Supporting Information

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SI Materials and Methods

Preparation of Bacteria Strains and Growth Conditions. Plasmid pVLT35 expressing avrPto-cyaA (pCPP3221) (1) was introduced into *Pseudomonas syringae* pv tomato DC3000 and DC3000 *hrcC*$^{-}$ (2) by triparental mating. Bacteria were grown on King's B medium (KBM) agar plates containing the appropriate selective antibiotics at concentrations of 30 μg/mL for rifampicin and 50 μg/mL for spectinomycin. Before use, bacteria were streaked onto agar plates from −80 °C glycerol stocks and were grown for 2–3 d at room temperature. For all experiments, bacteria were scraped from a KBM agar plate, resuspended in 1 mL of water, and then were washed once. Bacteria were pelleted at 16,000 × *g* for 1 min and were resuspended again in water. Bacteria inocula were adjusted before use by measuring the OD_{600} of the resuspended bacteria and diluting to the indicated concentrations using the formula OD_{600} of $1.0 = 1 \times 10^9$ cfu/mL.

Preparation of Plant Exudates. All solutions and exudates used in this study were prepared using Milli-Q purified ultrapure water (Millipore, Inc.). *Arabidopsis* loss-of-function transfer-DNA insertion mutants *mkp1* (ecotype Wassilewskija, Ws), *mkp1 mpk6* Ws, and *efr-2* (ecotype Columbia) have been described previously (3, 4). Seeds were sterilized with 0.5% sodium hypochlorite for 20 min, rinsed with water, and germinated on 0.6% agar plates (Phytotechnology Laboratories catalog no. A296) containing 4.33 g/L of Murashige and Skoog salts (Phytotechnology Laboratories catalog no. M524), 100 mg/L Murashige and Skoog vitamins (Phytotechnology Laboratories catalog no. M533), and 1% sucrose in disposable polystyrene Petri dishes sealed with micropore tape (3M, Inc.). After 2 wk of growth at 22 °C and 11-h daylight in a Percival incubator model CU-36L, plants were aseptically removed from the agar, rinsed for ∼20 s in sterile water to remove residual medium, and placed in sterile water within individual wells of a 24-well polystyrene microtiter plate (Corning, Inc.). Typically, three to five plants were placed in 1 mL of water in each well. Larger preparations of exudate also were prepared by immersing plants in water in 50-mL polypropylene conical tubes at a ratio of three plants/mL. After plants were incubated for 4 h to overnight in the Percival incubator, the water (the exudate) was removed, filtered (0.22 μM), and stored at −20 °C until needed.

GC-MS Analysis of Plant Exudates. ***Secreted metabolite extraction.*** Chloroform (20 mL) was mixed with 20 mL of plant exudate and centrifuged at 3,000 × *g* for 10 min to remove any lipophilic debris. The resulting aqueous phase was transferred to a clean polypropylene tube, flash frozen in liquid nitrogen, and lyophilized. The dried residue was resuspended in 2 mL of methanol and centrifuged at 16,000 × *g* for 5 min to remove insoluble material. The methanol-soluble fraction was transferred to a clean tube and evaporated to dryness in a speed vacuum concentrator. The dried metabolites in each sample then were dissolved in 1 mL of solution of Nanopure (Thermo Scientific) water and methanol (50:50, vol/vol), and the solution was divided into two fractions in glass vials for technical replicates. Then all the samples were dried completely in a speed vacuum concentrator.

Chemical derivatization of metabolites and GC-MS analysis. The exudates underwent a two-stage chemical derivatization as previously described (5). To protect carbonyl groups and reduce the number of tautomeric isomers, 20 μL of methoxyamine in pyridine (30 mg/mL) was added to each sample, followed by incubation at 37 °C with generous shaking for 90 min. To derivatize hydroxyl and amine groups to trimethylsilyated (TMS) forms, 80 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane was added to each vial, followed by incubation at 37 °C with shaking for 30 min. The samples were allowed to cool to room temperature and then were analyzed by GC-MS in random order. An Agilent GC 7890A coupled with a single quadrupole MSD 5975C (Agilent Technologies, Inc.) was used for all analyses, and separations were performed using an HP-5MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Inc.). The sample injection mode was splitless, and 1 μL of each sample was injected. The GC oven was held at 60 °C for 1 min after injection, and the temperature then was increased to 325 °C at 10 °C/min, followed by a 5-min hold at 325 °C. The injection port temperature was held at 250 °C throughout the analysis.

GC-MS data processing and analysis. GC-MS raw data files were processed using MetaboliteDetector (6). Retention indices of detected metabolites were calculated based on analyses of a mixture of fatty acid methyl esters (C8–C28), followed by their chromatographic alignment across all analyses after deconvolution. Metabolites then were identified by matching GC-MS features (characterized by measured retention indices and mass fragmentation spectra) to the Agilent Fiehn Metabolomics Retention Time Locked (RTL) Library (7), which contains validated spectra and retention indices for 700 metabolites [i.e., level 1 metabolite identifications (8)]. All metabolite identifications were manually validated to reduce deconvolution errors during automated data processing and to eliminate false identifications. The NIST08 GC-MS library was used to identify putatively those features that could not be matched to entries in the Agilent Fiehn Metabolomics RTL Library based on mass spectral similarity only, i.e., level 2 metabolite identifications (8). These putatively identified metabolites were annotated with "(NIST)". During the TMS derivatization of metabolites, partial derivatives having different numbers of TMS groups can be generated (9). In these instances, the most abundant GC-MS peak for each metabolite was selected, or the integrated areas of two or three peaks were combined when those peaks had relatively similar abundances.

