Pseudomonas sax Genes Overcome Aliphatic Isothiocyanate–Mediated Non-Host Resistance in *Arabidopsis*

Jun Fan,[1]*† Casey Crooks,[1,2]† Gary Creissen,[1] Lionel Hill,[1] Shirley Fairhurst,[1] Peter Doerner,[3,4]* Chris Lamb[1]‡

Most plant-microbe interactions do not result in disease; natural products restrict non-host pathogens. We found that sulforaphane (4-methylsulfinylbutyl isothiocyanate), a natural product derived from aliphatic glucosinolates, inhibits growth in *Arabidopsis* of non-host *Pseudomonas* bacteria in planta. Multiple *sax* genes (*saxCAB/F/D/G*) were identified in *Pseudomonas* species virulent on *Arabidopsis*. These *sax* genes are required to overwhelm isothiocyanate-based defenses and facilitate a disease outcome, especially in the young leaves critical for plant survival. Introduction of *saxCAB* genes into non-host strains enabled them to overcome these *Arabidopsis* defenses. Our study shows that aliphatic isothiocyanates, previously shown to limit damage by herbivores, are also crucial, robust, and developmentally regulated defenses that underpin non-host resistance in the *Arabidopsis-Pseudomonas* pathosystem.

Non-host resistance is the ability of most plant species to resist microbes or viruses that are successful pathogens on other plants. It is the most prevalent form of plant disease resistance, is durable and effective against a broad range of potential pathogens, but our understanding of its molecular basis is still poor (*1*–*3*). Plants generate a huge diversity of natural products, with multiple roles in defense, communication, and development (*4*). Preformed natural products provide chemical barriers to phytopathogenic fungi (*5*–*7*) and are deterrents in plant-herbivore interactions (*8*). However, their role in restricting bacterial host range remains obscure, as do the bacterial mechanisms involved in breaching natural product–mediated host defenses. To better understand fundamental host-pathogen biology and to inform the development of sustainable field resistance to major crop diseases (*9*), we sought to define plant components conferring non-host resistance as well as strategies used by virulent pathogens to overcome resistance barriers.

We observed that extracts from naïve *Arabidopsis* plants inhibited the growth of most pathovars of *Pseudomonas syringae* for which *Arabidopsis* is not a host. In contrast, *P. syringae* pathovars *maculicola* (*Psm*) ES4326 and *tomato* (*Pst*) DC3000, which are pathogenic on *Arabidopsis*, grew well on rich media supplemented with host extract from Col-0 (Table 1) or other accessions (table S1). Using *Arabidopsis–P. syringae* as a model system to dissect plant resistance to non-host pathogens, we screened a *Psm* ES4326 genomic library for genes conferring resistance in *Escherichia coli* to *Arabidopsis* extracts (Table 1) (*10*). A single operon, designated *sax* (survival in *Arabidopsis* extracts), allowed *E. coli* to grow on *Arabidopsis* extracts. In *E. coli*, *saxA* and *saxC* are together necessary for resistance: The absence of either resulted in susceptibility, whereas the absence of *saxB* reduced bacterial growth on extracts (fig. S1A). SaxA has a predicted secretory signal peptide (*11*) and, although related to class B β-lactamases (*12*, *13*), it is unable to confer resistance to eight representative β-lactam antibiotics (fig. S2A), indicating that SaxA activity is distinct. SaxB is related to isochorismatase. SaxC is a highly conserved member of the AraC/XylS family of transcriptional regulators found in diverse prokaryotes and involved in carbon metabolism, stress response, and pathogenesis (*14*). Analysis of 35 plant-associated *P. syringae* genomes showed that only *Arabidopsis* pathogens have *saxA*-like genes with ≥90% nucleic acid sequence identity to *Psm* ES4326 *saxA* (table S2 and fig. S3).

Non-host resistance is durable, and hence it is unlikely to be overwhelmed by a single mechanism. Indeed, deletion of *saxAB* genes in *Pst* DC3000 or *Psm* ES4326 had little impact on bacterial growth in *Arabidopsis* extracts (Table 1). To identify additional protective mechanism(s), we screened for compromised bacterial growth in *Arabidopsis* extracts after transposon mutagenesis of *PstΔsaxAB* (*10*). Two putative multidrug resistance (MDR) efflux genes were identified, with a third similar system revealed in *Pst* DC3000 by genome analysis (*10*); these were designated *saxF*, *saxD*, and *saxG*, respectively (fig. S1B). They form a subgroup among the nine resistance-nodulation-division (RND) efflux systems predicted in the *Pst* DC3000 genome (*15*), which function to extrude a wide range of substrates including antibiotics and host-derived molecules (*16*). We sequentially deleted *saxF/D/G* from the *PstΔsaxAB* background, which resulted in progressively increased sensitivity (Table 1); hence, these genes were required for robust resistance to *Arabidopsis* extracts. Deletion of *saxAB*, *saxF/D/G*, or *saxAB/F/D/G* did not impair growth in rich medium (Fig. 1A), indicating that they are not essential. Thus, *sax* genes have distinct but complementary roles in *Pseudomonas* resistance to *Arabidopsis* extracts.

