

Salicylate-mediated interactions between pathogens and herbivores

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Abstract. Plants employ hormone-mediated signaling pathways to defend against pathogens and insects. We tested predictions about the relative effect of jasmonate and salicylate pathways and how they mediate interactions between pathogens and herbivores. We employed two pathogens of tomato, *Pseudomonas syringae* (*Pst*) and tobacco mosaic virus (TMV), that are known to elicit distinct components of the two pathways, and we address the consequences of their induction for resistance in wild-type and salicylate-deficient transgenic plants in field experiments. We report that *Pst* infection induced jasmonic acid and proteinase inhibitors (PIs), and reduced the growth of *Spodoptera exigua* caterpillars on wild-type and salicylate-deficient plants. *Pst* and TMV both induced salicylic acid in wild-type but not salicylate-deficient plants. Although TMV did not affect jasmonic acid or PIs, infection increased caterpillar growth on wild-type plants, but not on salicylate-deficient plants. Aphid population growth was higher on salicylate-deficient compared to wild-type plants, and lower on salicylate-induced plants compared to controls. Natural aphid colonization was reduced on TMV-infected wild types, but not on salicylate-deficient plants. In sum, jasmonate-mediated resistance is induced by some pathogens, independent of salicylate, and salicylate-mediated induction by other pathogens results in induced susceptibility to a chewer and resistance to an aphid. We conclude with a predictive model for the expression of defense pathways and their consequences.

Key words: *BTH*, benzothiadiazole; chemical ecology; signaling cross talk; jasmonic acid; *nahG*; plant–insect interactions; *Pseudomonas syringae*; proteinase inhibitor; salicylic acid; systemic acquired resistance; tobacco mosaic virus; tomato *Solanum lycopersicum*.

INTRODUCTION

The recent elucidation of plant defense-signaling pathways has allowed for a novel level of predictability for the ecological outcomes of specific plant–enemy interactions (Howe 2004, Stout et al. 2006). Induced changes in plants following herbivore and pathogen attack can mediate interactions between any two organisms that share a host plant (McIntyre et al. 1981, Karban et al. 1987, Hatcher 1995). For example, early-season attack by one herbivore may elicit a plant physiological response that results in competition or facilitation with later-season herbivores (Van Zandt and Agrawal 2004, Ohgushi 2005, Viswanathan et al. 2005). Knowing the responses that particular enemies induce (specificity of elicitation) and the impact of these responses for various enemies (specificity of effect) can thus result in a mechanistic understanding of ecological interactions (Stout et al. 1998, 2006).

Several groups have been studying the jasmonate and salicylate responses of tomato plants *Solanum lycopersicum* (Thaler et al. 2002, 2004, O'Donnell et al. 2003, Cooper and Goggin 2005, Chen et al. 2006, Lin et al. 2008). In particular, early work established a role for

salicylate-mediated responses in plant resistance to pathogenic microbes (Raskin 1992, Brading et al. 2000) and jasmonate-mediated responses in resistance to insect herbivores (Farmer et al. 1992, Thaler et al. 2001). However, several lines of evidence suggest that this dichotomy is too simplistic. First, there is “cross talk” or reciprocal down-regulation of each of these responses by the other (Doares et al. 1995, Thaler et al. 1999, 2002, Ton et al. 2002, Cui et al. 2005, Zarate et al. 2007). Second, some herbivores induce a salicylate-dependent response (Kaloshian and Walling 2005, Musser et al. 2005) and some microbial pathogens induce jasmonate-mediated responses (Stout et al. 1998, Fidantsef et al. 1999, Thomma et al. 2001, Spoel et al. 2007). Finally, jasmonate-induced responses have been shown to be essential for plant resistance to several microbial pathogens (O'Donnell et al. 2003, Thaler et al. 2004, Stout et al. 2006) and salicylate has been implicated in resistance to some herbivores (Inbar et al. 1998, Walling 2000, Cooper et al. 2004). Thus, the dichotomy of jasmonate-induction by, and impact on, insect herbivores, and salicylate induction by, and impact on, pathogens, has given way to knowledge of greater complexity in such interactions.