Preparation of Metabolite Stocks. All metabolites tested for bioactivity were obtained from Sigma-Aldrich, Inc. and Acros Organics, Inc. with the exception of 2-(β-glucosyl)glycerol (Toronto Research Chemicals), glyceric acid (Tokyo Chemical Industry Co.), sinapinic acid (AB Sciex), glycine (Fisher Scientific), and triethanolamine (MP Biomedicals). Stocks (20 mM) were prepared in water and then were filter sterilized and maintained at −20 °C until use.

In Vitro Treatments of Bacteria with Plant Exudates and Metabolites. In vitro treatments of DC3000 with exudates and metabolites were performed in polystyrene microtiter plates (Corning, Inc.). For exudate treatments, 500 μL of plant exudate or water was mixed with 500 μL of a modified *hrp*-inducing minimal medium (10) [10 mM K_2HPO_4/KH_2PO_4 (pH 6.0), 7.5 mM $(NH_4)_2SO_4$, 3.3 mM $MgCl_2$, 1.7 mM NaCl] supplemented or not supplemented with 100 mM fructose. For metabolite treatments, 500 μL of a 2×-concentrated aqueous solution of an individual metabolite was substituted for plant exudate in the above assay. After adjustment to an OD_{600} = 2.0, 100 μL of bacterial suspension was added to each well in the assay plate. After the assay plate was incubated at 20 °C for the indicated times, the bacteria

were transferred to 1.5-mL Eppendorf tubes and were centrifuged at 16,000 × *g* at room temperature for 5 min. The resulting supernatant was removed, and the pelleted bacteria were flash frozen in liquid N_2. Samples were stored at −80 °C until use.

Isolation of Bacterial RNA and Protein. RNA and protein were isolated from treated bacteria using TRI reagent (Sigma-Aldrich, Inc.) following the manufacturer's protocol. Briefly, frozen bacterial pellets were resuspended in 500 μL of TRI reagent by pipetting. After 100 μL of chloroform was added to each, the samples were vortexed briefly and spun at 16,000 × *g* for 10 min at room temperature. The aqueous phase was removed to a clean tube, and RNA was precipitated from this solution by the addition of 250 μL of isopropanol. RNA pellets were washed twice with 70% ethanol and were stored at −20 °C until use. For protein isolation, 150 μL of ethanol was added to the remaining organic phase and was centrifuged at 2,000 × *g* for 5 min to pellet and remove DNA. The supernatant was transferred to a clean tube, and proteins were precipitated by the addition of 750 μL of isopropanol and incubation at −20 °C overnight. Protein precipitates then were pelleted by centrifugation at 16,000 × *g* for 5 min and were washed twice with 80% acetone. A sonicating water bath was used to break up the pellets after each washing and subsequent centrifugation step. Isolated proteins were stored in 80% acetone at −20 °C until use.

Immunoblot Detection of AvrPto. Bacterial protein pellets stored in 80% acetone were allowed to air-dry, resuspended in Laemmli buffer, incubated at 65 °C for 20 min and 100 °C for 2 min, and then were separated in 15% SDS/PAGE gels. Immunoblot analysis was performed using a polyclonal anti-AvrPto rabbit antibody (11) kindly provided by Greg Martin (Boyce Thompson Institute, Cornell University, Ithaca, NY) and chemiluminescence-based detection (Pierce SuperSignal, Thermo Scientific, Inc.) using a goat anti-rabbit secondary antibody (Sigma-Aldrich, Inc).

Quantitative RT-PCR Analysis of DC3000 Responses to Plant Exudates. Bacterial RNA was incubated with 0.5 μL of Dnase I (Fermentas, Inc.) at 37 °C in 25-μL reactions containing 0.5 μL RNaseOUT (Invitrogen) in the supplied DNase I buffer. After 1 h, 2.5 mM EDTA was added, and each reaction was incubated at 65 °C for 5 min. RNA was quantitated by absorbance at 260 nm using a spectrophotometer, and 350 ng of RNA per sample was reverse transcribed in 25-μL reactions containing 0.5 μL Molony murine leukemia virus (m-mlv) reverse transcriptase (Promega, Inc.), 0.5 μL RNaseOUT, and 0.5 μL of a 0.5 μg/μL random hexamer primer mix (Promega, Inc.) in the supplied m-mlv buffer at 42 °C for 1 h, followed by 5 min at 85 °C. Reactions then were diluted to 50 μL with water, and 1 μL of each reaction was mixed with 5 μL of SYBR Green PCR mix (Applied Biosystems, Inc.) and 4 μL of a solution containing 0.5 μM of each gene-specific primer. Sequences of primers used for quantitative RT-PCR (qRT-PCR) are

avrPto forward 5′- ATGACGGGAGCGTCAGGAATCAAT-3′,
avrPto reverse 5′-ATCCGTTCGGGTTCATAGTCGCAA-3′,
hrpL forward 5′-TCAGGAAAGCTGGGAAGACGAAGT-3′,
hrpL reverse 5′-ATGTTCGACGGCAGGCAATCAATG-3′,
gyrA forward 5′-TTCAATGCTGATCCCGGAAGAAGG-3′, and
gyrA reverse 5′-ATTTCCTCACCATCCAGCACCTGA-3′.

