

To define the congruence of human population distribution and *P. falciparum* transmission we used spatially linked databases of human population, limits of malaria risk and malaria endemicity within a Geographic Information System (GIS) as outlined in detail in Supplementary Information B. In brief, we first defined the spatial extent of *P. falciparum* risk by using the mapped global limits of malaria risk provided by the WHO²² and modified with contemporary descriptions of spatial risk used to inform antimalarial chemoprophylaxis regimes in travellers^{22,23}, to exclude the following: countries with only *P. vivax* transmission, areas above anopheline vector-specific altitude limits, and administrative areas defined as risk-free. These boundaries formed the limits of *P. falciparum* risk and were overlaid on the only available global map of malaria endemicity developed in 1968 (refs 6, 7). This map was part of a major synthesis of historical records, documents and maps of malaria endemicity (using the hypoendemic to holoendemic classifications) interpolated globally for malaria at the peak of its assumed historical distribution. We have assumed that this endemicity map is consistent with contemporary malaria risks in Africa, but development and intervention will have substantially reduced malaria risk elsewhere¹¹. Outside the African region we consider the historical (1968) hyperendemic to holoendemic areas as contemporary (2002) mesoendemic conditions, historically mesoendemic areas as hypoendemic, and hypoendemic risk areas at their historical descriptions within the revised 2002 spatial limits of risk.

Data from Gridded Population of the World (GPW3) version 3.0 beta (<http://sedac.ciesin.colombia.edu/gpw>) were projected to 2002 by using national inter-censal growth rates from the UN Population Prospects database (<http://esa.un.org/unpp>). Population totals were extracted by country for those residing in hypoendemic, mesoendemic and hyperendemic-to-holoendemic settings (Table 1, Fig. 2). These population totals were further adjusted for the suppressive effects of urbanization on malaria transmission²⁴ by identifying all urban areas with populations of more than 1 million. Urban population totals within these pixels were reclassified to the risk class below their original classification; thus, those located in hypoendemic areas were regarded as being at no infection risk. Populations in 2002 residing in the different urban-adjusted, *P. falciparum* endemicity risk zones are shown in Table 1. The endemicity-specific morbid risks were then applied to populations within their respective endemicity classes to estimate numbers of clinical events in 2002 (Table 2).

Received 10 November; accepted 30 December 2004; doi:10.1038/nature03342.

- Hay, S. I., Guerra, C. A., Tatem, A. J., Noor, A. M. & Snow, R. W. The global distribution and population at risk of malaria: past, present and future. *Lancet Infect. Dis.* **4**, 327–336 (2004).
- Snow, R. W., Marsh, K. & le Sueur, D. The need for maps of transmission intensity to guide malaria control in Africa. *Parasitol. Today* **12**, 455–457 (1996).
- Gallup, J. L. & Sachs, J. D. The economic burden of malaria. *Am. J. Trop. Med. Hyg.* **64**, 85–96 (2001).
- Kiszewski, A. *et al.* A global index representing the stability of malaria transmission. *Am. J. Trop. Med. Hyg.* **70**, 486–498 (2004).
- Russell, P. F. World-wide malaria distribution, prevalence and control. *Am. J. Trop. Med. Hyg.* **5**, 937–956 (1956).
- Lysenko, A. J. & Semashko, I. N. in *Itogi Nauki: Medicinskaja Geografija* (ed. Lebedew, A. W.) 25–146 (Academy of Sciences, Moscow, 1968).
- Lysenko, A. J. & Beljaev, A. E. An analysis of the geographical distribution of *Plasmodium ovale*. *Bull. World Health Organ.* **40**, 383–394 (1969).
- Murray, C. J. L. & Lopez, A. D. Mortality by cause for eight regions of the world: global burden of disease study. *Lancet* **349**, 1269–1276 (1997).
- World Health Organization. *The World Health Report 1999: Making a Difference* (World Health Organization, Geneva, 1999).
- Snow, R. W., Craig, M. H., Deichmann, U. & Marsh, K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bull. W.H.O.* **77**, 624–640 (1999).
- Carter, R. & Mendis, K. N. Evolutionary and historical aspects of the burden of malaria. *Clin. Microbiol. Rev.* **15**, 564–594 (2002).
- Mendis, K., Sina, L. J., Marchensini, P. & Carter, R. The neglected burden of *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg.* **64**, 97–106 (2001).
- Murray, C. J. & Lopez, A. D. *Global Health Statistics: a Compendium of Incidence, Prevalence and Mortality Estimates for over 200 Countries* (Harvard School of Public Health, Boston/World Health Organization, Geneva, 1996).
- International Network for the Continuous Demographic Evaluation of Populations and Their Health in Developing Countries. (<http://www.INDEPTH-network.org>).
- Snow, R. W. *et al.* Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* **349**, 1650–1654 (1997).
- Prusty, S. K. R. & Das, B. S. Low incidence of the severe complications of malaria and absence of malaria-specific mortality, in Tensa, Sundergarh district, Orissa State, India, an area hyper-endemic for malaria. *Ann. Trop. Med. Parasit.* **95**, 133–140 (2001).
- Maitland, K. *et al.* Absence of malaria-specific mortality in children in an area of hyperendemic malaria. *Trans. R. Soc. Trop. Med. Hyg.* **91**, 562–566 (1997).
- Alles, H. K., Mendis, K. N. & Carter, R. Malaria mortality rates in South Asia and in Africa: implications for malaria control. *Parasitol. Today* **14**, 369–375 (1998).
- Mayfong, M., Pukrittayakamee, S., Newton, P. N. & White, N. J. Mixed species malaria infections in humans. *Trends Parasitol.* **20**, 233–240 (2004).
- United Nations Development Programme. *Human Development Report 2003. Millennium Development Goals: a Compact among Nations to End Human Poverty* (Oxford Univ. Press, Oxford, 2003).
- Metselaar, D. & Van Theil, P. H. Classification of malaria. *Trop. Geogr. Med.* **11**, 157–161 (1959).
- World Health Organization. *International Travel and Health. Situation as on 1 January 2003* (World Health Organization, Geneva, 2003).
- International Association for Medical Assistance to Travellers. World malaria risk chart (including geographical distribution of principal vectors, geographical distribution of *P. falciparum* malaria, areas where *Plasmodium falciparum* is resistant to chloroquine and guidelines for suppressive medication by country). Status as of 15 March 2004. (<http://www.iamat.org>).

