Pseudomonas syringae manipulates systemic plant defenses against pathogens and herbivores

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Many pathogens are virulent because they specifically interfere with host defense responses and therefore can proliferate. Here, we report that virulent strains of the bacterial phytopathogen *Pseudomonas syringae* induce systemic susceptibility to secondary *P. syringae* infection in the host plant *Arabidopsis thaliana*. This systemic induced susceptibility (SIS) is in direct contrast to the well studied avirulence/*R* gene-dependent resistance response known as the hypersensitive response that elicits systemic acquired resistance. We show that *P. syringae*-elicited SIS is caused by the production of coronatine (COR), a pathogen-derived functional and structural mimic of the phytohormone jasmonic acid (JA). These data suggest that SIS may be a consequence of the previously described mutually antagonistic interaction between the salicylic acid and JA signaling pathways. Virulent *P. syringae* also has the potential to induce net systemic susceptibility to herbivory by an insect (*Trichoplusia ni*, cabbage looper), but this susceptibility is not caused by COR. Rather, consistent with its role as a JA mimic, COR induces systemic resistance to *T. ni*. These data highlight the complexity of defense signaling interactions among plants, pathogens, and herbivores.

Arabidopsis | induced susceptibility | coronatine | *Trichoplusia ni*

As immobile organisms, plants have little choice but to defend themselves against both pathogens and herbivores by mounting a variety of chemical, biochemical, and physiological defenses. Because defenses can be costly for plants in the absence of enemies (1), selection often favors the evolution of inducible rather than constitutive resistance (2). Plant defenses effective against one enemy may or may not confer resistance to other enemies (3). Moreover, some pathways involved in defense appear to have negative regulatory effects on pathways involved in resistance to other enemies (4, 5). Dissecting the mechanics of defense signaling "cross-talk" and its implications for calibrating phenotypic responses therefore is critical for understanding the ecology and evolution of plant resistance.

Plant microbial pathogens are referred to as virulent if they cause disease symptoms in susceptible hosts and avirulent if they elicit a strong defense response that blocks pathogenesis (6). One mechanism by which pathogens, such as the Gram-negative bacterium *Pseudomonas syringae*, activate an immune response is through the translocation of effector proteins (virulence factors) directly into host cells via a type III secretion system (6). Detection of type III effectors by resistance proteins encoded by *R* genes activates a signaling cascade leading to rapid, localized programmed cell death known as the hypersensitive response (HR), which is correlated with the restriction of pathogen growth at the infection site (6). In turn, the HR leads to a systemic response mediated by salicylic acid (SA) called systemic acquired resistance (SAR), in which noninfected leaves become resistant to a wide range of bacterial and fungal pathogens whose growth is limited by SA-dependent responses. Genes encoding type III effectors that are recognized through host *R* genes in an avirulent interaction are termed avirulence genes (*avr*) because of the phenotype they confer. Virulent pathogens, in contrast, cause disease either because they do not produce type III effectors that are recognized by the host or because they block the HR itself.

In addition to SA, another low molecular mass compound, jasmonic acid (JA), plays a role in modulating resistance against a variety of pathogens including *P. syringae* (7, 8). JA also plays a key role in conferring resistance to herbivorous insects (9, 10). Interestingly, depending on the particular experimental circumstances, SA- and JA-dependent pathways have been shown either to have additive effects on the induction of defense responses (11) or to be reciprocally antagonistic (12–14). In addition, the direction and degree of cross-talk can vary depending on the plant accession and/or enemies involved (12, 13, 15). Although SA/JA cross-talk presumably reduces fitness costs of inappropriate resistance and fine-tunes defense responses across biotic and abiotic environments (4, 12), the underlying molecular mechanisms of SA/JA cross-talk have not been elucidated, and it is not clear which components of each pathway affect each other and where and how the additive or antagonistic effects are exerted (4, 16).