Real-time PCR reactions were performed using an ABI7500 instrument (Applied Biosystems, Inc.), and expression levels were calculated as described (3). Levels of *avrPto* and *hrpL* transcripts were normalized to transcripts of the housekeeping gene *gyrA*.

qRT-PCR Analysis of *avrPto* Gene Expression in DC3000-Infected Plants. Fourteen-day-old plants grown on Murashige and Skoog agar plates were rinsed in sterile water and were transferred to 900 μL of 5 mM Mes (pH 5.7) in the wells of a 24-well microtiter plate (Corning, Inc.). Three plants were placed in each well, and six wells of plants were treated for each treatment condition. After 2 h, 100 μL of an OD_{600} = 5.0 solution of DC3000 was added to each well. For metabolite treatments, 2.5 μL each of 20 mM citric acid, aspartic acid, and 4-hydroxybenzoic acid stocks was added to the wells immediately before the bacteria were added. Four hours after infection, the plants were rinsed in water, blotted dry, and immediately frozen in liquid nitrogen. For each sample, three plants from an individual well were ground to a powder under liquid nitrogen, and RNA was extracted using 500 μL of TRI reagent according to the manufacturer's protocol. Isolated RNA was treated with DNase I, and 2 μg of total RNA was reverse-transcribed using random primers as described above, with the exceptions that SuperScript II enzyme (Invitrogen) and the supplied first-strand buffer with 1 mM DTT added were used for reverse transcription reactions. Reactions then were diluted to 100 μL with diethylpyrocarbonate-treated water, and 2 μL of each reaction was mixed with 5 μL of SYBR Green PCR mix (Applied Biosystems, Inc.) and 3 μL of a solution containing 1 μM of each primer. qPCR reactions and data analysis were performed as describe above. *avrPto* transcript levels were normalized to the levels of bacterial *RpoD* and 16S RNA transcripts. All primers were determined to be specific based on the lack of amplification in minus-reverse transcriptase reaction controls and in mock infection controls. *avrPto*-specific primers were the same as those described above; primers used for amplifying control transcripts were

rpoD forward 5′-GTTTCGGTGCGGTTTCCGATCAAA-3′ (described in ref. 12),
rpoD reverse 5′-ATCGGCATGAACAATTCGGCAAGG-3′ (described in ref. 12),
16S rRNA forward 5′-CTCAACCTGGGAACTGCATCC -3′, and
16S rRNA reverse 5′-TCAGTGTCAGTATCAGTCCAGGTGG-3′.

AvrPto-CyaA Delivery Assays. Fourteen-day-old plants grown on Murashige and Skoog agar plates were rinsed in sterile water and transferred to 900 μL of 5 mM Mes (pH 5.7) in the wells of a 24-well microtiter plate (Corning, Inc). For assaying AvrPto-CyaA delivery in soil-grown plants, 14-d-old plants in mesh-covered pots were cut at the base of the hypocotyl, and the aerial portions were rinsed briefly in water before transfer to 24-well plate as described above. For both agar- and soil-grown plants, two plants were placed in each microtiter plate well, and a minimum of 12 wells were prepared for each treatment condition. After 2 h, 100 μL of an OD_{600} = 1.0 solution of DC3000 expressing pCPP3221 (1) was added to each microtiter plate well. After incubation in the Percival incubator for times indicated, the infected plants were removed from each well, rinsed with water, blotted dry, and frozen in liquid nitrogen. For each sample, six plants pooled from three wells were ground to a powder under liquid nitrogen and extracted into 600 μL of 0.1 M HCl. Extracts were centrifuged at 16,000 × *g* for 15 min at 4 °C, and the amount of cAMP present in the supernatants was measured by the Direct cAMP ELISA kit (ENZO Life Sciences, Inc. catalog no. ADI-900-066) according to the manufacturer's protocol. cAMP levels were normalized to total protein present in each extract as determined by BCA assay (Thermo Scientific Pierce). To measure bacteria levels during infection with DC3000 AvrPto-CyaA, infected plants were surface-sterilized with ethanol for ∼10 s and rinsed with water, and serial-dilution plating was performed as described below.

Measurements of Bacteria Growth in Plants Grown on MS Agar. Fifteen-day-old plants were rinsed in sterile water and placed

into water in the wells of a 24-well microtiter plate. Two plants were placed into 2 mL of water in each well, and a minimum of six wells were prepared for each treatment condition. After 2 h, the water in each well was removed and replaced with 1.9 mL of 5 mM Mes (pH 5.7), supplemented or not supplemented with 100 μM citric acid, 100 μM 4-hydroxybenzoic acid, and 100 μM aspartic acid or 100 μM serine, 100 μM threonine, and 100 μM valine. Then 100 μL of an $OD_{600} = 0.02$ solution of DC3000 was added to each well. Plates containing infected plants were kept at the same environmental conditions as those used for plant growth. On the days indicated, the infected plants were rinsed in sterile water and blotted dry, and extracts were prepared by grinding two plants taken from the same assay well in 500 μL of 10 mM $MgCl_2$. Tenfold serial dilutions of the extracts were prepared, and 20 μL of each dilution was spotted onto KBM agar plates. Colonies were counted after 2 d growth at room temperature.