To unravel this complexity and work toward using knowledge of the specific plant responses and their effects, it would be useful to (1) categorize plant attackers by their elicitation of specific plant responses

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and (2) directly implicate plant-mediated defense responses in the interaction between potential enemies on the same host. To address the first issue, attempts have been made to understand whether herbivores and pathogens from specific feedings guilds are more likely to be associated with inducing jasmonate or salicylate (Walling 2000). Chewing lepidopteran herbivores have been associated largely with the jasmonate response, while herbivory by phloem-sucking hemipterans is often associated with the salicylate response and a somewhat weaker jasmonate response (Moran and Thompson 2001, Rodriguez-Saona et al. 2005, Thompson and Goggin 2006). For microbial pathogens, similar groups can be delineated. While biotrophic fungal pathogens and tobacco mosaic virus (TMV) primarily induce salicylate (Tornerio et al. 1997, Thomma et al. 2001, Stout et al. 2006), some necrotrophic pathogens including the bacterial speck *Pseudomonas syringae* (*Pst*; see Plate 1) induce both a jasmonate and salicylate response (Stout et al. 1998, Preston et al. 1999, Thaler et al. 2004).

The second issue is that pathogens and herbivores interact in many ways on plants. Both direct interactions (e.g., herbivores avoiding eating fungal hyphae) as well as indirect interactions (e.g., changes in host plant quality or volatile emissions) are possible. A useful tool to isolate the effects of particular defensive pathways is to use genetically modified plants that are disabled in individual signaling components (Cipollini et al. 2004, Kessler et al. 2004, Thaler et al. 2004, Zarate et al. 2007). If a particular interaction occurs on wild types but is altered on plants disabled in a specific signal pathway, it is reasonable to conclude that the pathway mediates the interaction.

We sought to address the role of a specific plant defense response, the salicylate pathway, in mediating the interaction between pathogens and herbivores. To study such interactions, we conducted field and laboratory experiments using wild-type and salicylate-deficient plants and two pathogens and two herbivores from divergent feeding guilds. TMV and *Pst* were chosen as plant attackers because of their known differential effect on induced responses. Both pathogens cause many changes in the plant physiology, with TMV primarily inducing the salicylate pathway in tomato plants (Tornerio et al. 1997), while *Pst* infection induces both a salicylate and jasmonate response (Stout et al. 1998, Fidantsef et al. 1999). Effects of these pathogen-induced responses were measured on the chewing herbivore, *Spodoptera exigua*, because it is susceptible to the jasmonate-, but not salicylate-dependent responses (Thaler et al. 1999). Thus, we predicted that *Pst* would induce resistance to *S. exigua* on both wild-type and salicylate-deficient plants, and that TMV would induce susceptibility to *S. exigua*, but only on wild-type plants (where the jasmonate signaling pathway would be suppressed by the induced salicylate response).

We also addressed the consequences of salicylate pathway-dependent responses for phloem-sucking hemipterans (aphids) because they have been associated with inducing salicylate responses, yet effects of jasmonate and salicylate responses on phloem feeders are relatively unknown and somewhat conflicting between plant systems (Kaloshian and Walling 2005). Jasmonate-dependent responses in *Arabidopsis* and tomato have a negative effect on *Myzus persicae* (green peach aphid) and *Macrosiphum euphorbiae* (potato aphid), respectively (Inbar et al. 1998, 2001, Thaler et al. 2001, Ellis et al. 2002), but sometimes this effect is weak (Boughton et al. 2006), depends on the plant cultivar (Cooper et al. 2004), or affects adult preference but not performance (Bhattarai et al. 2007). Inducing the salicylate pathway using the salicylate mimic benzothiadiazole (BTH) has also been shown to reduce fecundity of *M. persicae* (Boughton et al. 2006) and *M. euphorbiae* (Cooper et al. 2004) on tomato. *M. euphorbiae* has higher longevity on salicylate-deficient tomato plants (Li et al. 2006); together these studies show a different pattern than is seen on *Arabidopsis* (Thompson and Goggin 2006). Thus, our specific goal was to pinpoint any role for the salicylate pathway in resistance to two herbivores, a caterpillar and an aphid, and to show how effects of particular pathogens may be predicted from knowledge of the pathways they induce. Specifically, we conducted (1) field experiments to test the effect of the two pathogens on caterpillar performance and aphid abundance on wild-type and salicylate-deficient plants, (2) a greenhouse experiment to measure the plant hormonal response to each pathogen, and (3) a greenhouse experiment to measure aphid performance on plants that over- and under-express salicylate.

