MPMI Vol. 8, No. 1, 1995, pp. 165-171. 

# Role of the Phytotoxin Coronatine in the Infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato*

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Received 4 August 1994. Accepted 20 October 1994.

**The role of the phytotoxin coronatine in the virulence of *Pseudomonas syringae* pv. *tomato* in *Arabidopsis thaliana* was evaluated by comparing symptom development, *in planta* bacterial multiplication, and the induction of defense-related genes in *Arabidopsis* plants inoculated with the coronatine-producing (Cor$^+$) *P. s.* pv. *tomato* strain DC3000 and the coronatine-defective (Cor$^-$) strain DC3661 by either infiltration or dipping methods. The Cor$^+$ strain, *P. s.* pv. *tomato* DC3000, caused severe disease symptoms and multiplied by 4–6 logs after inoculation by either infiltration or dipping. *P. s.* pv. *tomato* DC3661 failed to produce any disease symptoms and multiplied by only 1–1.5 logs in dipped plants, whereas it caused mild symptoms and multiplied 6 logs over the 4-day experimental period in plants inoculated by infiltration. Parallel experiments using a natural host, tomato, yielded similar results. Analysis of the accumulation of mRNAs encoded by several distinct defense-related genes in *Arabidopsis* leaves infiltrated with either DC3000 or DC3661 demonstrated that the Cor$^-$ strain consistently induced higher levels of these transcripts. These results demonstrate that coronatine production is required under more natural inoculation conditions for the successful infection of *Arabidopsis* by DC3000, and that coronatine may play a critical role during the early stages of infection by suppressing the activation of defense-related genes.**

*Additional keywords:* disease resistance, ELI3, glutathione S-transferase, phenylalanine ammonia-lyase, virulence factors.

Many isolates of *Pseudomonas syringae* pv. *glycinea* and the closely related pathovars *tomato* and *maculicola* produce the non-host-specific phytotoxin coronatine (Völksch *et al.* 1989; Wiebe and Campbell 1993), a polyketide formed by the coupling of coronamic acid with coronafacic acid via an amide bond (Mitchell 1991). The biological effects of coronatine include induction of leaf chlorosis, induction of hypertrophic growth on potato tubers, inhibition of root growth in rice seedlings, and induction of increased 1-aminocyclopropane-1-carboxylic acid content and ethylene production in bean and tobacco leaves (Durbin 1991; Ferguson and Mitchell 1985; Kenyon and Turner 1992). Coronatine is also known to cause increased activities of several plant enzymes, such as amylase, polyphenoloxidase, peroxidase, ascorbate oxidase, cellulase, polygalacturonase, and polymethylgalacturonase (Mino *et al.* 1980; Perner and Schmauder 1993; Sakai *et al.* 1979, 1982). The role of coronatine in the virulence of toxin-producing phytopathogenic bacteria has been reviewed (Durbin 1991; Gross 1991; Mitchell 1991; Willis *et al.* 1991).

Previous studies with coronatine-producing (Cor$^+$) and coronatine-defective (Cor$^-$) mutants of *P. s.* pv. *tomato* (Bender *et al.* 1987; Jackson *et al.* 1992; Moore *et al.* 1989) have shown that coronatine synthesis contributes significantly to lesion expansion, the development of chlorosis, and bacterial multiplication in tomato leaves. The coronatine-producing strains *P. s.* pv. *tomato* DC3000 and *P. s.* pv. *maculicola* have recently been used to study bacterial speck disease of *Arabidopsis* (Davis *et al.* 1991; Debener *et al.* 1991; Dong *et al.* 1991; Whalen *et al.* 1991). Symptom development in *Arabidopsis* plants infected with these strains is characterized by the development of chlorotic lesions, which may be induced at least in part by coronatine. To further define the interaction of *Arabidopsis* with these *Pseudomonas* strains and to evaluate the role of coronatine in virulence in this pathosystem, we compared the virulence of *P. s.* pv. *tomato* DC3000 and *P. s.* pv. *tomato* DC3661, a Tn*5* mutant derivative that is Cor$^-$, on *Arabidopsis* and their ability to induce several defense-related mRNAs. Our results indicate that coronatine production is required during the early stages of infection, and that this requirement may be due in part to the suppression of host defense responses.

## RESULTS

### Symptom development and multiplication of bacterial strains *P. s.* pv. *tomato* DC3000 and DC3661 in *Arabidopsis* and tomato.

The development of disease symptoms and *in planta* multiplication of the bacterial strains *P. s.* pv. *tomato* DC3000 and DC3661 were monitored in *Arabidopsis* inoculated with a relatively high dose of bacteria by infiltration with a needleless syringe or by dipping plants in a bacterial suspension containing a surfactant, which provides a lower dose of inoculum.

When infiltrated into *Arabidopsis* leaves, the Cor$^+$ strain DC3000 produced severe disease symptoms within 2–3 days

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after inoculation. These symptoms were characterized by the development of water-soaked necrotic lesions surrounded by chlorotic tissue and were similar to those previously described (Wanner *et al.* 1993; Whalen *et al.* 1991). When the Cor$^-$ strain DC3661 was introduced into *Arabidopsis* by infiltration, the leaves exhibited milder symptoms characterized by the development of small, water-soaked lesions with some necrosis, but without significant chlorosis. In most cases the necrotic lesions dried out 2–3 days after infiltration and resembled lesions caused by avirulent *P. syringae* strains that cause a hypersensitive response in *Arabidopsis*. Studies of *in planta* bacterial growth in infiltrated leaves showed that there was no significant difference in the multiplication rates of these two bacterial strains. Both strains DC3000 and DC3661 multiplied by about 6 logs over a period of 4 days following inoculation (Fig. 1).