Measurements of Bacteria Growth in Soil-Grown Plants. Ecotype Wassilewskija (Ws) and *mkp1*(Ws) seeds were cold-treated for 2 d and were germinated directly on soil (Sunshine Mix; Sun Gro Horticulture Corp.) in 12 × 12 cm mesh-covered pots maintained at 22 °C and 11-h daylight in a Percival incubator model CU-36L. An inoculum of bacteria was prepared by scraping DC3000 from a KBM agar plate and resuspending to a final $OD_{600} = 0.02$ (2×10^7 cfu/mL) in 100 mL of 5 mM Mes (pH 5.7), supplemented or not supplemented with 100 μM each of citric acid, aspartic acid, and 4-hydroxybenzoic acid. Plants were infected by immersing the pots upside down in the inoculum for 4 h; then the pots were turned upright and placed in the same Percival chamber used for plant growth. Alternatively, 2×10^8 cfu/mL of DC3000 *lux-CDABE* (13) in 5 mM Mes (pH 5.7)/0.04% Silwet supplemented or not supplemented with 100 μM each of citric acid, aspartic acid and 4-hydroxybenzoic acid was sprayed onto plants using a small pump spray bottle. After infection, the plants were covered with plastic domes and placed back in the growth chamber for the duration of the assay. Bacterial growth was assessed by clipping plants at the base of the hypocotyl at the indicated times, rinsing them with water, and performing serial-dilution plating of extracts from the infected plants as described above.

Analysis of Metabolite Effects on MAPK Activation and Gene Expression Induced by Pathogen-Associated Molecular Patterns. Fourteen-day-old Ws plants grown on Murashige and Skoog agar were rinsed with water and transferred into 2 mL of sterile water in a 12-well plate. Six plants placed in each well, and two wells were treated for each treatment condition. After 4 h, water in the wells was replaced with 5 mM Mes (pH 5.7) buffer supplemented or not supplanted with 1 μM elf26 in the presence or absence of 50 μM each of citric, aspartic, and 4-hydroxybenzoic acid. At the indicated times, the treated plants were blotted dry and frozen in liquid nitrogen. Proteins and total RNA were extracted by TRI reagent (Sigma). Immunoblot detection of activated MAPKs and RT-qPCR measurements of *NHL10* and *FRK1* transcripts in isolated RNA were performed as described previously (3). Primers used for detecting *NHL10*, *FRK1*, and *AT2G28390* (control) were

FRK1 forward 5′-CGGTCAGATTTCAACAGTTGTC-3′,
FRK1 reverse 5′-AATAGCAGGTTGGCCTGTAATC-3′,
NHL10 forward 5′-TTCCTGTCCGTAACCCAAAC-3′,
NHL10 reverse 5′-CCCTCGTAGTAGGCATGAGC-3′,
AT2G28390 forward: 5′-AACTCTATGCAGCATTTGATC-CACT-3′, and
AT2G28390 reverse: 5′-TGATTGCATATCTTTATCGCCA-TC-3′.

Analysis of Callose Deposition. Fourteen-day-old plants were transferred from Murashige and Skoog agar into 1 mL of sterile water in a 24-well plate. One plant was placed in each well, and eight wells were treated for each genotype. After 2 h incubation in water, the plants were treated with 1×10^8 cfu/mL DC3000, or were mock-treated for 4 h, or were treated with 1 μM elf26 for 24 h. Callose staining was performed as described previously (14) with slight modifications. Briefly, plants were cleared and dehydrated in 2 mL 95% ethanol overnight with shaking. The cleared plants then were washed once in 1 mL 50% ethanol for 60 min and were incubated in 67 mM K_2HPO_4 (pH 12) for 60 min. For callose staining, each plant was incubated with 1 mL of 0.01% aniline blue in 67 mM K_2HPO_4 (pH 12) for 1 h and then was rinsed in 67 mM K_2HPO_4 (pH 12). For imaging callose, leaves were dissected from each plant and mounted in 70% glycerol, 67 mM K_2HPO_4 (pH 12), with the abaxial side up facing the cover slide. Callose deposits were detected using UV epifluorescence under a Leica M205F Stereoscope.

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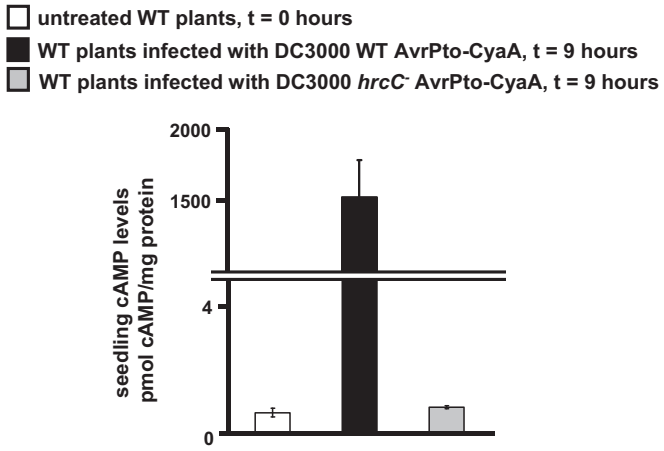

Fig. S1. Delivery of AvrPto-CyaA requires a functional type III secretion system (T3SS). Fourteen-day-old plants grown on agar plates were transferred to the wells of a microtiter plate. After overnight incubation in water, the plants were infected with 1×10^8 cfu/mL of DC3000 or DC3000 *hrcC*$^-$ expressing a fusion protein of AvrPto and adenylate cyclase (AvrPto-CyaA). After 9 h the plants were rinsed, frozen in liquid nitrogen, and ground in 0.1 M HCl. cAMP levels were measured by competitive ELISA and normalized by protein amount. Data are shown as means ± SE; $n \geq 6$.

