Staswick et al. 1992, 2002) (Fig. 3), suggesting that pollen development and some aspects of root inhibition do not require JA-Ile. In contrast, *JAR1* is re-

quired for resistance to several necrotrophic pathogens and full sensitivity to exogenous JA (Berrocal-Lobo and Molina 2004; Ferrari et al. 2003; Mellersh and Heath 2003; Staswick et al. 1992). MeJA also appears to be important for defense against *Botrytis cinerea*, and possibly other necrotrophic pathogens, because overexpression of the jasmonate methyl transferase (JMT) enzyme that forms MeJA results in decreased susceptibility to *B. cinerea* (Seo et al. 2001). *COI1* appears to mediate signaling through both of these branches because this gene is required for all aspects of jasmonate signaling. AXR1 is involved in proper activation of the SCF^{COI1} complex; therefore, it is likely to act at the same step in the pathway as COI1 and appears to affect all of the same responses, in addition to its independent effects on auxin-related signaling (Lincoln et al. 1990). *EDS8* is not included in our model because, presently, it is unclear what role it plays in jasmonate signaling or what form of jasmonate it may be responding to. The wild-type COR sensitivity of *eds8* mutants suggests that the gene is not involved in JA-Ile signaling.

We place *JIN1* in the *JAR1*-dependent (JA-Ile-responsive) signaling pathway downstream of *COI1* for the following reasons. First, *JIN1* and *JAR1* appear to act in the same pathway, leading to root sensitivity to exogenous jasmonates (Fig. 3). Second, *JIN1* is required for full susceptibility to infection by *P. syringae* (Fig. 1) (Nickstadt et al. 2004) and mediates jasmonate responses induced by the phytotoxin COR (Fig. 3). Given that COR is proposed to act as a molecular mimic of the endogenous jasmonate JA-Ile (Krumm et al. 1995; Staswick and Tiryaki 2004) and the recent observation that JAR1 catalyzes the formation of JA-Ile, it is likely that COR stimulates signaling through the *JAR1*-dependent pathway. Placement of *JIN1* downstream of *JAR1* in a pathway leading to *P. syringae* pv. *tomato* DC3000 susceptibility may initially appear contradictory, because *jar1* mutants retain full susceptibility to infection (Fig. 1) (Kloek et al. 2001; Nickstadt et al. 2004). However, this observation is consistent with the hypothesis that COR bypasses the requirement for production of JA-Ile to activate *JIN1*-dependent jasmonate responses leading to *P. syringae* growth and disease development. Thus, the presence of COR effectively complements the inability of the *jar1* mutant to produce JA-Ile. Furthermore, our observation that a *JMT*-overexpressing line that accumulates elevated levels of MeJA (Seo et al. 2001) does not exhibit enhanced susceptibility to *P. syringae* is consistent with the hypothesis that this process does not involve MeJA (N. Laurie-Berry, unpublished data).

To add to the complexity of this developing model, recent studies on *JIN1*, surprisingly, have demonstrated that the *JIN1*/AtMYC2 transcription factor negatively regulates several genes regarded as being positively regulated by jasmonates (Boter et al. 2004; Lorenzo et al. 2004). This finding is further supported by the accumulation of elevated transcript levels of *PDF1.2* in *jin1-1* mutants during *P. syringae* pv. *tomato* DC3000 infection (Fig. 5B). Recent findings that *jin1* mutants,

Table 1. Quantification of disease symptoms on wild-type, *jin1*, *sid2*, and *jin1 sid2* plants 4 days after infection^a

Disease symptoms	Arabidopsis genotype, number of plants (% of total)			
	Wild-type	<i>jin1-1</i>	<i>sid2-2</i>	<i>jin1 sid2</i>
No disease	0 (0)	8 (33)	0 (0)	0 (0)
Chlorosis only	0 (0)	16 (67)	0 (0)	3 (13)
Few individual lesions	1 (6)	0 (0)	0 (0)	18 (78)
Many lesions, often coalescent	17 (94)	0 (0)	32 (100)	2 (9)
Total plants examined	18	24	32	23