To investigate the consequences of SA/JA cross-talk, we previously developed a three-way interaction model involving the cruciferous plant *Arabidopsis*, the bacterial pathogen *P. syringae*, and the generalist insect herbivore *Trichoplusia ni* (cabbage looper) (17). Our assay involved infecting ("priming") (18) three lower leaves of *Arabidopsis* plants with virulent or avirulent strains of *P. syringae* (that is, expressing or not expressing an appropriate *avr*), removing the infected leaves, and quantifying secondary pathogen infection by *P. syringae* or herbivory by *T. ni* on uninfected leaves. We had predicted that induction of SAR (mediated by SA) by an avirulent pathogen would, through SA/JA antagonism (4, 12), make the plant more susceptible to *T. ni* herbivory. However, we found that infection by avirulent strains of *P. syringae* pv. *maculicola* (*Psm*) ES4326 resulted in a reduction in *T. ni* caterpillar weight gain (17), suggesting that *Psm* ES4326 also might up-regulate either JA-dependent resistance (15, 19) or an as-yet-unidentified pathway. Moreover, infection by virulent *Psm* ES4326 unexpectedly resulted in increased weight gain of caterpillars on uninfected leaves (17). These data do not support the model of a simple tradeoff between resistance to pathogens and insects and thus

Abbreviations: SA, salicylic acid; SAR, systemic acquired resistance; *avr*, avirulence gene; JA, jasmonic acid; MeJA, methyl JA; *Psm*, *Pseudomonas syringae* pv. *maculicola*; *Pst*, *P. syringae* pv. *tomato*; SIS, systemic induced susceptibility; COR, coronatine; Km^r, kanamycin resistance; cfu, colony-forming units; CFA, coronafacic acid; CMA, coronamic acid.

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require a more detailed dissection of the mechanisms underlying manipulation of host signaling by *P. syringae*.

In this article, we further investigate the phenomenon of systemic induced susceptibility (SIS) elicited by priming *Arabidopsis* plants with virulent pathogens. We demonstrate that priming with virulent *P. syringae* strains elicits SIS to subsequent *P. syringae* infection and that SIS in this case is a consequence of the production by the *P. syringae* priming strain of the phytotoxin coronatine (COR), a JA mimic. In contrast, we demonstrate that COR is not the cause of *P. syringae*-elicited enhanced susceptibility to *T. ni* feeding, but is instead an inducer of resistance to herbivory.

Materials and Methods

***Arabidopsis–P. syringae–T. ni* Model System.** WT *Arabidopsis* (Col-0), mutant *npr1-1*, and transgenic *nahG* plants were grown as described (17). Homozygous mutant *coi1-1* plants were selected on MS basal medium supplemented with vitamins, 1% sucrose, 0.5 g/liter Mes, and 10 μM methyl JA (MeJA) and then transferred to soil (20). The bacterial strains *Psm* ES4326 (21) and *P. syringae* pv. *tomato* (*Pst*) DC3000 (22) are virulent on Col-0; avirulent strains carried *avrRpt2* or *avrRpm1* on the plasmids pLH12 (23) or pAvrRpm1 (24), respectively. *T. ni* eggs were obtained from a highly inbred population (Entopath, Easton, PA) and hatched at 28°C for 2 days.

Construction of a *Psm* ES4326 COR Mutant. The *cfa6* gene, which encodes a polyketide synthase essential for COR biosynthesis (25), was disrupted at a *Bgl*II site by insertion of a 1.2-kb cassette encoding kanamycin resistance (Km^r). The *cfa6*::Km^r construct was integrated into the *Psm* ES4326 genome by homologous recombination, and the resulting mutant was verified by DNA blot analysis and PCR (data not shown). The *Psm* ES4326 *cfa6*::Km^r mutant did not produce detectable levels of COR (data not shown). Plasmid pLH12 (*avrRpt2*) was transferred into the *cfa6*::Km^r mutant as described (26) to generate ES4326 *cfa6*::Km^r (*avrRpt2*).

Bacterial Growth Assays. Three lower leaves of 5-week-old *Arabidopsis* plants were infiltrated (primed) as described (27) with a suspension of *Psm* ES4326 (±*avrRpt2*), *Psm* ES4326 *cfa6*::Km^r (±*avrRpt2*), or *Pst* DC3000 (±*avrRpt2*) in 10 mM $MgSO_4$ at a density of $\approx 10^6$ colony-forming units (cfu)/cm^2 of leaf area (equivalent to an inoculum concentration $OD_{600} = 0.2$ or $\approx 10^8$ cfu/ml). In parallel, 10 mM $MgSO_4$ was infiltrated as a procedural control. Four days later, infiltrated leaves were removed and a secondary infiltration was carried out in three upper (systemic) leaves at a dose of $\approx 10^3$ cfu/cm^2 of leaf area ($OD_{600} = 0.0002$ or $\approx 10^5$ cfu/ml). Four days later, bacterial growth was assayed as described (21, 27). Bacterial growth was analyzed in each plant genotype in an ANOVA using pathogen treatment and trial as the factors.