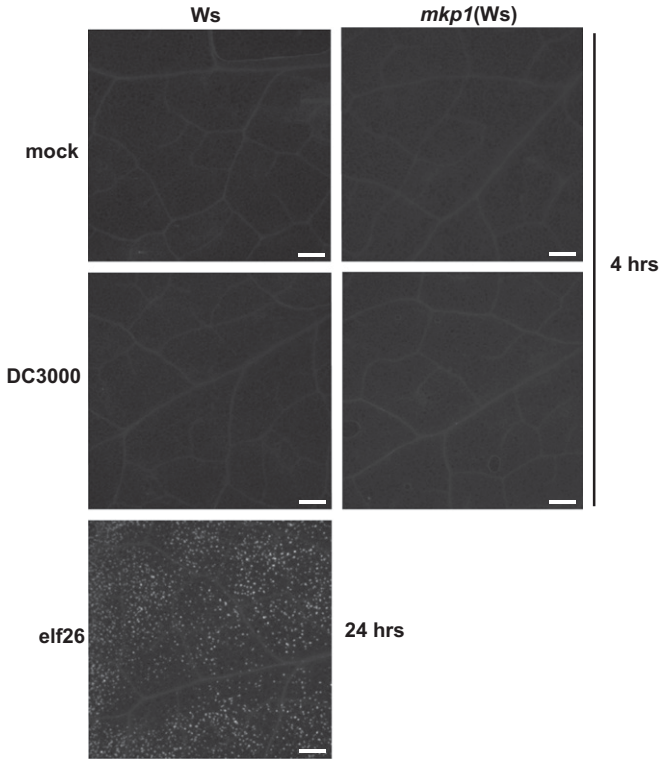

Fig. S2. Detectable levels of callose deposits are not present in *mkp1* at early stages of DC3000 infection. Ws and *mkp1*(Ws) plants were treated with 1×10^8 cfu/mL of *Pto* DC3000 or were mock treated (without bacteria) for 4 h, stained with aniline blue, and imaged under UV light to detect callose deposits. As a positive control, Ws plants also were treated with 1 μM elf26 for 24 h. Shown are images that represent the results of two independent experiments, each performed with eight plants per treatment condition. (Scale bars: 0.25 mm.)

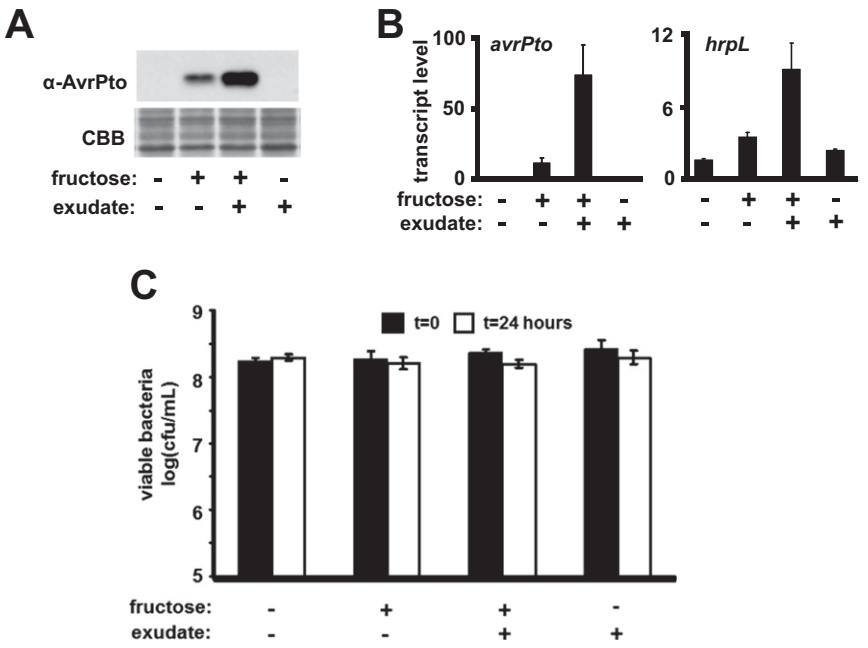

Fig. S3. Exudate treatment increases T3SS expression but does not increase growth of DC3000. *Arabidopsis* exudate was mixed with 2×10^8 cfu/mL of DC3000 in minimal medium with or without 50 mM fructose. (*A*) (*Upper*) Immunoblot of the T3SS effector AvrPto in DC3000 24 h posttreatment. (*Lower*) Coomassie Brilliant Blue (CBB) staining to confirm equal loading. (*B*) qRT-PCR analysis of *avrPto* and *hrpL* transcript levels in bacteria 24 h posttreatment. Graphed data are means from three experiments with technical replication of each; $n = 6$. (*C*) At 0 or 24 h posttreatment the liquid was removed from the wells, and the number of viable bacteria was measured by serial-dilution plating and colony counting. Graphed data are means ± SD; $n = 4$.