^a Plants were dip inoculated with *Pseudomonas syringae* pv. *tomato* DC3000 and symptoms were examined 4 days post inoculation. "Lesions" refers to the individual water-soaked lesions that typically develop following dip inoculation. Data presented reflect the number of plants of each genotype exhibiting each type of disease symptom. The percentage of the total plants of each genotype that this number represents is provided in parentheses. Similar results were seen in a second independent experiment.

unlike *jar1* and *coil* plants, exhibit reduced susceptibility to some necrotrophic fungal pathogens provides further evidence that *JIN1* negatively regulates some aspects of jasmonate signaling (Lorenzo et al. 2004; Nickstadt et al. 2004).

The abundance of new data and our appreciation for the ever increasing complexity of jasmonate signaling should be taken into account in future analyses of these processes. For example, *coil* mutants should be used in combination with other jasmonate-related mutants to fully examine the role of jasmonate in a process. Although the *COI1* F-box is required for most traditionally accepted jasmonate-mediated effects, the discrepancies between *coil* and *jin1* mutants with regard to fungal susceptibility (Lorenzo et al. 2004) and regulation of *PDF1.2* (Boter et al. 2004; Lorenzo et al. 2004; this work) demonstrate that the severe block in jasmonate signaling in *coil* mutants prevents analysis of different branches within the pathway. Thus, *coil* mutants can mask more subtle and complex regulation within this pathway. Likewise, caution should be used when interpreting the phenotypes of *jar1* mutants. *JAR1* is a biosynthetic enzyme that activates JA by conjugating it to isoleucine (Staswick and Tiryaki 2004). Although *jar1* plants do not produce JA-Ile, they do exhibit normal sensitivity to JA-Ile and COR, demonstrating that *jar1* mutants are not compromised in their ability to perceive and respond to these signaling molecules. Thus, although *jar1* mutants can be used to determine whether a jasmonate-dependent response requires the formation of JA-amino acid conjugates, data gathered using

these mutants alone cannot establish a requirement for intact jasmonate signaling in a process.

JA and SA signaling interactions and their role in *P. syringae* pathogenesis.

One of the unanswered questions in the study of *P. syringae* virulence mechanisms is why jasmonate signaling, which is known to be involved in defense, is required for disease susceptibility to *P. syringae*. In this study, we demonstrate that the reduced disease susceptible phenotype of *jin1-1* mutant plants is dependent on the accumulation of SA (Fig. 6) and is associated with elevated expression of SA-dependent defense responses (Fig. 5). This suggests that *JIN1*-dependent signaling is required for suppression of SA-mediated defenses during *P. syringae* infection. These data are consistent with the recent demonstration that COR is required to overcome SA-mediated defenses during *P. syringae* pv. *tomato* DC3000 infection of *A. thaliana* (Brooks et al. 2005). The overall picture that emerges from these studies suggests that *P. syringae* pv. *tomato* DC3000 uses COR as a jasmonate analog to manipulate host physiology in a manner that inhibits SA-mediated defenses, thereby providing the pathogen with an opportunity to grow to high levels and cause disease. This further supports the developing theory of mutual antagonism between jasmonate and SA pathways as a significant factor in regulating plant defense (Glazebrook et al. 2003; Kunkel and Brooks 2002; Reymond and Farmer 1998). It is important to note that this simple