COR, Coronafacic Acid (CFA), Coronamic Acid (CMA), and MeJA Treatment. For bacterial growth assays, serial dilutions of purified COR, CFA, and CMA (28, 29) were made in 10 mM $MgSO_4$ from methanol stock solutions. MeJA was diluted from a 95% pure stock (Sigma) in 10 mM $MgSO_4$. These compounds were infiltrated as above and *Psm* ES4326 growth (21) in systemic leaves was scored. Bacterial growth was analyzed in an ANOVA using chemical treatment and trial as the factors.

***T. ni* Feeding Assays.** In the assay involving preinoculation of *Psm* ES4326, three lower leaves of 5-week-old *Arabidopsis* plants were infiltrated with one of the *P. syringae* strains described above or the procedural control (10 mM $MgSO_4$). Four days later, infiltrated leaves were removed and single neonate *T. ni* caterpillars were caged on single whole plants. After 7 days of feeding, caterpillars were harvested, dried for 3 days at 70°C, and weighed (17). Because neonate *T. ni* caterpillars have an insignificant starting dry weight (≈ 10 μg), final weights accurately represent weight gain. Weight gain data were log-transformed for analysis to meet the assumptions of ANOVA. For clarity, untransformed data are presented in Figs. 2 and 3. In two cases (see Figs. 2*A* and 3), experimental trial was significant, but the trial × treatment interactions were never significant, indicating that despite differences in absolute caterpillar weight gains among trials, the relative effects of the treatments were consistent and interpretable.

In the *T. ni* feeding assays after elicitor treatment, stock solutions of MeJA, COR, and SA were made in 1% acetone, MeOH, and H_2O, respectively, and subsequently diluted in H_2O (where applicable). Procedural controls involved treatment with the solvent used to dissolve and dilute each elicitor. For Fig. 2*A*, three lower leaves of 5-week-old plants were infiltrated with 10 μM MeJA, 10 μM COR, or their corresponding controls, and *T. ni* performance was quantified 4 days later as above. Because plants were paired from the start, significance of differences between MeJA- or COR-treated plants and their corresponding control plants were assessed by using a paired *t* test. For Fig. 3, whole 5-week-old plants were sprayed to runoff on leaf undersides with 5 mM SA, 1 mM MeJA, or their corresponding controls. Two days later, *T. ni* performance was quantified as above. The plants were not covered with plastic domes during this experiment. Weight gain was analyzed in an ANOVA using chemical treatment and trial as the factors.

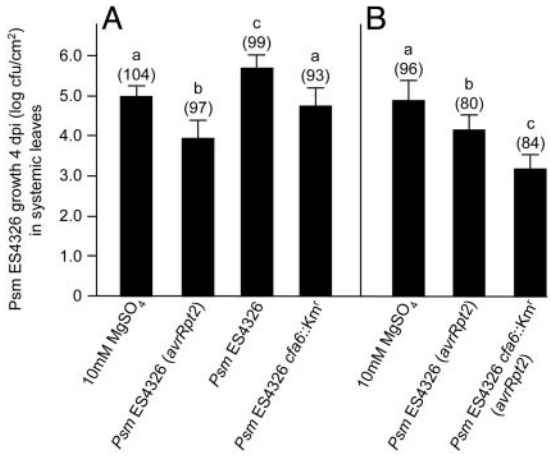

Fig. 1. *P. syringae* causes SIS by producing COR. *Psm* ES4326 growth was determined in secondarily infected leaves of *Arabidopsis* plants previously primed with *Psm* ES4326 strains with or without a cloned *avr* (*avrRpt2*) and with WT or mutant COR-producing capability (*cfa6*::Km^r), or control (10 mM $MgSO_4$) (see *Materials and Methods*). Results show means + SE and sample sizes (in parentheses above bars) for five pooled trials (*A*) (ANOVA, treatment: $P < 0.001$; trial: $P = 0.118$; treatment × trial: $P = 0.484$) and four pooled trials (*B*) (ANOVA, treatment: $P < 0.0001$; trial: $P = 0.102$; treatment × trial: $P = 0.337$). Letters above the bars signify statistically significant differences among groups (Bonferroni correction, $P < 0.05$).