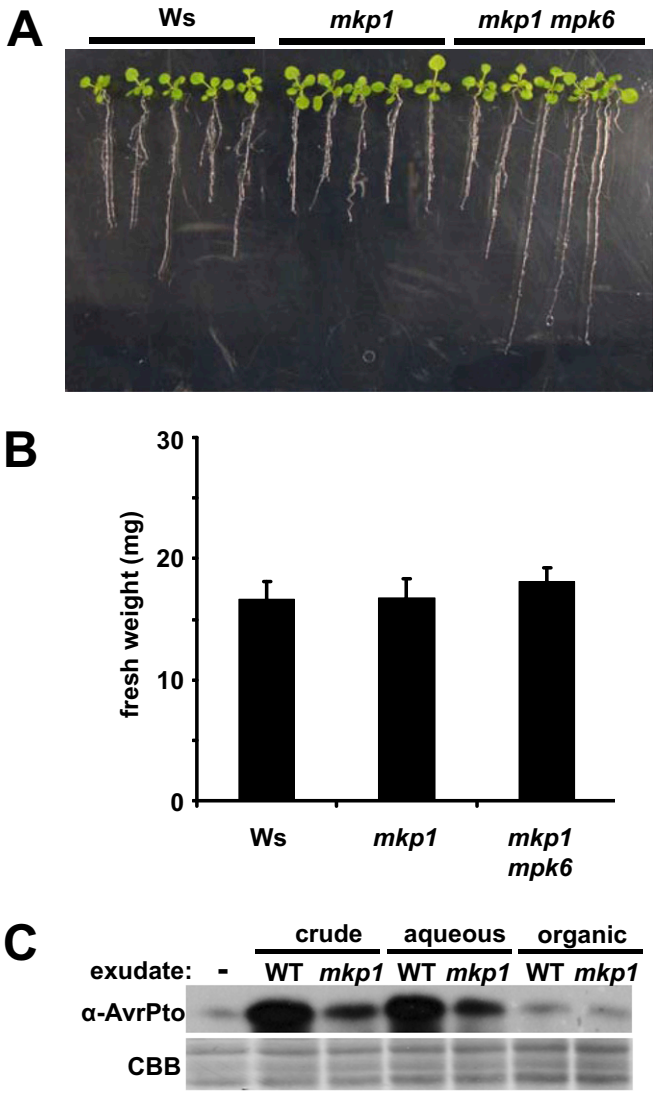

Fig. S4. Ws and *mkp1* plants do not show gross differences in morphology but produce different levels of hydrophilic T3SS-inducing signals. (*A*) Ws, *mkp1*(Ws), and *mkp1 mpk6*(Ws) seeds were germinated on Murashige and Skoog agar medium and were grown for 2 wk. The photograph of plants shows no major differences in morphology among genotypes except for the slightly elongated primary roots of *mkp1 mpk6* plants. (*B*) Fresh weight of 2-wk-old plants. Graphed data are means ± SD, $n = 8$. (*C*) T3SS-inducing components of plant exudates are hydrophilic. Exudates collected from 2-wk-old Ws and *mkp1*(Ws) plants were chloroform-extracted, and the resulting aqueous and organic fractions were tested for AvrPto-inducing activity in *hrp*-inducing minimal medium containing 50 mM fructose. Before the bioassay, the chloroform phase was evaporated to dryness in a vacuum centrifuge and was resuspended in an equivalent volume of water. (*Upper*) An immunoblot of AvrPto levels in DC3000 4 h posttreatment. (*Lower*) Coomassie Brilliant Blue (CBB) staining to confirm equal loading.