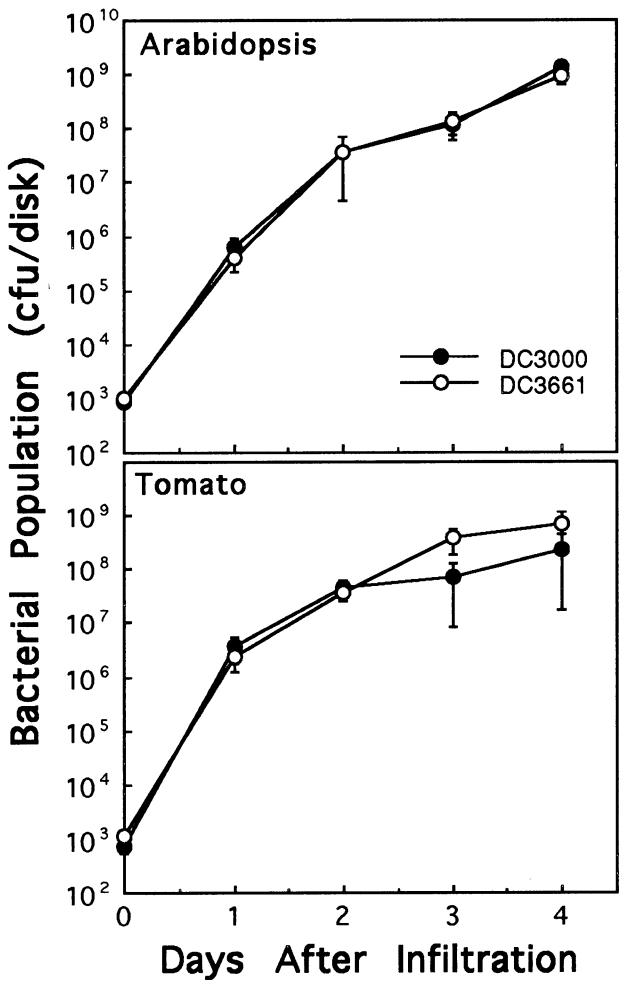


**Fig. 1.** Multiplication of *Pseudomonas syringae* pv. *tomato* strains DC3000 and DC3661 in *Arabidopsis* (land race Col-0) and tomato (Bonny Best) leaves inoculated by infiltration. A needleless syringe was used to infiltrate leaves with bacterial suspensions adjusted to $OD_{600}$ = 0.001 with 10 mM $MgCl_2$. At the indicated times, a leaf disk 0.6 cm in diameter (0.28 $cm^2$; the fresh weight of the *Arabidopsis* disks was 2.8–4.0 mg) was removed from three different inoculated leaves receiving the same treatment. The pooled leaf disks were homogenized in 10 mM $MgCl_2$, and serial dilutions were plated on King's B medium containing 50 μg $ml^{-1}$ rifampin to monitor bacterial multiplication. The results shown are the mean and standard deviations obtained from three different experiments.

In plants inoculated by dipping, *P. s.* pv. *tomato* DC3000 produced disease symptoms characteristic of this strain. Numerous round, water-soaked lesions with necrotic centers surrounded by a chlorotic halo developed within 2–3 days after inoculation. These lesions continued to expand over 4–5 days and sometimes coalesced. In contrast, *Arabidopsis* plants that were similarly inoculated with *P. s.* pv. *tomato* DC3661 did not develop any disease symptoms and were indistinguishable from mock-inoculated control plants (Fig. 2). Measurements of bacterial growth in plants inoculated by dipping demonstrated that symptom development was correlated with bacterial multiplication. *P. s.* pv. *tomato* DC3000 multiplied by 4–5 logs over a period of 4 days, whereas *P. s.* pv. *tomato* DC3661 multiplied by only about 1–1.5 logs (Fig. 3). Multiplication of DC3661 was limited to the first 1–2 days after dipping, after which the bacterial population declined, whereas DC3000 populations increased continuously over the 4-day experimental period.

Since tomato is the natural host of *P. s.* pv. *tomato* DC3000, we monitored the development of disease symptoms and *in planta* growth of *P. s.* pv. *tomato* DC3000 and the Cor$^-$ derivative DC3661 in this host to ascertain if the differences in symptom development and bacterial multiplication seen in *Arabidopsis* inoculated by either infiltration or dipping would be observed in tomato as well. When infiltrated into tomato leaves, *P. s.* pv. *tomato* DC3000 produced water-soaked, necrotic lesions surrounded by chlorotic tissue. Infiltration of *P. s.* pv. *tomato* DC3661 into tomato leaves resulted in mild symptoms typified by the development of pale chlorosis in the infiltrated area, with very little or no necrosis. Measurements of bacterial growth in infiltrated leaves demonstrated that both DC3000 and DC3661 multiplied by approximately 6 logs over a period of 4 days (Fig. 1). As was the case with *Arabidopsis,* no significant difference in the multiplication rates of the two bacterial strains was observed in infiltrated leaves. In tomato leaves inoculated by dipping, *P. s.* pv. *tomato* DC3000 produced disease symptoms characteristic of bacterial speck disease. Leaves dip-inoculated with DC3000 typically developed approximately 20 dark brown, necrotic lesions surrounded by chlorotic halos. In contrast, *P. s.* pv. *tomato* DC3661 did not produce significant disease symptoms in dip-inoculated tomato leaves. Leaves inoculated with *P. s.* pv. *tomato* DC3661 by dipping appeared similar to those of mock-inoculated plants except for the occasional development of two or three small, nonchlorotic lesions on a few of the inoculated leaves (Fig. 2). Bacterial growth in tomato leaves inoculated by dipping showed that *P. s.* pv. *tomato* DC3000 multiplied by 3–4 logs during the 8-day period after inoculation, whereas *P. s.* pv. *tomato* DC3661 multiplied by only about 2 logs during the first few days after inoculation, after which the bacterial population declined (Fig. 3).