Fig. 7. Revised model for the jasmonate and salicylic acid (SA) defense signaling pathways and their interactions during *Pseudomonas syringae* infection. The SA defense signaling pathway is shown in green. *ICS1* is directly involved in pathogen-induced synthesis of SA (Wildermuth et al. 2001). *NPR1* is required for most SA-dependent defenses, including expression of *PR-1* and induction of antimicrobial defense responses that limit growth and spread of biotrophic pathogens such as *P. syringae* (Cao et al. 1997). The jasmonate signaling pathway is shown in orange. Synthesis of jasmonic acid (JA) is dependent on *FAD3*, 7, and 8 (Browse et al. 1985). *JAR1* and *JMT* encode enzymes that catalyze the formation of modified forms of JA (e.g., JA-amino acid [JA-AA] conjugates and methyl jasmonate [MeJA]) that mediate different responses (Seo et al. 2001; Staswick and Tiryaki 2004; Staswick et al. 2002). The possibility of additional active jasmonates also is indicated. *COI1* and *AXR1* are placed downstream of both MeJA and JA-AA conjugates because *COI1* is required for all known JA responses and *AXR1* is required for proper activity of the SCF^{COI1} complex (Benedetti et al. 1995, 1998; del Pozo et al. 2002; Feys et al. 1994; Kloeck et al. 2001; Penninckx et al. 1996; Thomma et al. 1998; Tiryaki and Staswick 2002). *JIN1* is placed downstream of *COI1* because *jin1* mutants are impaired only in a subset of jasmonate-dependent responses (Berger et al. 1996; Boter et al. 2004; Lorenzo et al. 2004; Nickstadt et al. 2004; this work). *JIN1* is required for full susceptibility to *P. syringae* (Nickstadt et al. 2004; this study). *JAR1*, but not *JIN1*, is required for defense against the necrotrophic pathogens *Botrytis cinerea* and *Pythium* spp. (Ferrari et al. 2003; Staswick et al. 1998). *JIN1* appears to inhibit these defenses, based on decreased susceptibility of *jin1* mutants to *B. cinerea* (Lorenzo et al. 2004; Nickstadt et al. 2004) and elevated expression of *PDF1.2* in *jin1* mutants following JA treatment or *P. syringae* infection (Boter et al. 2004; Lorenzo et al. 2004; this work). MeJA appears to contribute to defense against necrotrophic pathogens because overexpression of *JMT* leads to resistance against *B. cinerea* (Seo et al. 2001). Inhibition of jasmonate signaling during SA-mediated defense responses is dependent on *NPR1* and appears to occur through inhibition of JA synthesis (Spoel et al. 2003). Inhibition of SA defenses by the jasmonate-signaling pathway is dependent on *JIN1* and may occur at the level of SA synthesis or accumulation as *jin1* mutants exhibit increased SA levels (Nickstadt et al. 2004). The jasmonate-signaling pathway also can be stimulated by wounding or the *P. syringae* phytotoxin coronatine, which is proposed to be a functional mimic of JA-Ile (Bender et al. 1999; Feys et al. 1994; Staswick and Tiryaki 2004; Weiler et al. 1994).

model does not account for all jasmonate-dependent events that occur during plant-microbe interactions. For example, we do not incorporate the signaling events that occur during induced systemic resistance (ISR), a plant defense response triggered by nonpathogenic rhizobacteria strains such as *P. fluorescens* WCS417-3 (Pieterse et al. 1998). ISR is mediated through a jasmonate-dependent pathway and induces resistance to *P. syringae* via a process that is independent of SA (Pieterse et al. 1998; Ton et al. 2002).

In our model for jasmonate signaling (Fig. 7), we have incorporated a possible explanation for how interactions between jasmonate- and SA-dependent signaling could result in the observed outcomes. It has been shown that SA-dependent signaling downregulates jasmonate signaling in an *NPR1*-dependent fashion (Spoel et al. 2003), whereas several recent reports suggest that the reciprocal occurs and is dependent upon *JIN1* and *COI1* (Fig. 6) (Kloek et al. 2001; Nickstadt et al. 2004; Zhao et al. 2003). The exact points of mutual inhibition between these pathways are not known; however, evidence suggests that *NPR1*-dependent SA signaling leads to the downregulation of JA synthesis within the plant (Spoel et al. 2003). Likewise, the finding that *jin1-1* mutants accumulate elevated levels of SA (Nickstadt et al. 2004) suggests that jasmonate signaling may interfere with SA synthesis or accumulation. This effect does not appear to occur at the level of *ICS1* transcript accumulation, because we did not observe elevated *ICS1* transcript levels in infected *jin1-1* plants compared with the wild type (N. Laurie-Berry, unpublished data).