Results

***P. syringae* Elicits SIS.** We observed that infection of *Arabidopsis* leaves with virulent strains of *Psm* ES4326 or *Pst* DC3000 resulted in significantly enhanced secondary growth of *Psm* ES4326 in uninfected leaves as compared with mock-treated plants (Fig. 1*A* and Table 1). We refer to this priming effect as SIS. Because the SIS effect was relatively small (approximately one-half log of increased growth in the secondarily infected leaves), it was necessary to take repeated measurements with relatively large sample sizes to confirm these results. Neverthe-

Table 1. Elicitation of SIS by *P. syringae*, COR, and MeJA

Infecting strain or chemical treatment in lower leaves	Dose	Host plant	Sample size, *n*	Enhanced (+) or diminished (−) *Psm* growth, log cfu/cm^{2†}
Psm ES4326	10^8 cfu/ml	WT	122	(+) 0.69 ± 0.07***
Psm ES4326 (*avrRpt2*)	10^8 cfu/ml	WT	120	(−) 0.45 ± 0.07***
Pst DC3000	10^8 cfu/ml	WT	120	(+) 0.62 ± 0.14***
Pst DC3000 (*avrRpt2*)	10^8 cfu/ml	WT	126	(−) 0.45 ± 0.13*
Psm ES4326 *cfa6*::Km^r	10^8 cfu/ml	WT	123	(+) 0.15 ± 0.07 (ns)
Psm ES4326 *cfa6*::Km^r (*avrRpt2*)	10^8 cfu/ml	WT	120	(−) 1.12 ± 0.08***
Psm ES4326	10^8 cfu/ml	*coi1-1*	64	(+) 0.22 ± 0.19 (ns)
Psm ES4326 (*avrRpt2*)	10^8 cfu/ml	*coi1-1*	47	(−) 0.42 ± 0.18*
Psm ES4326	10^8 cfu/ml	*npr1-1*	36	(+) 0.49 ± 0.14*
Psm ES4326	10^8 cfu/ml	*nahG*	62	(+) 0.12 ± 0.13 (ns)
COR	10 μM	WT	96	(+) 0.41 ± 0.09*
COR	1 μM	WT	96	(+) 0.57 ± 0.14**
COR	10^{-1} μM	WT	95	(+) 0.58 ± 0.10***
COR	10^{-2} μM	WT	96	(+) 0.81 ± 0.14***
COR	10^{-3} μM	WT	94	(+) 0.40 ± 0.10**
COR	10^{-4} μM	WT	95	(+) 0.13 ± 0.12 (ns)
MeJA	10^3 μM	WT	72	(+) 0.53 ± 0.12**
MeJA	10^2 μM	WT	72	(+) 0.62 ± 0.14***
MeJA	10 μM	WT	72	(+) 0.74 ± 0.11***
MeJA	1 μM	WT	71	(+) 0.53 ± 0.11**
MeJA	10^{-1} μM	WT	72	(+) 0.51 ± 0.14**
MeJA	10^{-2} μM	WT	72	(+) 0.24 ± 0.12 (ns)
CMA	1 μM	WT	84	(−) 0.22 ± 0.13 (ns)
CFA	1 μM	WT	87	(+) 0.06 ± 0.12 (ns)

Dose refers to the density of the bacterial suspension or the molarity of the solution that was infiltrated into *Arabidopsis* leaves to prime defense signaling (see *Materials and Methods*).

†Enhanced or diminished growth of a secondary infection of *Psm* ES4326 in previously primed plants (*e.g.* with *Psm* ES4326 *cfa6*::Km^r) compared with plants primed with 10mM $MgSO_4$. All experiments consisted of at least three independent trials. Data presented represent pooled values from all trials and no treatment × trial interaction was significant. (+) indicates plants exhibiting SIS; (−) indicates plants exhibiting SAR; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (ANOVA with Bonferroni correction). ns, not significant.

less, under the conditions of our experiments, the phenomenon of SIS elicited by virulent *P. syringae* strains was reproducible.

The observation of SIS was surprising because typically no systemic effect is observed after the infection of *Arabidopsis* plants with virulent bacterial pathogens (e.g., ref. 30), although there are several reports indicating that virulent as well as avirulent *P. syringae* strains elicit a SAR response (see, for example, refs. 31–33). In two of these experiments, the priming dose was ≈10-fold lower than in our experiments (31, 33), which may explain the discrepancy. Similar to the SAR response observed previously in a variety of species, including *Arabidopsis* (6), we observed that secondary *P. syringae* growth was significantly reduced in those plants preinoculated with strains of *Psm* ES4326 carrying either *avrRpt2* (Fig. 1*A*) or *avrRpm1* (data not shown). This result gave us confidence that our experimental conditions were not atypical and that SIS was a natural phenomenon that merited further study.