### Accumulation of defense-related mRNAs in *Arabidopsis* leaves infiltrated with *P. s.* pv. *tomato* DC3000 and DC3661.

The initial studies of symptom development and bacterial growth of the Cor$^+$ strain DC3000 and the Cor$^-$ strain DC3661 suggest that coronatine production may be required during the early stages of infection. To test the possibility that coronatine may function to inhibit the induction of a localized

defense response, we monitored the accumulation of three distinct mRNAs that have been shown to be induced in *Arabidopsis* during a resistance response to avirulent *Pseudomonas* strains. RNA blot analyses of total RNA isolated from leaves infiltrated with either DC3000 or DC3661 indicated that both strains induced the accumulation of mRNAs for phenylalanine ammonia-lyase (PAL), glutathione S-transferase, and ELI3 more than the $MgCl_2$-infiltrated controls (Fig. 4). However, the accumulation of these transcripts was consistently several times greater in leaves inoculated with DC3661 than in those inoculated with DC3000. The relative differences in mRNA accumulation in leaves infiltrated with DC3000 and DC3661 were larger for the PAL and GST transcripts than for the ELI3 transcript. PAL and GST mRNA

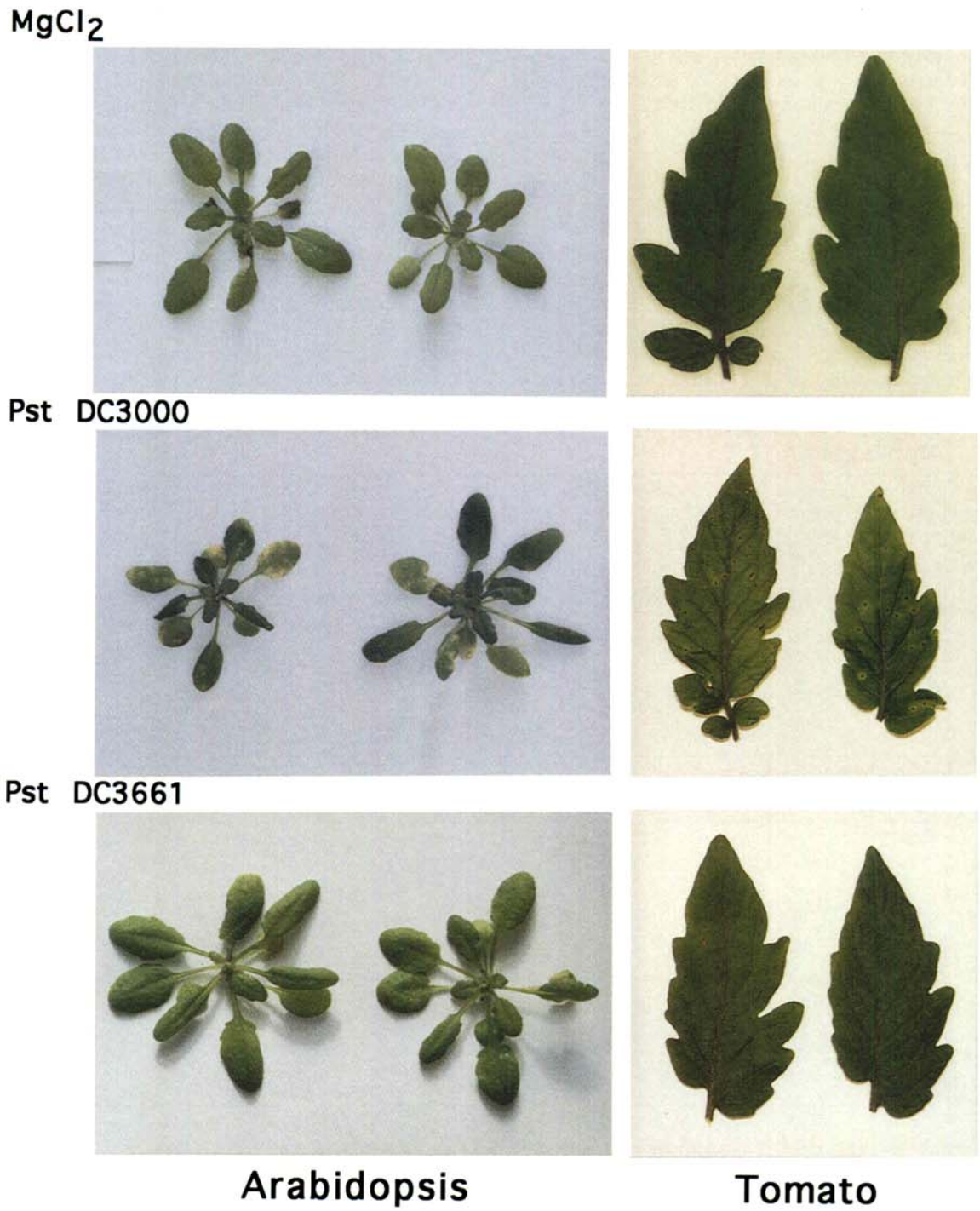


**Fig. 2.** Symptom development in *Arabidopsis* and tomato leaves inoculated with *Pseudomonas syringae* pv. *tomato* (Pst) strains DC3000 and DC3661. Leaves from 4- to 5-wk-old plants were dipped in a suspension of bacteria adjusted to $OD_{600}$ = 0.20 with 10 mM $MgCl_2$ containing 0.02% surfactant (Silwet L77). The leaves shown were photographed 4 days after dipping in either a control $MgCl_2$-surfactant solution, a suspension of *P. s.* pv. *tomato* DC3000 (coronatine-producing), or a suspension of *P. s.* pv. *tomato* DC3661 (coronatine-defective).

accumulation was transiently induced by DC3661 to levels two to five times higher than those induced by DC3000. The induction kinetics of the PAL and GST transcripts were very similar in leaves infiltrated with DC3661 and leaves infiltrated with DC3000 containing *avrB* from *P. s.* pv. *glycinea,* which is recognized by land race Col-0 of *Arabidopsis* (Wanner *et al.* 1993). ELI3 mRNA accumulation was induced by both DC3000 and DC3661; however, the levels observed in leaves infiltrated with DC3661 were consistently about two times higher during the first 3–6 hr after infiltration than in leaves infiltrated with DC3000. In all cases, DC3000 containing *avrB* induced higher levels of all three defense-related transcripts than the Cor$^-$ strain DC3661 (Fig. 4).