MATERIALS AND METHODS

Salicylate deficiency, pathogen infection, and S. exigua performance.—We hypothesized that if the salicylate pathway was involved in plant resistance to herbivores, then this would be revealed by differentially induced resistance in plants with and without an intact salicylate response. We employed wild-type tomatoes (*Solanum lycopersicum* cv Moneymaker) and a genetically engineered line of cv Moneymaker (with the nahG transgene) where salicylic acid is converted to catechol, a compound without salicylate function (Brading et al. 2000). Plants were germinated in the lab and transplanted to a plowed field at the Koffler Scientific Reserve at Joker's Hill, King City, Ontario, at the two-true-leaf stage. Permits were obtained to grow genetically modified plants in the field (Canadian Food Inspection Agency 02-UOT1-228-TOM). Plants of the two lines were randomized and grown in the field for 5 days and then were inoculated with either *Pst* or TMV, or were mock-treated as controls.

Pseudomonas syringae pv *tomato* DC3000 was incubated at 27°C for 48 h on King's B medium and colonies were suspended in sterile water. The terminal leaflet of

leaf two was lightly dusted with carborundrum, painted with 1 mL of an aqueous suspension of 10^7 colony-forming units, and gently rubbed. One leaf of Xanthi tobacco infected with TMV was ground in ice-cold buffer (0.1 mol/L KPhos buffer pH 7.2) and inoculated as previously described. Control plants were rubbed with carborundrum and 1 mL of water in the same manner as the inoculated plants. Two weeks after *Pst* inoculation, the degree of disease was measured on the plants as the number of leaves with signs of necrosis and the total necrotic leaf area (mm^2).

We examined whether the effect of pathogen infection is distinct on the two types of plants for induction of proteinase inhibitors (PIs, a marker of jasmonate-induced responses) and for the performance of *S. exigua* caterpillars. Seven days after pathogen inoculation, leaves were collected for chemical and biological assays. The terminal leaflet of the fourth leaf was excised with a razor blade, placed on ice, and stored in a -80°C freezer. Measurement of PI activity followed Thaler et al. (1996), modified for analysis in 96-well plates. The terminal leaflet of the third leaf was collected for bioassays in petri dishes lined with moist filter paper. A single newly hatched *S. exigua* caterpillar was allowed to feed on each leaflet for 3 days before it was weighed.

Two trials of this experiment were conducted, one in June and July. For each trial, there were 25–35 replicates of the TMV and *Pst* treatments and 15–20 of the control treatments per plant type (total $n = 315$ plants). We collected a subset (7–8 replicates per treatment) for chemical analysis. Factorial two-way ANOVA was used to assess the effects of plant line and pathogen infection on caterpillar growth and plant chemistry. Contrasts were conducted on treatments within plant type as the salicylate-deficient plants grew less than wild-type plants, independent of pathogen treatment. Residuals were examined for meeting the assumptions of ANOVA and data were transformed as indicated.

Pathogen infection and phytohormone induction.—Previous research suggested that *Pst* would induce both the jasmonate and salicylate pathways, whereas TMV would only induce the salicylate pathway. We confirmed this by inoculating wild-type and salicylate-deficient plants with the two pathogens and measuring subsequent JA and SA levels. Plants were grown for three weeks in the greenhouse and were then inoculated with *Pst*, TMV or controls as just described. Tissue from inoculated leaves was collected 24 and 48 h following inoculation ($n = 4$ –5 per treatment per plant type). Tissue was immediately placed in liquid nitrogen and then stored at -80°C until chemical analysis. Data were transformed as $\ln(x + 1)$ to equalize variance between treatments and were analyzed using one-way ANOVA on each plant type, followed by contrasts. Because there was no effect of collection time, the two time points were pooled for statistical analysis.