COR Mediates SIS to *P. syringae*. The small molecule COR is an important virulence factor for many strains of *P. syringae* and appears to function by suppressing host defenses in the early stages of infection (34, 35). Because COR can act as a JA mimic (20, 36, 37), we reasoned that COR may be involved in the elicitation of SIS to *P. syringae* by down-regulating SA-inducible defense response pathways via a SA/JA antagonism (38). Consistent with this hypothesis, a mutant of *Psm* ES4326 that does not produce detectable levels of COR, *Psm* ES4326 *cfa6*::Km^r, failed to elicit SIS (Fig. 1*A* and Table 1). To ascertain whether COR is sufficient to induce SIS in the absence of a priming infection, purified COR was infiltrated into lower leaves of *Arabidopsis*, and bacterial growth was assayed in upper leaves as before. Infiltration of as little as ≈0.50 ng (1.57×10^{-12} mol) of purified COR per g of leaf tissue in the lower leaves of *Arabidopsis* caused significant enhanced growth of *Psm* ES4326 in the untreated leaves (Table 1). As previously observed (25), infiltration of COR elicited the accumulation of anthocyanins in the lower leaves of *Arabidopsis*; however, COR did not elicit any observable chlorosis, even at the highest concentrations. Because purified COR elicits SIS without eliciting necrotic symptoms, it is highly unlikely that SIS is a trivial consequence of the plants being systemically weakened, which could occur as a result of necrotic lesion formation by the priming *P. syringae* strain or the infiltration of purified COR, thereby allowing enhanced growth in the secondarily infected leaves.

Application of MeJA can be used to induce the JA pathway (39–41). As with COR, infiltration of MeJA induced SIS to *P. syringae*, but the minimum amount of MeJA required was ≈50 ng (2.23×10^{-10} mol) per g of leaf tissue, which is 100 times higher than that for COR (Table 1). These results are consistent with the observation that COR functions as a JA mimic but is several orders of magnitude more active than JA (20, 36–38). The presumed final step of COR biosynthesis involves the linkage of the intermediates CFA and CMA (25). Infiltration of either 1 μM CFA or 1 μM CMA did not induce SIS (Table 1), suggesting that neither CFA nor CMA alone is sufficient to account for SIS. Recently published work has suggested that JA signaling is mediated primarily by JA amino acid conjugates, which resemble the structure of COR (42).

If COR mediates SIS, a further prediction would be that strains of *P. syringae* producing COR would not induce SIS in the *Arabidopsis coi1-1* mutant, which is deficient in signaling downstream of JA and was identified on the basis of COR insensitivity (4, 20). Indeed, the *coi1-1* mutant did not exhibit *P. syringae*-elicited SIS (Table 1).

Role of SA Signaling in SIS. We reasoned that if SIS were caused by COR antagonism of SA-mediated signaling pathways, *Arabidopsis* mutations that interfere with SA signaling might be insensitive to COR-mediated SIS. The NPR1 protein plays a key role downstream of SA in mediating SAR, but also appears to be required for some JA-mediated responses (16, 43, 44). However, we observed a similar level of SIS to *P. syringae* in *npr1-1* mutant plants as in WT plants (Table 1). That is, although *npr1* mutants are more susceptible to *P. syringae* than WT plants, *npr1* mutants exhibited approximately the same relative increase in growth in the secondary leaves as WT plants. Because *npr1* mutants are compromised in many SA-mediated responses, this result suggests that if SIS acts through SA/JA antagonism, then the SA-dependent defense responses down-regulated in SIS are *NPR1*-independent. SA-dependent, but *NPR1*-independent, pathways that confer enhanced *P. syringae* resistance and constitutive pathogenesis-related (*PR*) gene expression are well documented (5, 38, 45–51), and the data presented here are consistent with the possibility that SIS occurs through suppression of at least one of these pathways. A confounding factor in this experiment, however, is that there is more bacterial growth and thus more COR-producing cells in the primary *npr1-1* mutant leaves than in WT leaves. Nevertheless, the fact that SIS appears to be *NPR1*-independent may explain why the SIS effect is relatively modest (only 0.5 log enhanced growth in untreated leaves), because *NPR1*-dependent processes continue to confer some degree of pathogen resistance.