SA-dependent defenses are required for the plant to limit virulence of *P. syringae*, as evidenced by the increased disease susceptibility observed in SA-deficient plants (Delaney et al. 1994; Dewdney et al. 2000; Nawrath and Metraux 1999). Likewise, *JIN1*-dependent jasmonate signaling is required for full *P. syringae* growth in planta (Fig. 1) (Nickstadt et al. 2004). This growth-promoting effect could be accomplished in two ways. The ability of *JIN1* to mediate COR-dependent suppression of SA signaling, as indicated by accumulation of elevated SA levels (Nickstadt et al. 2004) and hyperactivation of SA-dependent defenses in *jin1-1* mutants (Fig. 5), raises the possibility that *JIN1* promotes bacterial growth indirectly by decreasing SA-dependent defenses that limit bacterial proliferation. Alternatively, *JIN1* may act more directly to actively promote bacterial growth through currently unknown mechanisms. These two proposed mechanisms are not mutually exclusive, and it is possible that *JIN1* acts to increase bacterial populations by both limiting SA-dependent defenses and inducing pathogen growth via some other mechanism.

Normally, mutual antagonism between SA and jasmonate signaling should allow a plant to properly regulate activation of defense responses against a given pathogen, allowing the plant to selectively induce effective defenses without stimulating inappropriate and possibly counterproductive responses (Felton and Korth 2000; Feys and Parker 2000; Kunkel and Brooks 2002; Reymond and Farmer 1998; Thomma et al. 2001). In the case of *P. syringae* infection, the appropriate plant defense response would be activation of SA-mediated defenses and a decrease in jasmonate-dependent signaling that would otherwise increase susceptibility. Activation of *NPR1*-dependent defenses would accomplish both of these goals. We propose that *P. syringae* evolved the ability to produce COR as a molecular mimic of JA-Ile to bypass this inhibition, thereby restoring *JIN1*-dependent signaling to downregulate the plant's SA-dependent defenses and increase its susceptibility. To most effectively accomplish this goal, COR would need to stimulate jasmonate signaling downstream of *NPR1*-mediated repression; hence, the placement of this inhibition upstream of *COI1* in our model.

Other *JIN1*-dependent processes may also contribute to *P. syringae* pathogenesis.

Although COR-activated suppression of SA-mediated defenses appears to be a critical factor for *P. syringae* growth in planta, promotion of disease symptom development is likely to occur through an SA-independent mechanism. Although *jin1 sid2* plants permit wild-type levels of bacterial growth (Fig. 6C), these plants do not develop the severe disease symptoms observed on wild-type or *sid2-2* plants. We observed a similar SA-independent reduction in symptoms in *coi1 nahG* plants (Kloek et al. 2001). These data suggest that the decreased symptom production observed in *coi1-20* and *jin1-1* plants is not simply due to decreased levels of pathogen growth resulting from hyperactivation of SA-dependent defenses, because impairment of SA synthesis during infection does not fully restore wild-type symptom development. Rather, *JIN1*-mediated signaling may lead to additional, SA-independent processes that promote chlorosis and formation of disease lesions in infected plants. It is likely that this pathway also is stimulated by COR because we have observed a similar decrease in symptom development despite full bacterial growth when examining *sid2-2* plants infected with bacteria unable to synthesize COR (Brooks et al. 2005).