Jasmonate and salicylate were extracted from the treated leaves as described in Pan et al. (2001), with the

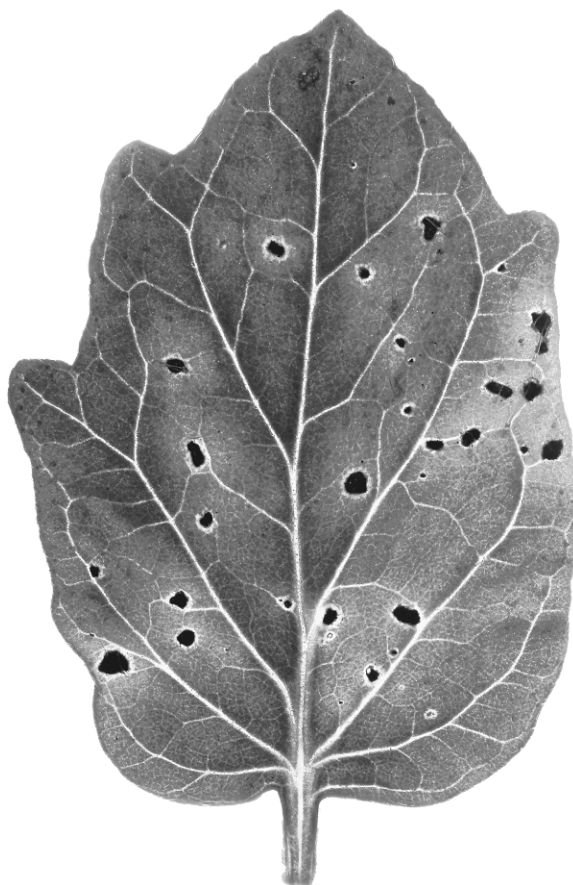


PLATE 1. *Pseudomonas syringae* infecting a tomato leaflet. Photo credit: Kent Loeffler.

following modification of the extraction procedure. Frozen samples were transferred into 2-mL screw cap tubes containing 900 mg zirconia/silica beads (BioSpec, Bartlesville, OK, USA) and 1 mL extraction buffer. d_4 -SA and d_5 -JA (CDN isotopes, Point-Claire, Canada) were added as internal standards and samples were homogenized in a FastPrep homogenizer (MP Biomedicals, Solon, Ohio, USA) at 6 m/s for 45 s. Samples were dissolved in 200 μL methanol after extraction with dichloromethane and solvent evaporation and 15 μL were analyzed on a triple-quadrupole LC-MS/MS system (Quantum Access; Thermo Scientific, Waltham, Massachusetts, USA). Analytes were separated on a C18 reversed-phase HPLC column (Gemini-NX, 3 μ , 150×2.00 mm; Phenomenex, Torrance, California, USA) using a gradient of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 300 $\mu\text{L}/\text{min}$. The initial condition of 10% B was kept for 2 min and increased to 100% solvent B at 20 min. Phytohormones were analyzed by negative electrospray ionization (spray voltage: 3.5 kV; sheat gas: 15; auxiliary gas: 15; capillary temperature: 350°C), collision-induced dissociation (argon CID gas pressure 1.3 mTorr [1.3 micron Hg], CID energy 16 V) and selected reaction