## DISCUSSION

Coronatine has been proposed as an important factor in the development of the chlorotic lesions that are typically caused by bacterial strains that produce this phytotoxin. Studies using wild-type and mutant *P. syringae* strains (Bender *et al.* 1987; Jackson *et al.* 1992) have shown that a Cor$^-$ mutant caused the production of smaller lesions and exhibited lower levels of *in planta* bacterial growth than the wild-type strain, suggesting that coronatine is required during the later stages of infection for enhancing the severity of the disease symptoms by aiding in lesion expansion and bacterial multiplication. These studies have led to the conclusion that coronatine is a virulence factor and is not required for infectivity or initial lesion formation (Bender *et al.* 1987; Willis *et al.* 1991). In the present study, we wished to determine the importance of coronatine in the virulence of *P. s.* pv. *tomato* on *Arabidopsis.*

To address this question, different doses of bacteria were introduced into leaves by means of two different inoculation procedures, infiltration and dipping, to infect plants with either the Cor$^+$ *P. s.* pv. *tomato* strain DC3000 or the Cor$^-$ derivative DC3661, in which the gene for coronatine biosynthesis has been disrupted by a Tn*5* transposon insertion (Jackson *et al.* 1992). In inoculation by infiltration, a concentrated dose of bacterial inoculum is forced into the mesophyll cells of the leaf in a localized area; hence the factors essential for the early establishment of the bacteria in nature may not be required, because of the relatively high dose of bacteria introduced into the leaf. In plants inoculated by dipping, a smaller number of bacteria enter the host via open stomata or other openings or wounds present on the leaf. This low level of inoculum is likely to be more representative of natural infections. Our previous studies have shown that the effects of