24. Hay, S. I., Guerra, C. A., Tatem, A. J., Atkinson, P. M. & Snow, R. W. Urbanization, malaria transmission and disease burden in Africa. *Nature Rev. Microbiol.* **3**, 81–90 (2005).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements This research was funded by the Wellcome Trust, UK. Part of the work on mapping population risk distributions was funded by the World Health Organization Roll Back Malaria Department. The World Health Organization/RBM Department Monitoring and Evaluation Team and staff from WHO regional offices provided passive case detection data, the WHO Evidence for Information and Policy Department provided SALB data and the WHO Public Health Mapping Group provided other geographical boundary files. We thank K. Marsh, D. Forster, C. Macintosh, K. Maitland and D. Rogers for comments on earlier drafts of the manuscript, D. Balk and G. Yetman for supplying alpha versions of GPW 3.0, and A. Tatem for the urban extractions. R.W.S. is a Wellcome Trust Senior Research Fellow and acknowledges the support of the Kenyan Medical Research Institute (KEMRI). S.I.H. is funded by a Research Career Development Fellowship from the Wellcome Trust. C.A.G. is partially funded by the Fundación para la Ciencia y Tecnología (FUNDACYT).

Competing interests statement The authors declare competing financial interests: details accompany the paper on www.nature.com.

Correspondence and requests for materials should be addressed to R.W.S. (rsnow@wtnairobi.mimcom.net).

Mediation of pathogen resistance by exudation of antimicrobials from roots

Harsh P. Bais¹, Balakrishnan Prithiviraj¹, Ajay K. Jha¹, Frederick M. Ausubel² & Jorge M. Vivanco¹

¹Department of Horticulture and Landscape Architecture, and Center for Rhizosphere Biology, Colorado State University, Fort Collins, Colorado 80523-1173, USA

²Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA

Most plant species are resistant to most potential pathogens. It is not known why most plant–microbe interactions do not lead to disease, although recent work indicates that this basic disease resistance is multi-factorial^{1,2}. Here we show that the exudation of root-derived antimicrobial metabolites by *Arabidopsis thaliana* confers tissue-specific resistance to a wide range of bacterial pathogens. However, a *Pseudomonas syringae* strain that is both at least partly resistant to these compounds and capable of blocking their synthesis/exudation is able to infect the roots and cause disease. We also show that the ability of this *P. syringae* strain to block antimicrobial exudation is dependent on the type III secretory system.

Recent work has shown that the Gram-negative bacterial pathogen *P. syringae* pv. *tomato* strain DC3000 (*Pst* DC3000) infects and colonizes *A. thaliana* roots, causing extensive necrosis and ultimately killing the plants³. In contrast, seven other *P. syringae* strains that were tested (*P. syringae* pv. *phaseolicola* strains NPS3121 and race 6 (*Psp* NPS3121 and *Psp* rc6), *P. syringae* pv. *glycinea* strain A29-2 race 4 (*Psg* A29-2), *P. syringae* pv. *syringae* strain B728a (*Pss* B728a) and *P. syringae* pv. *maculicola* strains ES4326, M₁ and M₄ (*Psm* ES4326, *Psm* M₁ and *Psm* M₄)) did not cause significant root necrosis or plant mortality when inoculated into liquid medium (Fig. 1a and Supplementary Fig. S1) or sterilized planting mix (Figs 1b and 2a, and Supplementary Figs S2A and S3A) in which *Arabidopsis* seedlings were growing. The seven non-pathogenic *P. syringae* strains also colonized *Arabidopsis* roots relatively poorly compared with *Pst* DC3000 (Fig. 2b and Supplementary Figs S3B and S4). One of the non-pathogenic strains, *Psp* NPS3121, has previously been described as an *Arabidopsis* ‘non-host’ pathogen

because it does not cause disease in *Arabidopsis* leaves⁴. However, even though *Psm* ES4326 and *Psm* M₄ failed to cause disease in either of the root infection assays described here, they are just as virulent as *Pst* DC3000 when infiltrated into *Arabidopsis* leaves^{5–7}.