It is unlikely that COR is the only *P. syringae* virulence factor manipulating jasmonate signaling during this interaction. Loss of COR is not sufficient to result in elevated *PR-1* expression during *P. syringae* pv. *tomato* DC3000 infection of *A. thaliana* (Brooks et al. 2005), suggesting that one or more additional virulence factors could be suppressing SA-mediated defenses. The strong elevation of the *PR-1* transcript in *jin1-1* plants indicates that the activity of any such additional factor would require intact jasmonate signaling (Fig. 5). As evidenced by the extremely reduced susceptibility of *coi1* mutants (Feys et al. 1994; Kloek et al. 2001; Zhao et al. 2003), activation of jasmonate signaling is a critical aspect of *P. syringae* virulence. It is not unreasonable to assume that the pathogen might have evolved more than one means to stimulate signaling through this pathway to insure its ability to colonize its plant hosts. This hypothesis is supported by evidence that some type three secreted effectors may require *COI1* function to activate a marker of susceptibility (He et al. 2004). Comparative analysis of gene expression during infection of plant mutants defective in jasmonate signaling and by bacteria unable to synthesize COR might offer insights into jasmonate-dependent processes necessary for proper infection that do not require the presence of COR. Presumably, any genes involved in such processes would show altered expression in *jin1* compared with wild-type plants but not in plants infected with COR-deficient bacteria.

Conclusion.

Overall, our data provide new insight into the physiological changes *P. syringae* fosters in *A. thaliana* in order to create a suitable environment for bacterial growth and disease development. The ability to co-opt the plant's own signaling networks to prevent it from mounting an effective defense suggests a combination of both complexity and subtlety in this interaction. Future studies in this area doubtless will yield more information about signaling interactions within the plant system as well as those between the plant and pathogen.

MATERIALS AND METHODS

Bacterial strains.

The bacterial pathogen *P. syringae* pv. *tomato* strain DC3000 has been described previously (Cuppels 1986). Bacteria were grown on King's B media (KB) (King et al. 1954) or NYG (Daniels et al. 1988) containing rifampicin at 50 µg ml⁻¹ at 28°C.

Plant materials, growth conditions, and inoculation procedures.

A. thaliana ecotype Colombia (Col-0) was used in this study. The *jin1-1 gl1* mutant line (Berger et al. 1996) was obtained from Susanne Berger and the *jar1-1* mutant line (Staswick et al. 1992) from the Arabidopsis Biological Resource Center (ABRC). The male sterile *coi1-20* (Kloek et al. 2001) line was maintained as a heterozygous stock. The *nahG* transgenic line (Reuber et al. 1998) was obtained from Peter Yorgey and Fred Ausubel, and the *sid2-2 (eds16)* line (Dewdney et al. 2000; Wildermuth et al. 2001) was obtained from Mary Wildermuth. The *axr1-12* line was obtained from ABRC. This allele was chosen because it is believed to be a null allele (Lincoln et al. 1990). The *JMT* overexpressor line (Seo et al. 2001) was obtained from Scigen Harvest Company, Ltd. (Seoul, Korea).

Plants were grown from seed in growth chambers with an 8-h photoperiod at 22°C and 75% relative humidity with light intensity of 140 to 160 $\mu\text{Ein s}^{-1} \text{m}^{-1}$. All plants used for virulence studies were approximately 4 weeks old at the time of infection. All infections were carried out using dip inoculations conducted by immersing whole rosettes into bacterial suspensions of approximately 5×10^8 CFU ml^{-1} containing 0.02% (vol/vol) Silwet L-77 (OSi Specialties Inc., Danbury, CT, U.S.A.) and 10 mM MgCl_2 , as described previously (Kunkel et al. 1993). To monitor bacterial populations within the plant, individual rosette leaves were removed 0, 2, and 4 days post inoculation. For the day 0 time point, leaf tissue was sampled approximately 2 h after inoculation. Leaves were weighed, surface sterilized in 15% (vol/vol) H_2O_2 for 5 to 10 min, and rinsed three times with sterile water. Leaves then were homogenized, and appropriate dilutions were plated on NYG medium containing rifampicin as described above. Plates were incubated at 28°C for 48 h before counting CFU.