We also tested for SIS elicitation in transgenic *Arabidopsis* plants expressing the *Pseudomonas putida nahG* gene, which encodes a salicylate hydroxylase that converts SA to catechol (52). Transgenic *nahG* plants produce low levels of SA, undetectable *PR* gene expression, and reduced resistance to a variety of pathogens (53). SIS was not observed in *nahG* plants infected with *Psm* ES4326 (Table 1). Interpretation of this result is complicated, however, by several considerations. First, it has been shown recently that catechol production in *nahG* plants may interfere with the plant defense response (54). Second, bacterial growth is higher in *nahG* plants than in *npr1* mutant plants, and it is possible that bacterial growth under these conditions may be saturated (45), making it difficult to observe enhanced growth caused by SIS. Finally, *P. syringae* induces very high levels of JA in *nahG* plants (55), which may elicit SIS independently of COR.

COR Counteracts the Elicitation of SAR. We hypothesized that COR-mediated SIS might counteract the elicitation of SAR. Based on this hypothesis we predicted that a *P. syringae* COR mutant expressing an *avr* gene would elicit a stronger SAR than an isogenic COR-producing strain. Indeed, priming *Arabidopsis* plants with *Psm* ES4326 *cfa6*::Km^r (*avrRpt2*) induced markedly stronger SAR than did the COR$^+$ strain *Psm* ES4326 (*avrRpt2*) (Fig. 1*B* and Table 1). SAR induced by avirulent strains of *Psm* ES4326 or *Pst* DC3000 is typically characterized by only 0.4–0.6 log reduced bacterial growth in untreated leaves, whereas chemically and genetically induced SAR is much stronger (31, 52, 56–58). These results provide an explanation for this phenomenon: released from the counteracting effects of COR, avirulent *P. syringae* COR mutants are much more efficient at activating SAR. On the other hand, consistent with previously reported results, the *coi1-1* mutant exhibited approximately the same level of SAR as WT plants (Table 1).

On the surface, this latter result seemingly contradicts the conclusion that COR counteracts SAR, because the *coi1-1* plants should not respond to COR. Indeed, to our knowledge, there is no published evidence that JA signaling is involved in SAR, and the fact that *coi1-1* exhibits the same level of SAR as WT plants has led to the conclusion that SAR does not involve JA signaling (44, 59). However, it has been reported that the *Arabidopsis* *jar1-1* mutant, which like *coi1-1* is also defective in JA signaling, is significantly more susceptible to *Psm* ES4326 (*avrRpt2*) than WT plants (60), indicating that JA is involved in local resistance. Therefore, *Psm ES4326* (*avrRpt2*) may elicit a weaker hypersensitive response in the *coi1-1* mutant than in WT, canceling out the effect of systemic COR insensitivity in the *coi1-1* mutant.

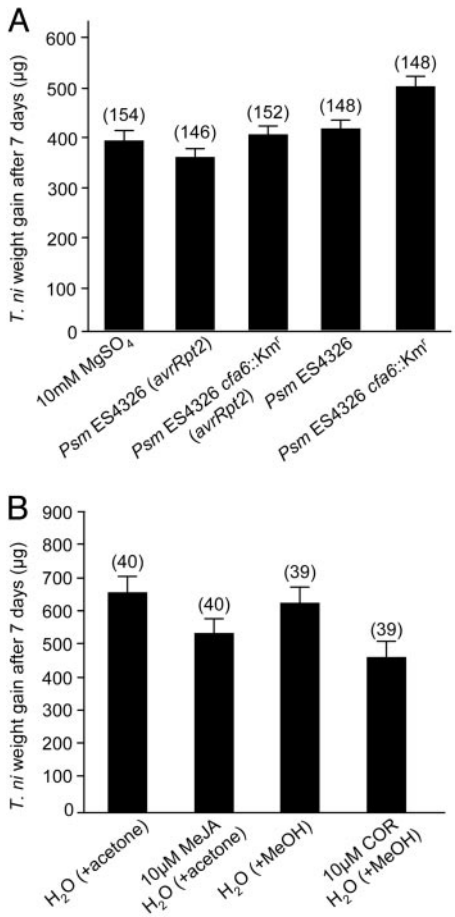

Fig. 2. COR induces systemic host resistance, not susceptibility, to the insect herbivore *T. ni*. (*A*) *T. ni* performance (weight gain) was determined on *Arabidopsis* plants previously infected with *Psm* ES4326 strains with or without *avrRpt2* and with WT or mutant COR-producing capability (*cfa6*::Km^r), or control (10 mM $MgSO_4$) (see *Materials and Methods*). Results show means + SE and sample sizes (in parentheses above bars) for four pooled trials. The presence of both *avrRpt2* and *cfa6* in infecting *Psm* ES4326 strains was a significant predictor of induced host resistance to *T. ni* (ANOVA, *avrRpt2*: $P < 0.001$; *cfa6*: $P < 0.0001$; trial: $P < 0.0001$, no higher order interactions were significant). Importantly, *P. syringae* COR mutants induced greater susceptibility to *T. ni* than did isogenic COR-producing strains, suggesting that under WT conditions pathogen elicited susceptibility and resistance signals compete in the host. (*B*) *T. ni* performance was determined on *Arabidopsis* plants previously infiltrated with 10 μM MeJA, 10 μM COR, or control solvents (H_2O with trace acetone or MeOH, respectively) (see *Materials and Methods*). Results show means + SE and sample sizes (in parentheses above bars). See text for statistical analysis.