Our previous studies showed that *Arabidopsis* roots exude a variety of antimicrobial compounds^{8,9}. To test whether these compounds confer resistance to the seven non-pathogenic *P. syringae* strains, we repeated the root pathogenicity assays in the presence of activated charcoal, which adsorbs root-exuded secondary compounds^{10,11}. Figures 1c and 2a, b (and Supplementary Figs S2A and S3A, B) show that disease symptoms, plant mortality and root colonization increased markedly in plants inoculated with the seven non-pathogenic *P. syringae* strains in the presence of activated charcoal. Activated charcoal in the absence of the bacterial strains was not toxic to *Arabidopsis* (Fig. 1c and Supplementary Fig. S2A). Washing the activated charcoal off the roots restored resistance to two non-pathogenic strains tested (*Psp* NPS3121 and *Psg* A29-2; Supplementary Fig. S2B). Confocal scanning laser microscopy analysis showed that *Psp* NPS3121 and *Psg* A29-2 efficiently colonized activated charcoal-treated roots (Supplementary Fig. S4). In contrast, *Sinorhizobium meliloti*, a well-characterized non-pathogenic soil bacterium, did not elicit any disease symptoms in the presence of activated charcoal (Fig. 1b, c). These results support the hypothesis that root exudates have a function in rhizosphere-mediated resistance to specific bacterial pathogens.

We used high-performance liquid chromatography (HPLC) to profile low-molecular-mass compounds in root exudates of *Arabidopsis* seedlings that had been infected *in vitro*, to determine whether there is a correlation between the exudation of antimicrobial compounds and resistance to particular *P. syringae* strains. The kinetics of accumulation of a representative antimicrobial compound (butanoic acid) in root exudates from plants infected with *Pst* DC3000 (pathogenic) or *Psp* NPS3121 (representative non-pathogenic strain) compared with non-infected plants is shown in Fig. 3a. The kinetics of accumulation of nine additional antimicrobial compounds (see Table 1 and Supplementary Table S1) as well as the entire chromatographic profiles of root exudates after infection with a variety of *P. syringae* strains are shown in

Supplementary Figs S5–S7. The exudates from plants infected with the non-pathogenic strains *Psp* NPS3121 and *Psg* A29-2 contained more low-molecular-mass compounds than exudates from non-infected plants. At later time points the exudates from plants infected with *Pst* DC3000 contained even lower concentrations of compounds than exudates from non-infected plants (Fig. 3a and Supplementary Figs S5 and S6). Because the same compounds are exuded by *Arabidopsis* roots in both the presence and absence of bacterial infection, it is very unlikely that they are synthesized by the *P. syringae* strains. Much higher concentrations of some of these compounds (up to 800-fold higher for *p*-coumaric acid) were found in the root exudates of plants inoculated with *Psp* N3121 and *Psg* A29-2 than in the exudates of plants inoculated with *Pst* DC3000 (Supplementary Figs S5–S7; see Supplementary Fig. S7F).

We compared the bacteriostatic activity of total root exudates isolated from non-infected plants to exudates isolated from plants infected with *Pst* DC3000, *Psp* NPS3121 or *Psg* A29-2. We also tested the growth inhibitory activity of a cocktail containing a mixture of the ten identified compounds (Supplementary Table S1), as well as the activity of each of the ten individual compounds. The root exudates from non-infected plants had moderate bacteriostatic activity against all seven of the non-pathogenic *P. syringae* strains but did not show growth inhibition against *Pst* DC3000 (Table 1 and Supplementary Fig. S8). The root exudates elicited by *Psp* NPS3121 and *Psg* A29-2 had more bacteriostatic activity against the seven non-pathogenic strains than the exudates from non-infected plants (Table 1 and Supplementary Fig. S8). *Pst* DC3000 had a significant amount of resistance to these latter exudates (Table 1 and Supplementary Fig. S8). Similarly, the cocktail of the ten identified compounds also had bacteriostatic activity against all of the non-infectious strains but not against *Pst* DC3000 (Table 1 and Supplementary Fig. S8). When tested individually, each of the ten identified compounds, with the exception of ferulic acid, had a differential bacteriostatic activity against the seven non-infectious *P. syringae* strains (but not against *Pst* DC3000) at a concentration similar to that found in the exudates of control plants not exposed to microbes (Table 1 and Supplementary Fig. S8). The root exudates from *Arabidopsis* plants infected with *Pst* DC3000 did not contain