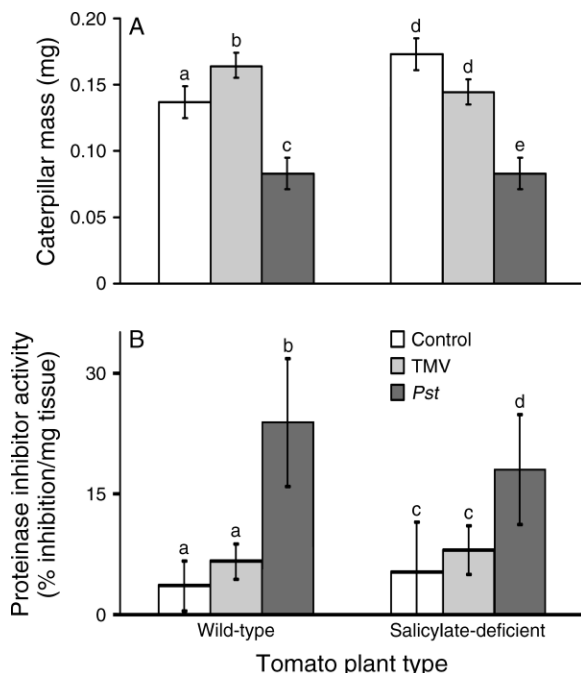


FIG. 1. (A) Growth of *Spodoptera exigua* caterpillars and (B) proteinase inhibitor activity (measured as the percentage inhibition of chymotrypsin activity relative to a control run with chymotrypsin and substrate only), comparing wild-type and salicylate-deficient tomato plants (*Solanum lycopersicum*) treated with either tobacco mosaic virus (TMV) or *Pseudomonas syringae* (*Pst*) in the field vs. control. Values are means \pm SE. Different letters indicate significant differences ($P < 0.05$) within plant type using contrasts.

monitoring (SRM) of compound-specific parent/product ion transitions: SA 137 \rightarrow 93; d₄-SA 141 \rightarrow 97; JA 209 \rightarrow 59; d₅-JA 214 \rightarrow 62.

Impacts of salicylate responses on aphids.—In a separate field experiment, we treated wild-type and salicylate-deficient plants with or without TMV and examined subsequent colonization by *Myzus persicae* (total $n = 121$ plants). All colonizing insects were counted, but aphids were the only insects that reached appreciable numbers. We predicted that TMV would induce resistance to aphids, but only on wild-type plants because resistance is known to be salicylate-mediated. Plants were grown and treated as previously described. Plant height and aphid abundance were determined for each plant after 7 days. Analysis was with two-way ANOVA, with plant height included as a covariate.

An additional laboratory experiment was conducted to test the specific effects of the salicylate pathway on aphid reproduction, as the field experiment combined effects on preference (colonization) and reproduction. We employed salicylate-deficient plants, wild-type plants, and wild-type plants induced with BTH to increase salicylate expression (Inbar et al. 1998) (total $n = 96$ plants). Wild-type and salicylate-deficient plants were grown in the greenhouse until the four-leaf stage. Wild-type plants were divided into two groups: the third

leaf of one group was treated with 1.2 mmol/L BTH and the third leaf of the other group was treated with 1.2 mmol/L of the BTH carrier as a control. Salicylate-deficient plants were treated with carrier only. Two days after treatment, the third leaf of each plant was enclosed in a polyester sleeve and two 7-day-old *Macrosiphum euphorbiae* were placed in each sleeve. Seven days later, we counted the number of aphids. Because some plants with the nahG transgene spontaneously induce necrotic lesions (in the absence of infection), necrosis was measured, and only plants with less than 5% leaf tissue necrosis were used.

RESULTS

Salicylate deficiency, pathogen infection, and *S. exigua* performance.—*Pst* infection reduced the growth of *S. exigua* larvae $>50\%$ on both the wild-type and salicylate-deficient plants compared to controls and TMV-infected plants (Fig. 1A and Appendix). This effect occurred despite greater *Pst* symptoms on the salicylate-deficient plants, with twice the number of infected leaves (wild-type 1.2 ± 0.107 leaves, mean \pm SE; salicylate-deficient 2.3 ± 0.239 leaves; $F_{1,29} = 18.93$, $P < 0.001$) and twice the infected area (wild-type 78.13 ± 12.8 mm², mean \pm SE; salicylate-deficient 142.7 ± 19.83 mm²; $F_{1,29} = 7.41$, $P = 0.011$) on salicylate-deficient compared to wild-type plants.

The effect of TMV infection on *S. exigua* growth was reversed on the salicylate-deficient plants compared to wild types. Across the two trials of the experiment, TMV caused a 20% increase effect on *S. exigua* growth compared to controls on the wild types, but there was a 17% decrease when caterpillars were on the salicylate-deficient plants. Although the 17% decrease was not significant in the contrast within plant type, the interaction term between plant type and treatment was significant ($P = 0.011$), indicating that plant types differentially responded to infection (see Appendix). In a separate analysis using only control and TMV-infected plants, the interaction term for plant type and TMV persisted ($F_{1,200} = 8.46$, $P = 0.004$). No visual symptoms of systemic TMV infection were observed.