Role of COR in Insect Herbivory. Our previous work showed that infection with virulent *P. syringae* also resulted in enhanced feeding of the cabbage looper, *T. ni*, on uninfected leaves (17). When this experiment was repeated in the current study by priming *Arabidopsis* with *Psm* ES4326 (at Harvard University rather than at Massachusetts General Hospital), we did not observe a significant increase in *T. ni* feeding compared with controls (Fig. 2*A*). We attribute the failure to replicate the

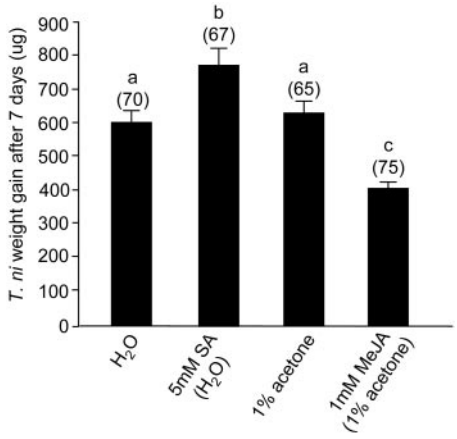

Fig. 3. Inverse relationship between SA- and JA-dependent resistance to *T. ni* is correlated with COR activity. *T. ni* performance was determined on *Arabidopsis* plants previously sprayed to runoff with 5 mM SA, 1 mM MeJA, or control solvents (H_2O and 1% acetone, respectively) (see *Materials and Methods*). Results show means + SE and sample sizes (in parentheses above bars) for two pooled trials (ANOVA, treatment: $P < 0.0001$; trial: $P < 0.0001$; treatment × trial: $P = 0.80$). Letters above the bars signify statistically significant differences among groups (Bonferroni correction, $P < 0.01$).

elicitation by *Psm* ES4326 of enhanced susceptibility to *T. ni* to a relatively weak SIS effect that is modulated by a variety of environmental factors. On the other hand, infiltration of *Arabidopsis* with *Psm* ES4326 *cfa6*::Km^r resulted in significantly enhanced susceptibility to subsequent *T. ni* feeding compared with the isogenic COR$^+$ strain (*Psm* ES4326) (Fig. 2*A*). Further, infiltration of purified 10 μM COR in *Arabidopsis* lower leaves was sufficient to induce resistance to *T. ni* in untreated leaves (paired *t* test, $t = 2.97$, df = 38, $P = 0.003$; Fig. 2*B*). Similarly, infiltration of 10 μM MeJA showed the same trend in inducing resistance to *T. ni* in untreated leaves (paired *t* test, $t = 1.44$, df = 39, $P = 0.079$; Fig. 2*B*). Thus, in contrast to the experiments in which we demonstrated SIS to *P. syringae*, the experiments with *T. ni* feeding show that COR produced by *P. syringae* enhances the systemic resistance of *Arabidopsis* to *T. ni*, rather than decreasing it. Our data support the well established model that COR acts as a mimic of JA (20, 36, 37) capable of inducing resistance to insects (61).

We interpret the data described in this section as follows: priming by WT *P. syringae* elicits enhanced resistance to *T. ni* by the production of COR but simultaneously elicits enhanced susceptibility to *T. ni* by an as-yet-unexplained process. These two counteracting signaling pathways make it difficult to observe either enhanced resistance or susceptibility. In contrast, priming with a COR-deficient *P. syringae* mutant only elicits enhanced susceptibility to *T. ni*, which is readily measured.

To provide further confirmation that JA confers resistance to *T. ni* feeding, whereas SA antagonizes basal resistance (presumably mediated by JA), whole *Arabidopsis* plants were sprayed with 1 mM MeJA or 5 mM SA, and *T. ni* weight gain was subsequently quantified. As expected, the MeJA treatment augmented resistance to *T. ni*, whereas the SA treatment diminished resistance to *T. ni* (Fig. 3).