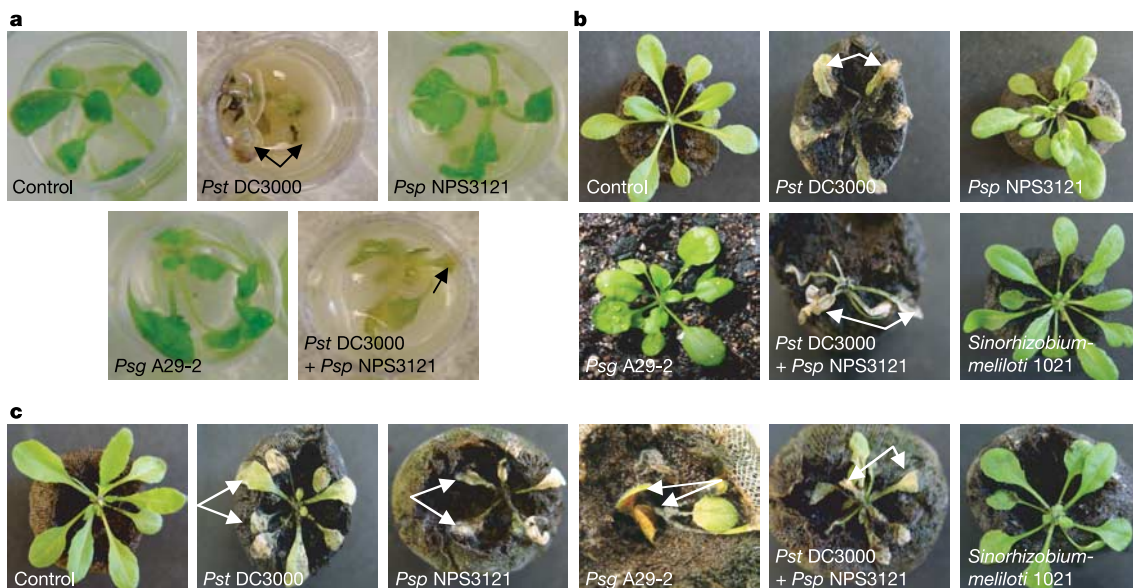


Figure 1 Pathogenicity of various *P. syringae* pathovars against *A. thaliana*. *Arabidopsis* plants grown in liquid medium (**a**), sterilized planting mix (**b**) or sterilized planting mix supplemented with activated charcoal (30 mg per gram of planting mix) (**c**) were infected with the indicated *P. syringae* strains. The appearances of disease symptoms on roots and

leaves infected with specific *P. syringae* strains are described in Supplementary Figs S1 and S2. In all cases, disease symptoms were photographed on the seventh day after inoculation. Experiments were repeated twice, each experiment containing three replicates of 20 plants each. Arrows indicate chlorosis and plant mortality.

significant bacteriostatic activity against any of the *P. syringae* strains used in this study (Table 1 and Supplementary Fig. S8).

These data suggest two potentially additive reasons why *Pst* DC3000 is pathogenic in the root infection assays. First, very low levels of antimicrobial compounds are exuded after infection with *Pst* DC3000, possibly because DC3000 escapes detection by the plant, blocks the synthesis or exudation of antimicrobials or actively degrades them. Second, *Pst* DC3000 is relatively resistant to the antimicrobial compounds, possibly because of inherent resistance, active detoxification or potent efflux systems¹².

It seems unlikely that *Pst* DC3000 simply kills the root cells before they can exude compounds. As shown in Fig. 3 and Supplementary Figs S5–S7, significant amounts of the antimicrobial compounds accumulate by 3 days after infection with the non-pathogenic strain *Psp* NPS3121, at about the time that necrotic lesions first appear on the roots of plants infected with *Pst* DC3000.

We performed co-inoculation experiments with equal mixtures of *Pst* DC3000 and *Psp* NPS3121 or *Pst* DC3000 and *Psg* A29-2 to determine whether *Pst* DC3000 actively blocks the synthesis or exudation of antimicrobial compounds. In both cases the mixed inoculum resulted in disease symptoms (Fig. 1a, b and

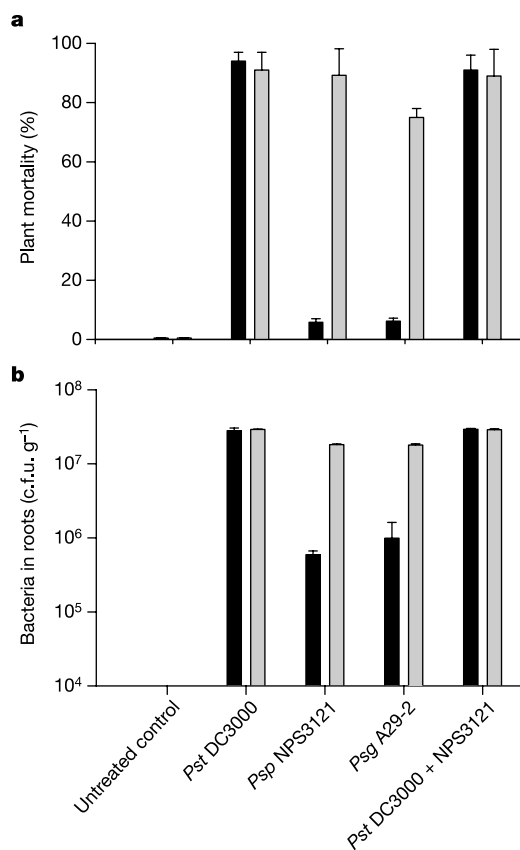


Figure 2 Activated charcoal enhances virulence of non-host pathogens. **a**, Plant mortality at 7 days after infection. Two-way analysis of variance for plant mortality:

$F_{\text{treatment}}=14.12$; $df = 1,56$; $P < 0.005$. **b**, Growth of *P. syringae* in the rhizosphere. Data are averages of two independent experiments. Experiments were conducted in the presence (grey bars) and absence (black bars) of activated charcoal. In panel **b** for the mixed inoculation treatment (*Pst* DC3000 + *Psp* NPS3121), the group bars depict c.f.u. numbers for *Pst* DC3000. The c.f.u. numbers for *Psp* NPS3121 in untreated and activated charcoal treatment under the mixed inoculation regime were about 3.9×10^5 and about 3.1×10^5 bacteria per gram of roots respectively. Error bars indicate s.e.m. Two-way analysis of variance for total bacterial counts: $F_{\text{treatment}}=12.56$; $df = 1,38$; $P < 0.001$.

Supplementary Figs S1 and S2A), plant mortality (Fig. 2a and Supplementary Fig. S3A), proliferation of both of the inoculating *P. syringae* strains in the rhizosphere (Fig. 2b and Supplementary Fig. S3B; data not shown), and a lack of antimicrobial compounds in root exudates (Fig. 3a and Supplementary Figs S6 and S7). These data suggest that *Pst* DC3000 either actively blocks the synthesis or exudation of antimicrobials or actively degrades these compounds.