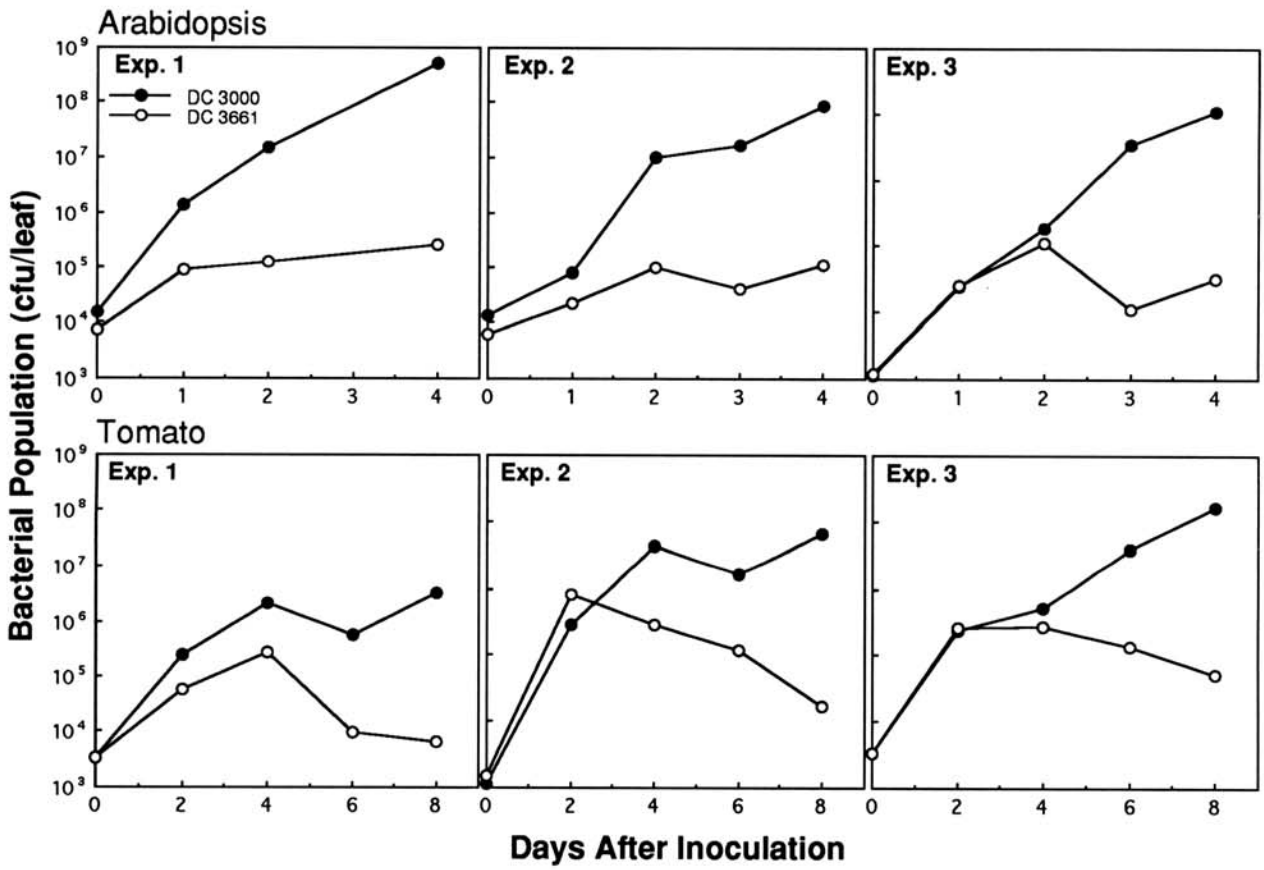


**Fig. 3.** Multiplication of *Pseudomonas syringae* pv. *tomato* strains DC3000 and DC3661 in *Arabidopsis* (land race Col-0) and tomato leaves inoculated by dipping. Leaves were dipped in a suspension of bacteria adjusted to $OD_{600}$ = 0.20 with 10 mM $MgCl_2$ containing 0.02% surfactant (Silwet L77). At the indicated times, single whole leaves were removed from four individual infected plants receiving the same treatment and pooled (the fresh weight of the *Arabidopsis* leaves was 10–30 mg each). The pooled leaves were homogenized in 10 mM $MgCl_2$, and serial dilutions were plated on King's B medium containing 50 μg ml$^{-1}$ rifampin to monitor bacterial multiplication. Experiments 1 and 3 were done with tomato cultivar Bonny Best, while experiment 2 used VFNT Cherry tomato.

avirulence genes in attenuating virulence are more pronounced when plants are inoculated by dipping (Wanner *et al.* 1993).

In infiltration experiments, the Cor$^+$ and the Cor$^-$ strains multiplied at similar rates and attained similar population densities in inoculated *Arabidopsis* and tomato leaves, although the disease symptoms produced by the Cor$^-$ strain were clearly less severe than those produced by the Cor$^+$ strain. In *Arabidopsis* leaves inoculated by dipping, the Cor$^+$ strain produced typical disease symptoms and multiplied by 4–5 logs, whereas the Cor$^-$ strain did not produce significant disease symptoms and did not multiply by more than 2 logs. The limited growth of the Cor$^-$ strain occurred only during the first several days after dipping and was similar to that observed for virulent *P. s.* pv. *tomato* and *P. s.* pv. *maculicola* strains harboring cloned *avr* genes that are recognized by *Arabidopsis* (Wanner *et al.* 1993). Thus, in dipping experiments, DC3661 appears to be avirulent, which suggests that coronatine may be required for lesion formation and virulence in this host.

Dip inoculation of tomato leaves also demonstrated that DC3661 had clearly attenuated virulence, but not to the extent of that observed in *Arabidopsis*. Our results with tomato are similar to those obtained by others (Bender *et al.* 1987; Jackson *et al.* 1992), with the exception that we found that the Cor$^-$ strain DC3661 caused fewer lesions to develop in tomato leaves inoculated by dipping than the Cor$^+$ strain did. Bender *et al.* (1987) reported that a Cor$^-$ derivative of *P. s.* pv. *tomato* PT23 caused lesions on spray-inoculated plants in numbers similar to those produced by the toxin-producing wild-type strain; however, the lesions caused by the Cor$^-$ derivative were clearly reduced in size. The differences between the two studies may be due to several factors, including differences in the inoculation procedures, differences in the aggressiveness of DC3000 and PT23, and the fact that different tomato cultivars were used.

The observation that infiltrating relatively high doses of bacteria into the mesophyll overcomes the requirement for coronatine in both *Arabidopsis* and tomato indicates that coronatine is required for the initial establishment of infection sites which can then support high levels of bacterial growth in leaves. One function of coronatine during the initial stages of infection could be to suppress the induction of plant defense responses until the bacterial population increases to a level at which it is no longer possible for the plant to limit the infection. This hypothesis is supported in part by our observations that mRNAs encoded by three distinct defense-related genes were induced to levels several times higher in *Arabidopsis* leaves inoculated with the Cor$^-$ strain than in leaves inoculated with the Cor$^+$ strain; however, the levels induced by DC3661 were not as high as those observed in leaves infiltrated with DC3000 containing an avirulence gene recognized by *Arabidopsis*.