Discussion

In this article, we have examined the consequences of a virulent bacterial infection in *Arabidopsis* on subsequent attack by a bacterial pathogen or an insect herbivore. Although we found that *P. syringae* can induce systemic susceptibility both to a second *P. syringae* infection (i.e., SIS) and *T. ni* herbivory, the molecular mechanisms by which it does so are different: whereas the first is COR-dependent, the second is not caused by COR, but is counteracted by it. It remains unclear how *P. syringae* induces susceptibility to *T. ni*.

Although our results are consistent with previous reports demonstrating that SA-mediated defense responses are antagonized by JA and COR via a *COI1*-dependent process (4, 5, 9, 10, 12–16, 38), as explained above, the data obtained with *npr1-1* and *nahG* transgenic plants do not provide definitive evidence for an SA-dependent mechanism. Further, our results showing that plants primed with a *P. syringae* COR-defective mutant are more susceptible to *T. ni* feeding suggest that, in addition to JA- and SA-mediated responses, other as-yet-uncharacterized pathways contribute to the plant's ability to defend itself against and/or be manipulated by its enemies.

In general, there is a shortage of information on the mechanism of action for plant hormones. Clearly they must interact with receptors or protein complexes, but the nature of plant hormone receptors and the subsequent downstream signaling events have remained elusive for many of these compounds. Octadecanoid target proteins have not been characterized, and virtually nothing is known about signal transduction downstream of JA (9, 62). The precise mode of action for COR will remain unknown until the proteins that interact with this phytotoxin are identified and characterized. Further, it is unknown whether COR itself acts as the systemic signal transported throughout the plant or if COR merely activates JA signaling.

The ability of *P. syringae* to manipulate plant defense pathways via the production of COR has important evolutionary implications. For example, it is assumed that the mutually antagonistic interactions between the SA and JA pathways reflect selection for plants to optimize defense responses to particular enemies (e.g., pathogen vs. herbivore) and/or reduce metabolic costs of inappropriate defense (4, 14). Once such a system was in place, *P. syringae* may have evolved COR as a means to exploit this important regulatory node in defense signaling (63). What is most surprising is that the activity of COR results in systemic susceptibility. It is unclear what benefit it would provide individual pathogen strains to open up distal plant resources to competitors, unless those strains themselves were the primary colonizers of distal tissue in the same host plant. Indeed, significant local migration of genetically marked *P. syringae* strains has been documented (64), confirming the feasibility of this evolutionary strategy. A SIS-like phenomenon might also function over relatively short distances within a leaf, thereby promoting colonization of a larger area of the leaf after a localized infection. This finding is consistent with the observation that COR is an important virulence factor (25). Thus, systemic SIS and COR-mediated disease enhancement at an infection site may have a common underlying molecular basis.

The ability of *P. syringae* to manipulate plant resistance against insect herbivores is also of evolutionary interest. Although *P. syringae* induces net susceptibility to the cabbage looper *T. ni* (17), COR is not its cause. In fact, consistent with its role as a structural and functional mimic of JA, COR induces systemic resistance to *T. ni*. It is likely that the effect of COR in inducing resistance to herbivory is a by-product of correlated selection favoring subversion of pathogen defenses. On the other hand, many pathogens are vectored to new host tissue by insect herbivores (65), and it would be expected for these pathogens that selection should favor pathogen traits that induce plant susceptibility to pathogen-vectoring herbivores. It is possible that the ability of *P. syringae* to induce susceptibility to *T. ni*, despite the counteracting effect of COR, reflects selection for improved vector competency. Indeed, *P. syringae* can be vectored by insects, although the specificity of its interaction with particular insect species appears to be weak (64).

Taken together, the data reported here suggest that ecological outcomes of species interactions among *Arabidopsis* and its

enemies can be products of multiple, often competing, signaling interactions (e.g., *avr*/*R* gene resistance vs. COR-dependent susceptibility, on the one hand, or pathogen-induced susceptibility to herbivory vs. COR-dependent resistance, on the other hand). Clearly, the interactive effects of the SA and JA pathways are important, yet they cannot fully explain the interactions described here. Knowledge of the mechanics of such complex interactions is necessary to understand the evolution of resistance and the future development of successful solutions to pest problems in agriculture. For example, it has been suggested that JA (66, 67) or a COR analog (61) could be applied to crops in advance of attacking herbivores to preinduce resistance. Although this treatment may be practical in some situations, our results suggest that such treatments could result in inadvertent susceptibility to bacterial pathogens, many of which are serious agricultural pests.

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