When root exudates or the ten individual compounds were tested for their ability to inhibit the growth of a mixture of *Pst* DC3000 and *Psp* NPS3121 or *Pst* DC3000 and *Psg* A29-2, strains *Psp* NPS3121 or *Psg* A29-2 were inhibited to levels that were similar to those when tested without *Pst* DC3000, whereas *Pst* DC3000 was able to grow (see Supplementary Methods; data not shown). This makes it unlikely that *Pst* DC3000 is actively causing the degradation of antimicrobial compounds in the exudates.

Additional experiments were performed to determine whether the *Pst* DC3000 type III secretion system (TTSS), which involves the translocation of bacterial-encoded virulence factors directly into host cells, has a function in blocking the synthesis or exudation of antimicrobial compounds. The TTSS is encoded by *hrp* (for hypersensitive response and pathogenesis) and *hrc* (for hypersensitive response conserved) genes¹³ and is required for pathogenicity in a variety of infection assays¹⁴. As shown in Fig. 3b and Supplementary Figs S5 and S6, *Pst* DC3000 *hrcC* and *hrpL* mutants elicited profiles of compounds similar to those generated by *Psp* NPS3121 and *Psg* A29-2 inoculation. The HrcC protein is a structural component of the TTSS machinery. The HrpL protein is required for the transcription of *hrcC* and of most of the *hrp* genes as well as a variety of type III effectors^{15,16} and virulence genes, including genes

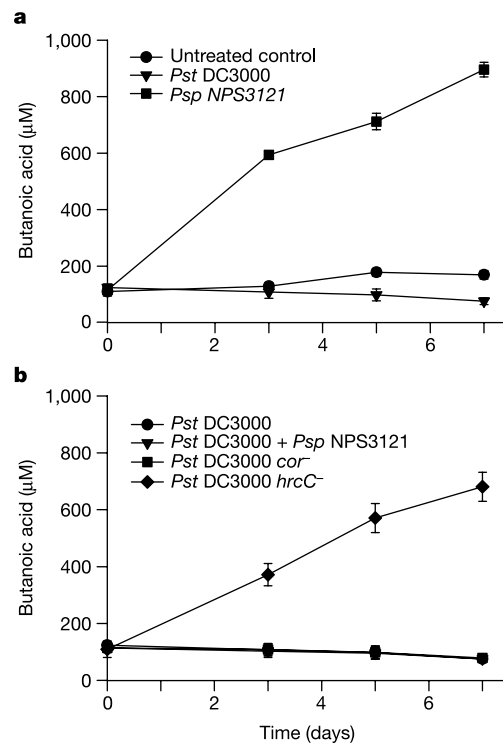


Figure 3 Kinetics of accumulation of a representative antimicrobial compound (butanoic acid) in *Arabidopsis* root exudates. **a**, **b**, *Arabidopsis* plants infected with *Pst* DC3000, *Psp* NPS3121 (representative non-pathogenic strain), a mixture of *Pst* DC3000 and *Psp* NPS3121, *Pst* DC3000 *cor*⁻ or *Pst* DC3000 *hrcC*⁻ are compared with non-infected plants on days 0, 3, 5 and 7 after inoculation. Data are the average of ten independent experiments. Error bars indicate s.e.m. Two-way analysis of variance for each metabolite concentration: $F_{\text{treatment}}=12.41$; $df = 1,58$; $P < 0.001$.

Table 1 Bacteriostatic activities of total root exudates and secondary metabolites isolated from *Arabidopsis* root exudates

Compounds	Bacterial growth inhibition (%)			
	<i>Pst</i> DC3000	<i>Psp</i> NPS3121	<i>Psg</i> A29-2	<i>Pst</i> DC3000 <i>hrc</i> ⁻
Total root exudates (Untreated)	1.52 ± 0.07	84.51 ± 2.52	74.61 ± 4.83	19.22 ± 1.24
Total root exudates (<i>Pst</i> DC3000)	1.54 ± 0.09	2.13 ± 0.09	1.82 ± 0.02	1.41 ± 0.022
Total root exudates (<i>Psp</i> NS3121)	7.13 ± 0.15	91.24 ± 2.81	81.91 ± 3.52	36.91 ± 3.91
Total root exudates (<i>Psg</i> A29-2)	8.91 ± 0.21	95.31 ± 4.12	83.21 ± 4.83	41.82 ± 2.14
Cocktail of compounds listed below	0.82 ± 0.03	78.93 ± 8.94	69.12 ± 7.14	34.91 ± 4.04
1. Butanoic acid	0.74 ± 0.02	46.91 ± 8.02	48.14 ± 12.13	17.12 ± 3.91
2. <i>trans</i> -Cinnamic acid	0.41 ± 0.01	44.21 ± 8.24	22.21 ± 9.13	31.64 ± 4.23
3. <i>o</i> -Coumaric acid	0.33 ± 0.05	51.21 ± 7.14	41.31 ± 3.81	9.83 ± 1.94
4. <i>p</i> -Coumaric acid	0.12 ± 0.03	18.12 ± 4.03	18.24 ± 2.14	16.91 ± 3.82
5. 3-Indolepropanoic acid	0.63 ± 0.04	89.14 ± 11.01	4.13 ± 0.02	28.84 ± 5.21
6. Ferulic acid	0.14 ± 0.04	0	0	14.81 ± 4.12
7. Vanillic acid	0.21 ± 0.04	1.23 ± 0.04	46.61 ± 5.12	11.53 ± 1.52
8. Methyl <i>p</i> -hydroxybenzoate	0.94 ± 0.04	21.23 ± 6.12	7.11 ± 1.14	18.91 ± 2.90
9. <i>p</i> -Hydroxybenzamide	0.73 ± 0.07	91.14 ± 3.41	6.41 ± 1.22	21.84 ± 1.93
10. Syringic acid	1.71 ± 0.08	19.21 ± 6.32	3.21 ± 0.03	19.82 ± 1.82