The mode of action of coronatine is currently not well defined, and thus a detailed model of how coronatine might suppress the activation of defense responses remains to be developed. It is possible that coronatine inhibits the induction of plant defense responses indirectly, simply by damaging plant cells to an extent that they are incapable of responding to elicitor signals. In addition, toxin damage may cause the release of nutrients that stimulate bacterial growth. Alterna-

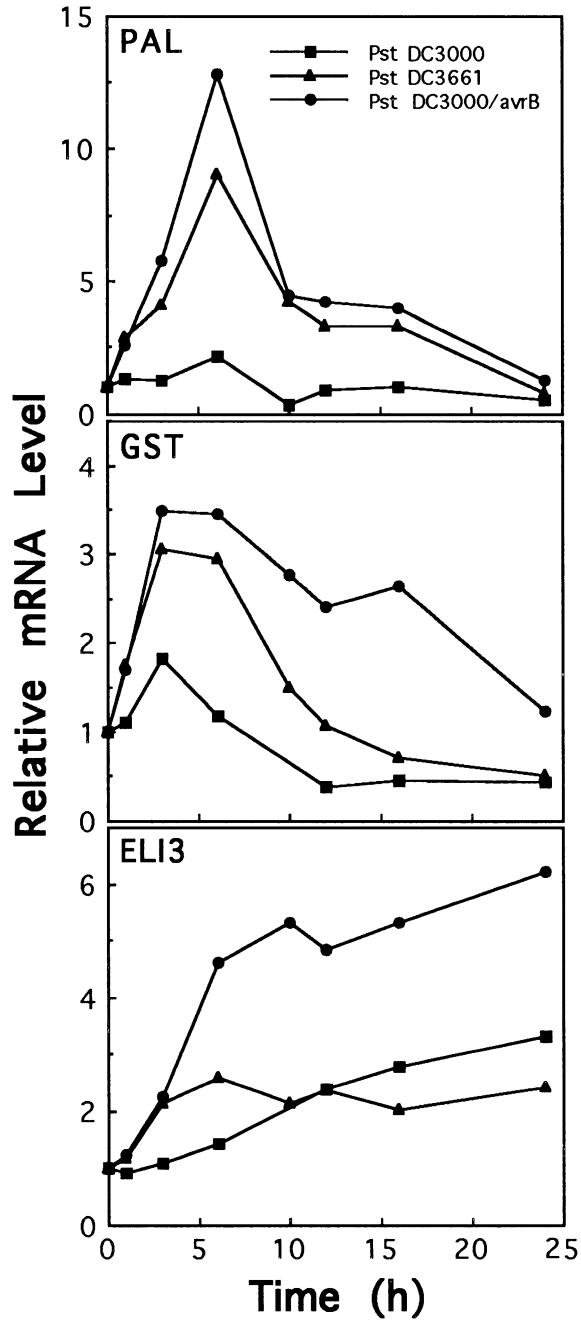


**Fig. 4.** Accumulation of the defense-related mRNAs in *Arabidopsis* leaves inoculated with *Pseudomonas syringae* pv. *tomato* (Pst) strains DC3000 and DC3661 and with strain DC3000 containing *avrB* from *P. s.* pv. *glycinea*. A needleless syringe was used to infiltrate entire leaves with bacterial suspensions adjusted to $OD_{600}$ = 0.10 with 10 mM $MgCl_2$. At the indicated times, eight to 10 leaves were harvested from five to 10 different plants receiving the same treatment and frozen in liquid nitrogen. Total RNA was prepared from the pooled leaves and analyzed by RNA blot analyses using probes specific for phenylalanine ammonia-lyase (PAL), glutathione S-transferase (GST), or ELI3. Hybridization signals were analyzed with a PhosphorImager. The data shown are from a representative experiment and are expressed as the ratio between mRNA levels in the bacterial treatments and mRNA levels in an $MgCl_2$ control, and thus represent the fold-induction observed. Similar results were observed in three additional experiments.

tively, coronatine may have a direct effect on defense gene activation by interfering with a specific step or steps of defense signal-response pathways. Previous studies have indicated that coronatine induces ethylene production (Ferguson and Mitchell 1985; Kenyon and Turner 1992), although studies of the response of *Arabidopsis* ethylene-response mutants to infection with *P. s.* pv. *tomato* DC3000 or treatment with coronatine indicate ethylene is not required for coronatine-induced effects (Bent *et al.* 1992). In addition, ethylene production is normally associated with the activation of defense-related genes, and thus it is not likely that the apparent suppression of defense gene activation by DC3000 is mediated through ethylene. A recent study of coronatine-resistant *Arabidopsis* mutants suggests that coronatine may function by mimicking methyl jasmonate (Feys *et al.* 1994). This suggestion is somewhat at odds with our results, since methyl jasmonate has generally been found to be an inducer of defense responses (Dittrich *et al.* 1992; Farmer and Ryan 1992; Gundlach *et al.* 1992; Melan *et al.* 1993) rather than a suppressor. Interestingly, the coronatine-resistant mutant was found to be resistant to a $Cor^+$ *P. s.* pv. *atropurpurea* strain that was virulent on wild-type *Arabidopsis*, which indicates that coronatine is required for the virulence of this *Pseudomonas* strain and is consistent with the hypothesis that coronatine may suppress plant defense responses. Clearly, further studies are required to clarify the molecular mechanisms involved in the interaction of coronatine with plant cells.