MeJA and COR root inhibition assays.

The sensitivity of seedlings to MeJA and COR was assayed by germinating sterilized seed on one-half strength Murashige and Skoog (0.5× MS) (Murashige and Skoog 1962) plates (pH 6.0, 1% [wt/vol] agar, 1% [wt/vol] sucrose) containing 10 μM MeJA (Sigma Aldrich, St. Louis) or 0.1 μM COR (C. Bender, Oklahoma State University, Stillwater, OK, U.S.A.). Seedlings were grown vertically on square plates. To ensure that the roots remained completely within the agar, an approximately 1-in.-thick section of agar was removed from the top of each plate, and seeds were placed on the resulting cut surface. After 2 days of cold treatment in the dark, plates were placed vertically in a growth chamber such that roots grew downward through the agar. Digital images of the plates were taken after 10 days of growth in continuous light, and roots were measured using NIH Image (Research Services Branch of the National Institute for Mental Health, Bethesda, MD, U.S.A.).

COR sensitivity in leaf tissue.

COR sensitivity of leaf tissue was measured in leaves of 4-week-old plants. Leaves were syringe-infiltrated with either 5 nM COR dissolved in 20% (vol/vol) methanol or a mock solution containing 20% (vol/vol) methanol in water. Infiltration was conducted so that approximately half of the leaf area was saturated with the solution. Seven days later, the leaves were harvested and weighed, and areas of anthocyanin production were noted. Pigments were extracted by shaking overnight at 4°C in 500 μl of methanol containing 1% (vol/vol) HCl (Rabino and Mancinelli 1986). Absorbance of the extracted solution was measured at 530 and 657 nm (A_{530} and A_{657} , respectively). Anthocyanin levels for each leaf were calculated as $A_{530} - (0.25 \times A_{657})/(\text{g fresh weight})$ to correct for absorption by chlorophyll (Rabino and Mancinelli 1986).

Creation of *jin1 GL1* lines.

Homozygous *jin1-1 gl1* plants were crossed to wild-type Col-0 plants. The resulting F1 plants were allowed to self-pollinate and their seed was harvested and planted. From this population, 112 of the resulting F2 plants were examined for the presence of trichomes and assayed for disease susceptibility by dip inoculation as described above. Of these plants, 60 exhibited wild-type levels of susceptibility and trichomes, 20 were susceptible and lacked trichomes, 21 exhibited both reduced disease susceptibility and the presence of trichomes, and 6 exhibited reduced disease susceptibility while lacking trichomes. This is as predicted for Mendelian segregation of two independent loci (χ^2 1.524, $P > 0.5$). F3 progeny from plants with trichomes that exhibited reduced susceptibility then were scored for the presence of trichomes and for disease susceptibility and plants that were *jin1 GL1* were identified based on reduced disease susceptibility and presence of trichomes.

To verify cosegregation of reduced disease susceptibility with JA insensitivity, F2 seed from this cross was grown on 0.5× MS media containing MeJA as described above. Seedlings were scored for JA insensitivity and then 50 exhibiting JA insensitivity and 50 with wild-type sensitivity were transplanted to soil for disease susceptibility assays. All (100%) of the JA-insensitive plants also exhibited reduced susceptibility to *P. syringae* pv. *tomato* DC3000, whereas 100% of those with wild-type sensitivity also developed wild-type disease symptoms. Thus, the JA-insensitivity and reduced-disease-susceptibility phenotypes cosegregate in this population.

Identification of *jin1 jar1* double mutants.