Net bacterial growth was calculated by subtracting the final OD₆₀₀ from the initial OD₆₀₀ readings after 24 h of incubation. Percentage inhibition was calculated from net bacterial growth based on OD₆₀₀ readings by the following formula: 100 × [(untreated - treated)/untreated]. Results are means ± s.e.m. for five independent experiments with five replicates each. Two-way analysis of variance for percentage inhibition: $F_{\text{treatment}}=13.23$; $df = 1,41$; $P < 0.005$. The concentration of antimicrobial compounds in all the treatments approximates the biological concentration of the same compounds (see Supplementary Figs S5–S7) in uninfected *Arabidopsis* root exudates. A complementary graph with all the non-pathogenic *P. syringae* strains is provided in Fig. 3 and Supplementary Figs S5–S7. The compounds numbered 1–10 here are referred to by these numbers in Supplementary Table S1 and Supplementary Figs S5–S7.

involved in the biosynthesis of the phytotoxin coronatine¹⁷. However, a *Pst* DC3000 mutant deficient in coronatine production elicited disease symptoms (Supplementary Fig. S1) and suppressed the antimicrobial compounds in the root exudates similarly to *Pst* DC3000 (Fig. 3b, Supplementary Figs S5–S7). Moreover, coronatine at concentrations as high as 20 µg ml⁻¹ in the liquid medium failed to suppress—and in fact stimulated—the exudation of antimicrobial compounds (data not shown). These results suggest that the TTSS and perhaps other virulence factors under HrpL control (but not coronatine) are required for blocking the synthesis or exudation of antimicrobial compounds.

The data in Table 1 and Supplementary Fig. S8 show that the *hrcC* and *hrpL* mutants are more susceptible than their *Pst* DC3000 parent to the root exudates from non-infected plants as well as to the exudates elicited by *Psp* NPS3121 and *Psg* 29-2. The *hrcC* and *hrpL* mutants were also more susceptible to the cocktail of ten isolated compounds and the individually administered compounds (Table 1, Supplementary Fig. S8). These data suggest that the TTSS in *P. syringae* is also important in conferring resistance to plant-derived antibiotics.

Many studies have described the existence of preformed and induced low-molecular-mass compounds referred to as phyto-anticipins or phytoalexins, respectively, that have antimicrobial activity^{18–22}. Here we have shown that in addition to constitutive active defences, infection of *Arabidopsis* with a variety of *P. syringae* strains elicits the exudation of significantly higher concentrations of these antimicrobials. The consequence of both the constitutive and inducible production of antimicrobials is that *Arabidopsis* roots are resistant to seven of eight diverse *P. syringae* strains tested. However, the eighth strain, *Pst* DC3000, is a potent *Arabidopsis* root pathogen under our experimental conditions. In *Pst* DC3000, the TTSS not only seems to block the exudation of antimicrobial compounds but also confers at least partial resistance to these compounds. How this occurs awaits further explanation. A note of caution is that *Pst* DC3000 is not generally considered a genuine root pathogen and that further studies are required to determine whether our laboratory observations are relevant to natural habitats where many microbes compete in the rhizosphere. Recently, in results reminiscent of our study, *Magnaporthe grisea*, a fungal leaf pathogen of rice, was also shown to be capable of infecting rice roots²³.

It therefore seems that *Pst* DC3000 has a fail-safe mechanism for overcoming host defences. We suggest that because *Pst* DC3000 is inherently more resistant to exuded antimicrobial compounds, it is

able to proliferate in the rhizosphere and to initiate a root infection. This enables *Pst* DC3000 in a second step to block the further production of antimicrobials by the translocation of type III effectors into root cells. These findings might offer a general explanation for the observation that very few bacterial pathogens are able to cause disease on any particular host plant. □

Methods

Plant and bacterial strains, and growth conditions

Wild-type *Arabidopsis thaliana* ecotype Columbia (Col-0) seeds (Lehle Seeds) were surface-sterilized with sodium hypochlorite (0.3% v/v) and germinated on solidified Murashige and Skoog basal medium (MS)²⁴ in a growth chamber at 25 °C. *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000)²⁵, *P. syringae* pv. *phaseolicola* NPS3121 (*Psp* NPS3121)²⁶, *P. syringae* pv. *glycinea* 29-2 race 4 (*Psg* A29-2)²⁵, *P. syringae* pv. *phaseolicola* race 6 (*Psp* rc6)²⁷, *P. syringae* pv. *syringae* B728a (*Pss* B728a)²⁸ and *P. syringae* pv. *maculicola* strains ES4326 (*Psm* ES4326)⁵, M₁ (*Psm* M₁)⁶ and M₄ (*Psm* M₄)⁷ have been described previously. *Pst* DC3000 *hrpL*⁻ and *Pst* DC3000 *hrcC*⁻ (ref. 16) mutants were obtained from S.-Y. He. Purified coronatine and a coronatine non-producing mutant of *Pst* DC3000 with Tn5 inserted in *cfpA-7* (ref. 29) (strain DB5A6) were obtained from C. Bender. *Sinorhizobium meliloti* 1021 strain was obtained from A. M. Hirsch and was cultured in Luria-Bertani (LB) medium.