The effects of infection on PI activity were significant for both plant types, and the effects of *Pst* on chemistry matched ecological effects on *S. exigua* (Fig. 1B and Appendix). PI activity in the uninfected control plants was equal on the wild-type and salicylate-deficient plants. *Pst* induced PI activity equally on both plant types, consistent with the negative effects of *Pst* on *S. exigua* on both plant types (effect of *Pst*, $F_{1,36} = 11.61$, $P = 0.002$; *Pst* \times plant type, $F_{1,36} = 0.057$, $P = 0.81$). TMV did not induce PI activity on either plant type (effect of TMV, $F_{1,33} = 0.14$, $P = 0.71$; TMV \times plant type, $F_{1,33} = 0.58$, $P = 0.96$), but did increase growth of *S. exigua* on the wild types and decreased it on salicylate-deficient plants. This latter result indicates that much of the effect of salicylate pathway induction by TMV on caterpillars is independent of PIs. Many

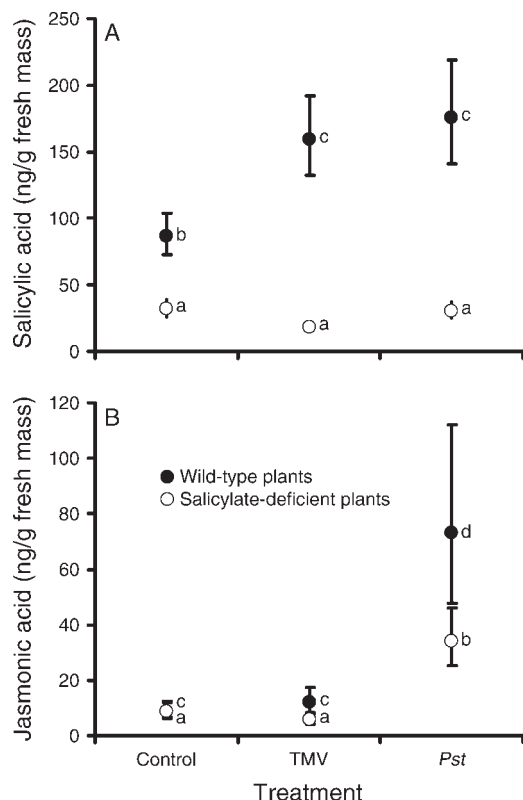


FIG. 2. The impact of tobacco mosaic virus (TMV) or *Pseudomonas syringae* (*Pst*) infection on (A) salicylic acid (SA) and (B) jasmonic acid (JA) pools in wild-type or salicylate-deficient plants. Shown are back-transformed means \pm SE. Different letters indicate significant differences ($P < 0.05$) within plant type using contrasts.

other important JA-regulated responses that could be differentially affected in TMV-infected wild-type and salicylate-deficient plants were not measured.

Pathogen infection and phytohormone induction.—As expected, *Pst* strongly induced jasmonic acid in wild-type plants (effect of infection, $F_{2,28} = 8.71$, $P = 0.001$) and salicylate-deficient plants ($F_{2,26} = 9.46$, $P = 0.001$; Fig. 2). *Pst* and TMV each induced salicylic acid twofold in wild-type plants ($F_{2,28} = 4.36$, $P = 0.022$), but there was no impact of infection on salicylate-deficient plants ($F_{2,26} = 0.87$, $P = 0.432$). TMV did not induce JA in either plant type (Fig. 2).

Salicylate alteration and aphids.—Aphid colonization of field plants was reduced by TMV infection on wild-type plants but was unaffected by TMV on salicylate-deficient plants (plant \times infection interaction, $F_{1,115} = 6.97$, $P = 0.009$; height covariate, $F_{1,115} = 6.75$, $P = 0.011$; main effects not significant; Fig. 3A). In the laboratory, aphids were negatively affected by salicylate pathway induction, as reproduction was 23% lower on wild-type plants treated with BTH, and 23% higher on salicylate-deficient plants compared to untreated wild types ($F_{2,93} = 12.2$, $P < 0.001$; Fig. 3B).