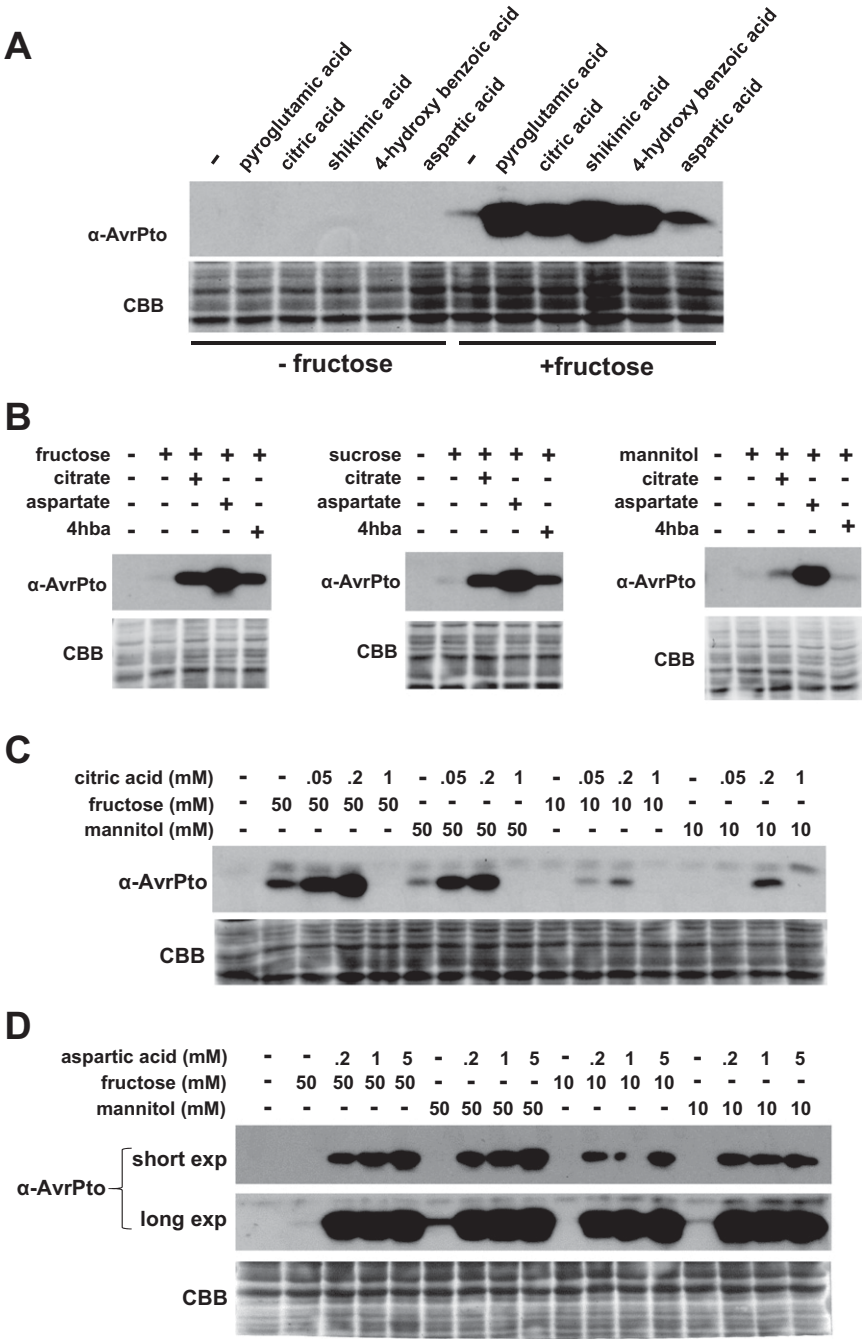

Fig. S5. Concentration-dependent effects of sugars and metabolites on inducing AvrPto accumulation in DC3000. (*A*) A subset of metabolites decreased in *mkp1* exudates enhances AvrPto accumulation in DC3000. DC3000 (2×10^8 cfu/mL) was incubated with 100 μM of individual metabolites as indicated in minimal medium supplemented or not supplemented with 50 mM fructose. (*Upper*) Immunoblot of AvrPto in bacteria 4 h posttreatment. (*Lower*) Coomassie Brilliant Blue (CBB) staining to confirm equal loading. (*B*) Different sugars and sugar alcohols act synergistically with T3SS-inducing metabolites to induce AvrPto accumulation. DC3000 (2×10^8 cfu/mL) was incubated in minimal medium with or without 10 mM fructose, sucrose, or mannitol in the presence or absence of 200 μM of citric acid, aspartic acid, or 4-hydroxybenzoic acid (4hba) for 4 h. Shown are anti-AvrPto immunoblots to detect AvrPto in protein extracts prepared from treated bacteria and images of Coomassie Brilliant Blue (CBB)-stained blots to confirm equal protein loading in each lane. (*C* and *D*) Citric acid, but not aspartic acid, inhibits AvrPto accumulation at higher concentrations. DC3000 (2×10^8 cfu/mL) was incubated for 4 h in minimal medium supplemented or not supplemented with fructose or mannitol in the presence or absence of citric acid (*C*) or aspartic acid (*D*) at the concentrations indicated. (*Top*) Anti-AvrPto immunoblots of protein extracts from treated bacteria. (*Bottom*) Coomassie Brilliant Blue (CBB)-stained blots to confirm equal loading. (*Middle*) A longer exposure of the bottom blot is included to show the induction of AvrPto by fructose or mannitol in the absence of aspartic acid.

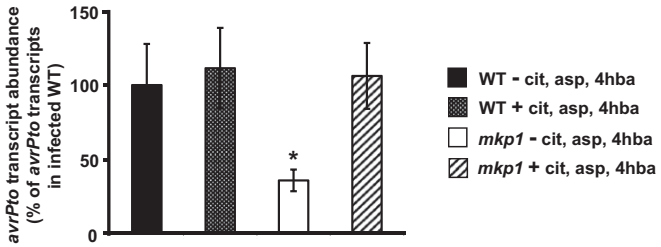

Fig. S6. Type III-inducing metabolites restore *avrPto* transcript levels in DC3000-infected *mkp1* to the levels observed in infected WT plants. qRT-PCR analysis of *avrPto* transcripts in plants infected with 5×10^8 cfu/mL of DC3000 for 4 h. *avrPto* transcript levels were normalized to the levels of bacterial *RpoD* and 16S rRNA transcripts detected in each sample. Each sample was RNA extracted from three infected plants. Six samples were analyzed for each genotype with technical replication of qRT-PCR performed for each. Graphed data are means ± SE of the percent *avrPto* transcripts relative to mean *avrPto* transcript levels in infected WT plants; $n = 12$. Data shown for WT plants and *mkp1* plants infected without metabolites are the same as in Fig. 1*C*. $^*P < 0.05$ based on Student *t* test.