Root pathogenicity assays *in vitro* and in planting mix

Arabidopsis seedlings 25 days old, growing in MS medium in 12-well tissue culture plates (see above), were inoculated with *P. syringae* strains that had been grown to an OD₆₀₀ of 0.02–0.04 in LB medium at 28 °C to give an initial dose of about 2.5 × 10⁷ colony-forming units (c.f.u.) ml⁻¹. Twenty plants per treatment were used for the analysis of disease symptoms and mortality rates. Pots of sterilized planting mix, each containing a single 4-week-old *Arabidopsis* plant, were flooded with 10 ml of a *P. syringae* or *S. meliloti* culture grown to an OD₆₀₀ of 0.2–0.4 in LB medium at 28 °C to give an inoculum of about 10⁸ to 5 × 10⁸ c.f.u. per gram of planting mix. Twenty plants per treatment were used for the analysis of mortality rates.

In some experiments, activated charcoal (30 mg per gram of planting mix; Merck) was added to sterilized planting mix by mixing it with water (1.5 g in 5 ml of water for 50 g of planting mix) and pipetting the suspension around 4-week-old *Arabidopsis* rosettes.

Media extraction and HPLC analysis

Liquid medium samples from *Arabidopsis* plants grown *in vitro* with well-differentiated roots were freeze-dried (Genesis 25LL; Virtis), extracted and analysed by HPLC as described previously⁹.

Growth inhibition assays

Broth microdilution antimicrobial susceptibility testing was performed in accordance with published methods of the National Committee for Clinical Laboratory Standards³⁰.

Other protocols

Detailed protocols for growing *Arabidopsis* in liquid MS medium or in planting mix, for performing the root pathogenicity assays in liquid or planting soil, for determining the bacterial colonization of roots, for confocal microscopy, for activated charcoal supplementation of planting mix, for HPLC analysis and for *P. syringae* growth inhibition assays are provided in Supplementary Methods.

Received 13 May 2004; accepted 10 January 2005; doi:10.1038/nature03356.

- Nishimura, M. & Somerville, S. Plant biology. Resisting attack. *Science* **295**, 2032–2033 (2002).
- Thordal-Christensen, H. Fresh insights into processes of nonhost resistance. *Curr. Opin. Plant Biol.* **6**, 351–357 (2003).
- Bais, H. P., Fall, R. & Vivanco, J. M. Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiol.* **134**, 307–319 (2004).
- Yu, G. L., Katagiri, F. & Ausubel, F. M. *Arabidopsis* mutations at the *RPS2* locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Mol. Plant Microbe Interact.* **6**, 434–443 (1993).
- Davis, K. R., Schott, E. & Ausubel, F. M. Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. *Mol. Plant Microbe Interact.* **4**, 477–488 (1991).
- Dong, X., Mindrinos, M., Davis, K. R. & Ausubel, F. M. Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. *Plant Cell* **3**, 61–72 (1991).
- Debener, T., Lehnackers, H., Arnold, M. & Dangl, J. L. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. *Plant J.* **1**, 289–302 (1991).
- Walker, T. S., Bais, H. P., Grotewold, E. & Vivanco, J. M. Root exudation and rhizosphere biology. *Plant Physiol.* **132**, 44–51 (2003).
- Walker, T. S., Bais, H. P., Halligan, K. M., Stermitz, F. R. & Vivanco, J. M. Metabolic profiling of root exudates of *Arabidopsis thaliana*. *J. Agric. Food Chem.* **51**, 2548–2554 (2003).
- Callaway, R. M. & Aschehoug, E. T. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290**, 521–523 (2000).
- Bais, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M. & Vivanco, J. M. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* **301**, 1377–1380 (2003).
- Nikaido, H. Multiple antibiotic resistance and efflux. *Curr. Opin. Microbiol.* **1**, 516–523 (1998).
- Galan, J. E. & Collmer, A. Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* **284**, 1322–1328 (1999).
- Roine, E. *et al.* Hrp pilus: an hrp-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl Acad. Sci. USA* **94**, 3459–3464 (1997).
- Xiao, Y., Heu, S., Yi, J., Lu, Y. & Hutcheson, S. W. Identification of a putative alternate sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. *J. Bacteriol.* **176**, 1025–1036 (1994).
- Zwiesler-Vollick, J. *et al.* Identification of novel hrp-regulated genes through functional genomic analysis of the *Pseudomonas syringae* pv. *tomato* DC3000 genome. *Mol. Microbiol.* **45**, 1207–1218 (2002).
- Bender, C. L., Alarcon-Chaidez, F. & Gross, D. C. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiol. Mol. Biol. Rev.* **63**, 266–292 (1999).
- Dixon, R. A. Natural products and plant disease resistance. *Nature* **411**, 843–847 (2001).
- Papadopoulou, K., Melton, R. E., Leggett, M., Daniels, M. J. & Osbourn, A. E. Compromised disease resistance in saponin-deficient plants. *Proc. Natl Acad. Sci. USA* **96**, 12923–12928 (1999).
- Haralampidis, K. *et al.* A new class of oxidosqualene cyclases directs synthesis of antimicrobial phytoprotectants in monocots. *Proc. Natl Acad. Sci. USA* **98**, 13431–13436 (2001).
- Reimann, C. & VanEtten, H. D. Cloning and characterization of the PDA6–1 gene encoding a fungal cytochrome P-450 which detoxifies the phytoalexin pisatin from garden pea. *Gene* **146**, 221–226 (1994).
- Bouarab, K., Melton, R., Peet, J., Baulcombe, D. & Osbourn, A. A saponin-detoxifying enzyme mediates suppression of plant defenses. *Nature* **418**, 889–892 (2002).
- Sesma, A. & Osbourn, A. E. The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature* **431**, 582–586 (2004).
- Murashige, T. & Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**, 473–497 (1962).
- Whalen, M. C., Innes, R. W., Bent, A. F. & Staskawicz, B. J. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *Plant Cell* **3**, 49–59 (1991).
- Lindgren, P. B., Peet, R. C. & Panopoulos, N. J. Gene cluster of *Pseudomonas syringae* pv. 'phaseolicola' controls pathogenicity of bean plants and hypersensitivity of nonhost plants. *J. Bacteriol.* **168**, 512–522 (1986).
- Fillingham, A. J. *et al.* Avirulence genes from *Pseudomonas syringae* pathovars *phaseolicola* and *pisi* confer specificity towards both host and non-host species. *Physiol. Mol. Plant Pathol.* **40**, 1–15 (1992).
- Rich, J. J., Hirano, S. S. & Willis, D. K. Pathovar-specific requirement for the *Pseudomonas syringae lemA* gene in disease lesion formation. *Appl. Environ. Microbiol.* **58**, 1440–1446 (1992).
- Brooks, D. M. *et al.* Identification and characterization of a well-defined series of coronatine biosynthetic mutants of *Pseudomonas syringae* pv. *tomato* DC3000. *Mol. Plant Microbe Interact.* **17**, 162–174 (2004).
- National Committee for Clinical Laboratory Standards (NCCLS). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard M7–A6 5th edn* (NCCLS, Wayne, Pennsylvania, USA, 2003).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank W. Songnuan and J. Dangl for critical reading of the manuscript, and E. Wortman-Wunder for editing suggestions. This work was supported by the Colorado State University Agricultural Experiment Station (J.M.V.) and by NIH and NSF grants to F.M.A. J.M.V. is a NSF-CAREER Faculty Fellow.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to J.M.V. (j.vivanco@colostate.edu) or F.M.A. (ausubel@molbio.mgh.harvard.edu).