In conclusion, we have shown that coronatine appears to be a major virulence factor that allows *P. s.* pv. *tomato* strain DC3000 to infect *Arabidopsis* and tomato. These results indicate that coronatine production may be a required virulence determinant for some coronatine-producing pseudomonads on specific plant hosts.

## MATERIALS AND METHODS

### Plant growth and inoculations.

Seeds of *Arabidopsis thaliana,* land race Col-0, were planted in pre-wetted Metromix 200 (Hummert Seed Co., St. Louis, MO) in flats (for infiltration) or in pots covered with a nylon screen (for dipping). Covered flats of newly planted seeds were kept at 4° C for 2 days, and then transferred to a 20–22° C controlled-environment room with a 12-hr photoperiod supplied by fluorescent lights (50–150 $\mu mol\ m^{-2}\ sec^{-1}$). After 2 wk, the clear plastic covers were removed. Plants with fully expanded rosette leaves were used for inoculation 4–5 wk after planting. Tomato varieties Bonny Best and VFNT Cherry were grown either in growth chambers as described above or in a greenhouse at 22–25° C with supplemental lighting to maintain a photoperiod of at least 12 hr. The results obtained with tomato plants grown in chambers and with those grown in the greenhouse were not significantly different.

*P. s.* pv. *tomato* strains DC3000 and DC3661 were obtained from Diane Cuppels (Agriculture Canada Research Centre, London, Ontario) and grown in King's B medium with 50 $\mu g\ ml^{-1}$ rifampin containing 0.15% glucose as carbon source (King *et al.* 1954). Inoculum was prepared by collecting bacteria from overnight cultures ($OD_{600}$ = 1.6–2.2) by centrifugation and resuspending in 10 mM $MgCl_2$ at the desired dilution. Plants were inoculated by two different methods. In the first method, bacteria were resuspended to $OD_{600}$ = 0.001 ($1 \times 10^6$ cfu $ml^{-1}$) and infiltrated into the undersides of leaves by means of a needleless syringe as previously described (Davis *et al.* 1991). Alternatively, plants growing through nylon screen in pots were dipped in a suspension of bacteria at $OD_{600}$ = 0.2 ($2 \times 10^8$ cfu $ml^{-1}$) containing 0.02% Silwet L77, a surfactant (Wanner *et al.* 1993; Whalen *et al.* 1991). The *in planta* growth of bacteria in infected plants was measured as previously described (Wanner *et al.* 1993).

### RNA isolation and RNA blot hybridization.

Eight to 10 leaves were harvested from five to 10 different plants receiving the same treatment and frozen in liquid nitrogen. Total RNA was prepared, and 5- or 10-μg samples of RNA were separated by electrophoresis on 1.1% agarose-formaldehyde gels as previously described (Davis *et al.* 1991). The fractionated RNA was transferred overnight to Duralon-UV nylon membranes (Stratagene, La Jolla, CA) using 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (Sambrook *et al.* 1989). The RNA was fixed onto the filters with 12 mJ of UV irradiation supplied by a Stratalinker (Stratagene). Blots were prehybridized for 1–2 hr and hybridized in a solution containing 5× SSC, 1% sodium dodecyl sulfate (SDS), and denatured fish sperm DNA (70 $\mu g\ ml^{-1}$) at 42° C. A random primer labeling kit (Life Technologies, Gaithersburg, MD) was used to prepare probes from gel-purified plasmid inserts, to specific activities of $5 \times 10^8$ to $2 \times 10^9$ cpm $\mu g^{-1}$. Labeled DNA was separated from unincorporated $^{32}P$-nucleotides in a 1-ml Sephadex G-50 spin column (Sambrook *et al.* 1989), heat-denatured, and added to hybridization solutions at approximately $10^6$ cpm $ml^{-1}$. Filters were hybridized for 20–24 hr and then washed twice in a solution of 2× SSC and 0.8% SDS for 10–15 min at 65° C. Filters to be rehybridized to a second probe were stripped by pouring a boiling hot solution of 0.02× SSC and 0.5% SDS over the filters. The damp filters were exposed to Kodak XAR5 film at −80° C with a Dupont Cronex intensifying screen and/or imaged with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Quantitative data were obtained from PhosphorImager scans by the use of Molecular Dynamics ImageQuant software to sum total pixel values in equal-sized areas placed over individual hybridizing bands. The GST and PAL probes were made from a 0.9-kb *Bam*HI fragment of the *AtGST1* cDNA clone and the 0.52-kb *Hin*dIII fragment of the *AtPAL1* genomic clone as previously described (Sharma and Davis 1994). The ELI3 probe was prepared by labeling a 1.3-kb full-length *Eco*RI cDNA fragment (Kiedrowski *et al.* 1992). To correct for any differences in the amount of RNA present in each lane, blots were reprobed with a 28S ribosomal RNA gene from pea (Wanner and Gruissem 1991). In the data shown, the counts obtained with the rRNA probe have been used to correct for any loading differences.

## ACKNOWLEDGMENTS

We thank Diane Cuppels for supplying *P. s.* pv *tomato* strains and helpful discussions and Frederick Ausubel and Jeff Dangl for providing the glutathione S-transferase and ELI3 cDNA clones, respectively. We also thank Yogesh Sharma and David Coplin for critically reading the manuscript. This research was supported by National Institute of General Medical Sciences grant number GM45570.