Approximately 80 F2 progeny from a cross between homozygous *jin1-1* and *jar1-1* mutants were assayed for disease susceptibility using dip inoculations as described above. Of these, 24 F2 plants (approximately 25%) were homozygous for the *jin1-1* mutation, based on their reduced susceptibility to infection. F3 progeny from several of these homozygous *jin1-1* plants were screened to identify *jar1-1* homozygotes via derived cleaved amplified polymorphic sequence analysis (Neff et al. 1998) using the following polymerase chain reaction (PCR) primers: *jar1*For (5' CAA TGG AAA CGC TAC TGA CCC TGA 3') and *jar1*Rev (5' ATA AAC TTT GGA CGG CTT TGA CTA GTT CTA 3'). The resulting 250-bp fragment then was cleaved by *Xba*I to reveal a polymorphism present in wild-type *JAR1* and absent in *jar1-1* mutant plants.

Identification of *jin1 nahG* lines.

Homozygous *jin1-1 gl1* plants were crossed to Col-0 *nahG* plants in which the *nahG* transgene had been inserted in a T-DNA construct also containing kanamycin resistance. Segregating F2 seed was plated on 0.5× MS agar containing kanamycin (50 $\mu\text{g ml}^{-1}$) and 10 μM MeJA. Of the 142 seedlings plated, 100 individuals (approximately 75%) remained green on 0.5× MS Kan plates, indicating the presence of the kanamycin resistance gene present in the T-DNA construct carrying the *nahG* gene. Of these green seedlings, 22 individuals (approximately 25%) exhibited insensitivity to MeJA and were transplanted and grown for seed. F3 progeny from these plants were assayed on 0.5× MS agar containing Kan to distinguish *nahG* homozygotes from heterozygous lines segregating for this trait. F3 seedlings were also grown on 10 μM MeJA to confirm the *jin1-1* phenotype of JA insensitivity.

Identification of *jin1 sid2* double mutants.

F2 seedlings of a cross between *jin1-1* and *sid2-2* homozygous plants were grown on 0.5× MS plates containing 0.01 μM COR, as described above. Seedlings that exhibited JA

insensitivity were transplanted to soil and allowed to self-fertilize. F3 populations derived from each of these individuals were grown on 10 μ M MeJA, as described above, to confirm the presence of the *jin1-1* mutation. The *sid2-2* homozygous plants were identified from these lines by PCR designed to amplify a region in exon IX of the *ICS1* gene that contains a 50-bp deletion in *sid2-2* mutant plants (Wildermuth et al. 2001). This was done using primers ICS1F (5' GCT CTG CAG CTT CAA TGC TT 3') and ICS1R (5' CGA AGA AAT GAA GAG CTT GGA AAT G 3'). PCR products were resolved on a 3% (wt/vol) agarose gel using Tris-borate-EDTA running buffer. Wild-type plants yielded a product of approximately 250 bp, whereas *sid2-2* mutants yielded a product of approximately 200 bp. Plants heterozygous for the *sid2-2* mutation were identified by the presence of both bands.

RNA isolation and Northern analysis.

Leaf tissue harvested from approximately six individual inoculated *A. thaliana* plants was pooled for each time point and stored at -80°C until all samples were obtained. Total RNA was isolated using RNAwiz (Ambion, Austin, TX, U.S.A.). RNA gel-blot analysis was carried out according to Sambrook and associates (1989). Total RNA (7 μ g in Fig. 5A, 10 μ g in Fig. 5B) was loaded in each lane. Hybridization probes were prepared using the Prime-it II kit (Stratagene, La Jolla, CA, U.S.A.). The *A. thaliana* cDNAs corresponding to the *LOX2*, *COR11*, *PDF1.2*, and *PR-1* genes were used as probes (Bell and Mullet 1993; Benedetti et al. 1998; Penninckx et al. 1996). The RNA blots were analyzed using a phosphorimager (BioRad Personal Molecular Imager FX; BioRad, Hercules, CA, U.S.A.).

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