DISCUSSION

It is clear that the dichotomy between jasmonate responses as a defense against herbivores and salicylate responses as a defense against pathogens is oversimplified, and we are still lacking a predictive framework for understanding the specificity of induction and ecological impacts (Fig. 4). Here we have tested hypotheses for the role of salicylate responses in mediating interactions and how specific pathogens may differentially affect chewing herbivores and aphids. For example, growth of *S. exigua* caterpillars was reduced on wild-type and salicylate-deficient plants infected with *Pst*, indicating a salicylate-independent mechanism of induced resistance. This effect is probably due to induction of a jasmonate response including PI activity by *Pst*. Thus, although the traditional view of chewing herbivores being impacted by jasmonate appears to hold, here resistance was induced by bacteria (Fig. 4). Other pathogens also activate and are impacted by jasmonate responses (Ton et al. 2002, Thaler et al. 2004).

In contrast to the effects of *Pst*, TMV infection positively affected caterpillar growth on wild-type plants capable of inducing salicylate responses, but a neutral to

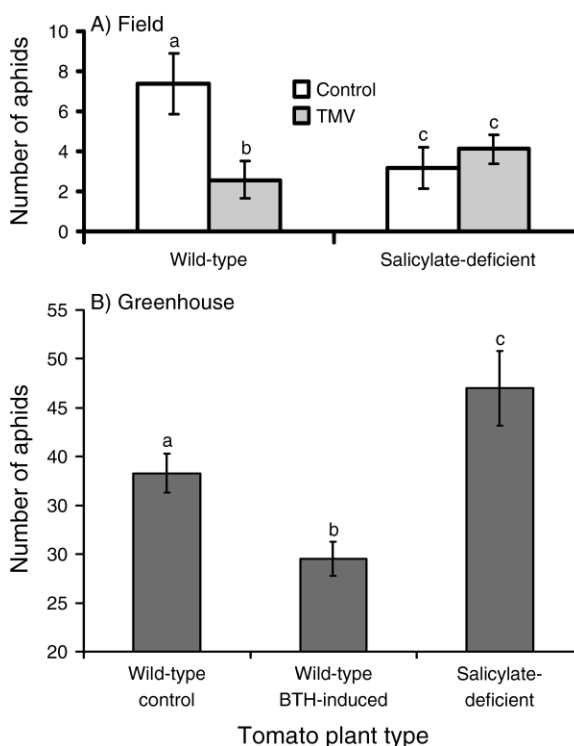


FIG. 3. (A) Abundance of naturally colonizing aphids on wild-type and salicylate-deficient plants with or without induction by tobacco mosaic virus (TMV) in the field, and (B) aphid reproduction on greenhouse grown wild-type plants (control), wild-type plants treated with BTH to induce the salicylate pathway (see *Methods: Impacts of salicylate responses on aphids*), and salicylate-deficient plants. BTH is benzothiadiazole, a salicylate mimic. Different letters indicate significant differences ($P < 0.05$) using contrasts. Shown are means \pm SE.

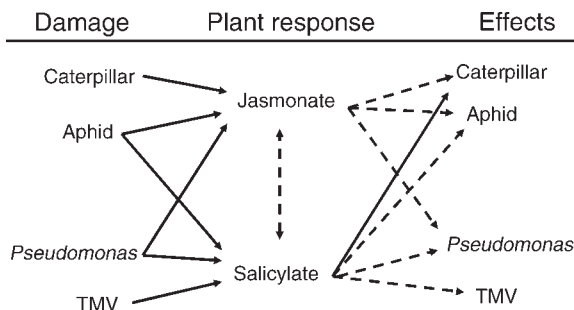


FIG. 4. A conceptual diagram for specificity in plant responses to insect and pathogen attackers and their effects on the various organisms via jasmonate and salicylate pathways. Solid arrows indicate positive effects, and dashed arrows indicate negative effects. The arrows are based primarily on work in this study and other work conducted on tomato and other Solanaceae (Raskin 1992, Fidantsef et al. 1999, Preston et al. 1999, Thaler 1999, Zhao et al. 2003). Effects independent of jasmonate and salicylate were evident but are not depicted here.