## LITERATURE CITED


Bender, C. L., Stone, H. E., Sims, J. J., and Cooksey, D. A. 1987. Reduced pathogen fitness of *Pseudomonas syringae* pv. *tomato* Tn*5* mutants defective in coronatine production. Physiol. Mol. Plant Pathol. 30:272-283.

Bent, A. F., Innes, R. W., Ecker, J. R., and Staskawicz, B. J. 1992. Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent *Pseudomonas* and *Xanthomonas* pathogens. Mol. Plant-Microbe Interact. 5:373-378.

Davis, K. R., Schott, E., and Ausubel, F. M. 1991. Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 4:477-488.

Debener, T., Lehnackers, H., Arnold, M., and Dangl, J. L. 1991. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. Plant J. 1:289-302.

Dittrich, H., Kutchan, T. M., and Zenk, M. H. 1992. The jasmonate precursor, 12-oxo-phytodienoic acid, induces phytoalexin synthesis in *Petroselinum crispum* cell cultures. FEBS Lett. 309:33-36.

Dong, X., Mindrinos, M., Davis, K. R., and Ausubel, F. M. 1991. Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. Plant Cell 3:61-72.

Durbin, R. D. 1991. Bacterial phytotoxins—Mechanisms of action. Experientia 47:776-783.

Farmer, E. E., and Ryan, C. A. 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4:129-134.

Ferguson, I. B., and Mitchell, R. E. 1985. Stimulation of ethylene production in bean leaf discs by the pseudomonad phytotoxin coronatine. Plant Physiol. 77:969-973.

Feys, B. J. F., Benedetti, C. E., Penfold, C. N., and Turner, J. G. 1994. *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell 6:751-759.

Gross, D. C. 1991. Molecular and genetic analysis of toxin production by pathovars of *Pseudomonas syringae*. Annu. Rev. Phytopathol. 29: 247-278.

Gundlach, H., Muller, M. J., Kutchan, T. M., and Zenk, M. H. 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc. Natl. Acad. Sci. USA 89:2389-2393.

Jackson, D. P., Gray, D. A., Morris, V. L., and Cuppels, D. A. 1992. Identification of a DNA region required for growth of *Pseudomonas syringae* pv. *tomato* on tomato plants. Can. J. Microbiol. 38:883-890.

Kenyon, J. S., and Turner, J. G. 1992. The stimulation of synthesis in *Nicotiana tabacum* leaves by the phytotoxin coronatine. Plant Physiol. 100:219-224.

Kiedrowski, S., Kawalleck, P., Hahlbrock, K., Somssich, I. E., and Dangl, J. L. 1992. Rapid activation of a novel plant defense gene is strictly dependent on the *Arabidopsis* RPM1 disease resistance locus. EMBO J. 11:4677-4684.

King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of phycocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.

Melan, M. A., Dong, X., Endara, M. E., Davis, K. R., Ausubel, F. M., and Peterman, T. K. 1993. An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. Plant Physiol. 101:441-450.

Mino, Y., Sakai, R., Uchino, K., and Sasabuchi, T. 1980. Effect of coronatine on the metabolism of phenolics in the discs of potato tuber. Ann. Phytopathol. Soc. Jpn. 46:510-516.

Mitchell, R. E. 1991. Implications of toxins in the ecology and evolution of plant pathogenic microorganisms: Bacteria. Experientia 47:791-803.

Moore, R. A., Starratt, A. N., Ma, S. W., Morris, V. L., and Cuppels, D. A. 1989. Identification of a chromosomal region required for biosynthesis of the phytotoxin coronatine by *Pseudomonas syringae* pv. *tomato*. Can. J. Microbiol. 35:910-917.

Perner, B., and Schmauder, H. P. 1993. Effect of coronatine of plant cell cultures. J. Phytopathol. 138:98-104.

Sakai, R., Mino, Y., and Hosoi, E. 1982. Effect of coronatine on the induction of cell wall degrading enzymes in potato tuber discs. Ann. Phytopathol. Soc. Jpn. 48:52-57.

Sakai, R., Mino, Y., Takachi, M., and Enoki, S. 1979. Effect of coronatine on the decomposition of starch grains in the discs of potato tuber. Ann. Phytopathol. Soc. Jpn. 45:596-602.

Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sharma, Y. K., and Davis, K. R. 1994. Ozone-induced expression of stress-related genes in *Arabidopsis thaliana*. Plant Physiol. 105:1089-1096.

Völksch, B., Friedemann, B., and Fritsche, W. 1989. Coronatine production by *Pseudomonas syringae* pathovars: Screening method and capacity of product formation. J. Basic Microbiol. 29:463-468.

Wanner, L. A., and Gruissem, W. 1991. Expression dynamics of the tomato *rbcS* gene family during development. Plant Cell 3:1289-1303.

Wanner, L. A., Mittal, S., and Davis, K. R. 1993. Recognition of the avirulence gene *avrB* from *Pseudomonas syringae* pv. *glycinea* by *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 6:582-591.

Whalen, M. C., Innes, R. W., Bent, A. F., and Staskawicz, B. J. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell 3:49-59.

Wiebe, W. L., and Campbell, R. N. 1993. Characterization of *Pseudomonas syringae* pv. *maculicola* and comparison with *P. s. tomato*. Plant Dis. 77:414-419.

Willis, D. K., Barta, T. M., and Kinscherf, T. G. 1991. Genetics of toxin production and resistance in phytopathogenic bacteria. Experientia 47:765-771.