Spike-timing-dependent synaptic plasticity depends on dendritic location

Robert C. Froemke, Mu-ming Poo & Yang Dan

Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley, California 94720-3200, USA

In the neocortex, each neuron receives thousands of synaptic inputs distributed across an extensive dendritic tree. Although postsynaptic processing of each input is known to depend on its dendritic location^{1–8}, it is unclear whether activity-dependent synaptic modification is also location-dependent. Here we report that both the magnitude and the temporal specificity of spike-timing-dependent synaptic modification^{9–17} vary along the apical dendrite of rat cortical layer 2/3 pyramidal neurons. At the distal dendrite, the magnitude of long-term potentiation is smaller, and the window of pre-/postsynaptic spike interval for long-term depression (LTD) is broader. The spike-timing window for LTD correlates with the window of action potential-induced suppression of NMDA (N-methyl-D-aspartate) receptors; this correlation applies to both their dendritic location-dependence and pharmacological properties. Presynaptic stimulation with partial blockade of NMDA receptors induced LTD and occluded further induction of spike-timing-dependent LTD, suggesting that NMDA receptor suppression underlies LTD induction. Computer simulation studies showed that the dendritic inhomogeneity of spike-timing-dependent synaptic modification leads to differential input selection at distal and proximal dendrites according to the temporal characteristics of presynaptic spike trains. Such location-dependent tuning of inputs, together with the dendritic heterogeneity of postsynaptic processing, could enhance the computational capacity of cortical pyramidal neurons.

Whole-cell recordings were made from layer 2/3 pyramidal neurons in rat cortical slices¹⁴. To activate synapses selectively at each dendritic location, two extracellular stimulating electrodes were placed <10 μm away from the apical dendrite, one <50 μm from the soma ('proximal') and one >100 μm from the soma ('distal', Fig. 1a). Both electrodes reliably evoked excitatory postsynaptic potentials (EPSPs) from separate populations of synapses, as indicated by linear EPSP summation^{3,7} (Fig. 1b) and the absence of paired-pulse depression between the inputs. To induce long-term synaptic modification, postsynaptic action potentials (APs) were paired with presynaptic stimulation of one input (60 pairs, 0.2 Hz). At the paired input, pre → post pairing (presynaptic stimulation followed by postsynaptic stimulation) induced long-term potentiation (LTP) and post → pre pairing (postsynaptic stimulation followed by presynaptic stimulation) induced long-term depression (LTD) (Fig. 1c), consistent with spike-timing-dependent plasticity (STDP) of these synapses¹⁴. Interestingly, there are quantitative differences in the temporal window for proximal and distal inputs (Fig. 1d). Although the magnitude of LTP was smaller distally than proximally, the main difference was in the width of the LTD window, with the distal window markedly broader than the proximal window. In particular, with a pre-/postsynaptic spike interval of $-100 < \Delta t < -50$ ms (Fig. 1d, dotted box), LTD was absent at proximal synapses ($-1.9 \pm 2.7\%$, $n = 8$; $P > 0.2$, t -test), but was still observed at distal synapses ($-22.6 \pm 3.4\%$, $n = 13$, $P < 0.00001$). This difference in the LTD window was also found with the GABA_A receptor antagonist picrotoxin (10 μM) in the bath (see Supplementary Fig. 1).

Induction of LTP by pre → post pairing may depend on supra-linear summation of EPSPs and back-propagating APs^{9,15,18}. The