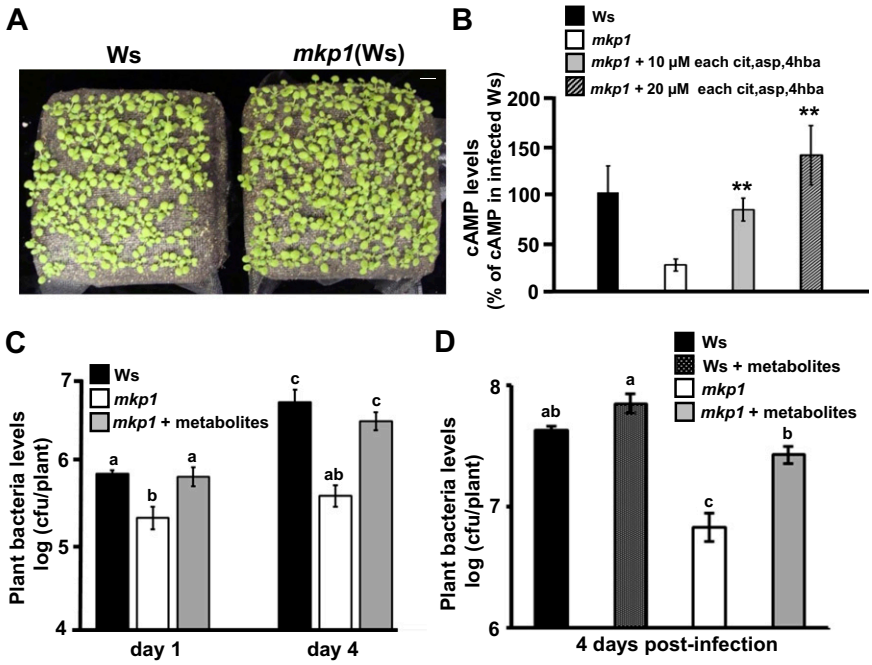

Fig. S7. Soil-grown plants show *mkp1*-dependent block in type III effector delivery and enhanced resistance to DC3000 infection, and these phenotypes are suppressed by cotreatment with T3SS-inducing metabolites. (*A*) Photographs of 14-d-old plants germinated and grown in soil in mesh-covered pots. (Scale bar: 1 cm.) (*B*) cAMP levels in soil-grown Ws and *mkp1*(Ws) plants infected with 1×10^8 cfu/mL DC3000 expressing AvrPto-CyaA for 4 h in the presence or absence of 10 μM each of citric acid, aspartic acid, and 4-hydroxybenzoic acid or 20 μM each of citric acid, aspartic acid, and 4-hydroxybenzoic acid. Graphed data are means ± SE of percent cAMP in infected plants relative to cAMP in Ws plants infected in the absence of metabolites, $n = 6$. Asterisks indicate a significant difference based on Student *t* test comparison with *mkp1* treated without metabolites: $^{**}P < 0.01$. (*C*) Measurements of bacteria in soil-grown Ws and *mkp1*(Ws) plants infected by dip inoculation with 2×10^7 cfu/mL of DC3000 in the presence or absence of 100 μM each of citric acid, aspartic acid, and 4-hydroxybenzoic acid. On the indicated days extracts were prepared from infected plants, and bacteria levels were determined by serial-dilution plating. Graphed data are means ± SE of bacteria in infected plants; $n = 12$. Each sample is an extract prepared from two plants. Data were pooled from two independent experiments; $n = 6$ from each. (*D*) Ws and *mkp1*(Ws) plants infected by spray inoculation with 2×10^8 cfu/mL of DC3000 *luxCDABE* strain and 0.04% Silwet in the presence or absence of 100 μM each of citric acid, aspartic acid, and 4-hydroxybenzoic acid. Extracts were prepared from infected plants 4 d postinfection, and bacteria levels were determined by serial-dilution plating. Graphed data are means ± SE of bacteria in infected plants; $n = 4$. Each sample is an extract prepared from two plants. Lower-case letters in *C* and *D* indicate significance grouping based on ANOVA with pairwise Student *t* test and Tukey's post hoc test; $P < 0.05$.

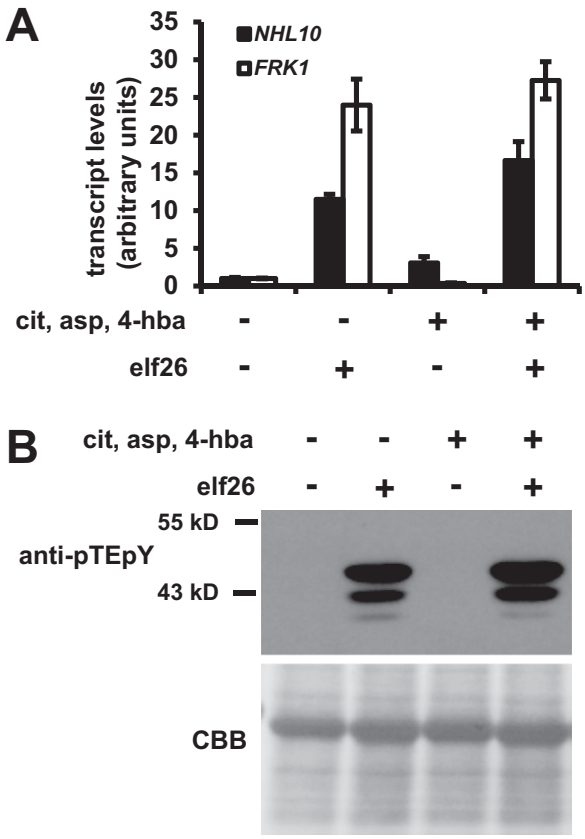

Fig. S8. T3SS-inducing metabolites do not alter molecular responses induced by pathogen-associated molecular patterns (PAMPs). Fourteen-day-old Ws plants were treated with or without 1 μM elf26 in the presence or absence of 50 μM each of citric acid (cit), aspartic acid (asp), and 4-hydroxybenzoic acid (4hba). (*A*) qRT-PCR measurements of PAMP-inducible *NHL10* and *FRK1* transcripts 30 and 90 min, respectively, after elicitation with elf26. Graphed data are means ± SE of expression levels relative to the control gene *At2g28390* in two biological replicates with technical replication; $n = 4$. Transcript abundances in mock-treated plants were set arbitrarily to a value = 1, and all data are graphed relative to these values. (*B*) Immunoblot analysis of MAPK activation 10 min posttreatment. (*Upper*) Immunoblot with antibody against the phosphorylated MAPK motif pTEpY. Results shown are representative of four biological replicates. (*Lower*) Coomassie Brilliant Blue (CBB) staining of blot to confirm equal loading.

Other Supporting Information Files

Dataset S1 (XLSX)