negative effect on caterpillar growth on the salicylate-deficient plants. Other manipulations of the salicylate pathway have also revealed positive effects of salicylate induction on *S. exigua* (Thaler 1999, Thaler et al. 1999, 2002). The reversal in the direction of the effect of TMV infection on wild-type and salicylate-deficient plants in our study emphasizes the indirect role of the salicylate pathway in interactions between pathogens and herbivores. When the salicylate pathway is disabled, the balance of pathogen-induced responses is negative for insects (Zarate et al. 2007). The benefit to caterpillars of being on a plant with a functional salicylate pathway is likely due to a weaker ability of this plant to induce the jasmonate pathway, which can clearly reduce caterpillar performance (Thaler et al. 1999). Here again, we offer a revision to the traditional dichotomous view of jasmonate and salicylate, with a pathogen–insect interaction being dependent on the salicylate pathway (Fig. 4).

Recent research demonstrates that aphids *M. persicae* and *M. euphorbiae* are negatively affected by both the jasmonate and salicylate pathways on tomato plants. The effect of the salicylate pathway appears to be more consistently negative than the effect of the jasmonate pathway (Thaler et al. 2001, Thaler 2002, Cooper et al. 2004, Cooper and Goggin 2005, Li et al. 2006; J. Thaler, *personal observations*). In field colonization experiments, we found that TMV infection had a negative effect on aphids on wild-type plants, whereas on salicylate-deficient plants there was no effect of infection. Similarly, in the lab, genetic (salicylate deficiency) and phenotypic (BTH application) manipulations showed nearly identical impacts, positive and negative, respectively, on aphids. The comparison of uninfected wild-type and salicylate-deficient plants in the field did not support this pattern; and this is likely due to the larger size and therefore greater attractiveness of wild-type plants.

In other plant systems, induction of the salicylate pathway by pathogens can have varying effects on aphid performance (McIntyre et al. 1981, Stout et al. 2006, Goggin 2007). Given that aphids are known to be inducers of, and are impacted by, both pathways, they evade the traditional jasmonate–salicylate dichotomy (Fig. 4). Like spider mites and other cell content feeders, phloem-sucking hemipterans may function as part herbivore and part pathogen (Walling 2000).

Other studies using signaling-pathway mutants have found evidence for the importance of jasmonate–salicylate signaling cross talk on the outcome of interactions between divergent plant parasites (Cui et al. 2005). For example, Zarate et al. (2007) showed that cross talk is an important determinant of the success of whiteflies feeding on *Arabidopsis*. Similarly, Spoel et al. (2007) showed that cross talk was important for the interaction between *Pst* and a fungal pathogen *Alternaria brassicicola* on *Arabidopsis*. How to grapple with this seeming complexity? We will build on some general modifications to the dichotomous view of jasmonate and salicylate (Moran and Thompson 2001, Ton et al. 2002, Cui et al. 2005, Musser et al. 2005).

Most arthropod herbivores probably will be classified as inducers of jasmonate only, or of jasmonate and salicylate together. Indeed, the joint activation of pathways may benefit the inducer because of cross-pathway suppression, as has been demonstrated for a hemipteran (Zarate et al. 2007). In other systems it is more complicated, but salicylate-regulated products are still involved (Musser et al. 2005). Most microbial pathogens will be inducers of salicylate or of salicylate and jasmonate together. Again, some pathogens are clearly taking advantage of jasmonate–salicylate cross talk (e.g., Cui et al. 2005). We are not aware of any completely stealthy plant parasites evading both defense pathways, herbivores that only induce salicylate, or pathogens that only induce jasmonate. The differential impact of salicylate and jasmonate on guilds of herbivores and pathogens is more difficult to predict at this stage, as we have less knowledge about all of the responses cued by salicylate and jasmonate, and what makes plant parasites susceptible. Nonetheless, a priori predictions can be derived from our framework (Fig. 4) and tested with both genetic and phenotypic manipulations such as we have initiated here. Although the generality of this model across plant, herbivore, and pathogen systems is unclear, it appears that enemies can be predictably categorized along axes of the responses that they induce and the resistance traits to which they are susceptible.

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APPENDIX

Effects of various factors on mass of *Spodoptera exigua* and proteinase inhibitor activity (*Ecological Archives* E091-075-A1).