We purified the *Arabidopsis* antimicrobial compound restricting non-host *Pseudomonas*

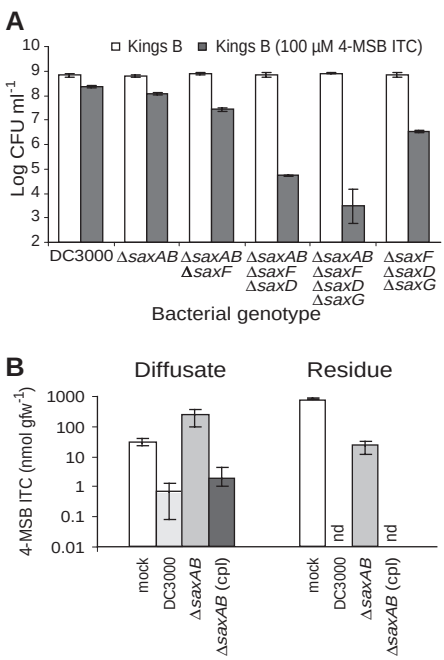

Fig. 1. *sax* genes in *Pst* DC3000 protect against *Arabidopsis*-derived isothiocyanates. (**A**) *sax* genes are synergistically required for bacterial resistance to isothiocyanates. Bacterial strains were inoculated into Kings B medium with or without sulforaphane and grown overnight. (**B**) Bacterial infection released sulforaphane (4-MSB ITC) into apoplastic diffusates. *Arabidopsis* leaf discs were vacuum-infiltrated with bacteria [optical density at 600 nm (OD_{600}) = 0.1] and incubated at 23°C for 48 hours before analysis. Data are means ± SD; cpl, episomal complementation with the *sax* operon; gfw, gram fresh weight; nd, not detected.

[1]John Innes Centre, Norwich NR4 7UH, UK. [2]USDA Forest Products Laboratory, 1 Gifford Pinchot Drive, Madison, WI 53726, USA. [3]Institute of Molecular Plant Sciences, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, UK. [4]Laboratoire de Physiologie Cellulaire Végétale, CNRS, CEA, INRA, and Université Joseph Fourier, F-38000 Grenoble, France

*To whom correspondence should be addressed. E-mail: jun.fan@bbsrc.ac.uk (J.F.); peter.doerner@ed.ac.uk (P.D.)
†These authors contributed equally to this work.
‡Deceased.

from Col-0 extracts (*10*) and identified it as sulforaphane [4-methylsulfinylbutyl isothiocyanate (4-MSB ITC)] (fig. S5). Aliphatic isothiocyanates are breakdown products derived from glucosinolates, a class of sulfur- and nitrogen-containing natural products widely distributed in crucifers; *Arabidopsis* plants produce a variety of glucosinolates, with extensive variation in leaf and root content across different accessions (*17*). In Col-0, 4-MSB glucosinolate predominantly accumulates in rosette leaves (*18*); however, *Arabidopsis* plants with loss-of-function alleles of MYB28 and MYB29 transcription factors, but not Col-0 plants transformed with 35S::*saxA*, lack aliphatic glucosinolates (*19*, *20*) (table S3). A panel of virulent and non-host bacteria showed a higher median inhibitory concentration (IC_{50}) for sulforaphane in the former (table S5).

Antimicrobial activity of sulforaphane in vitro has been reported (*21*), but its role in disease resistance in planta is unknown. We tested the protective effect of the *sax* operon against a panel of isothiocyanates derived from glucosinolates prevalent in *Arabidopsis* and other crucifers. *E. coli* carrying the *sax* operon was more tolerant than the vector control to all five tested compounds (fig. S2B), indicating that *saxCAB* genes are active against a broad spectrum of isothiocyanates. Deletion of *saxAB*, *saxF/D/G*, or *saxAB/F/D/G* progressively impaired growth in rich medium supplemented with sulforaphane, or in extracts from *myb28/29* plants supplemented with sulforaphane, but not in extracts from Col-0 plants transgenic for 35S::*saxA* (Fig. 1A and Table 1); these findings indicate that SaxA is sufficient for detoxification of sulforaphane and other aliphatic isothiocyanates (Fig. 2A and fig. S2B). Treatments with *Arabidopsis* extracts or with sulforaphane induced *saxA* and *saxF* expression (fig. S4). These findings indicate a broader role for aliphatic isothiocyanates in plant-biotic interactions than previously understood.

Glucosinolate breakdown is activated by tissue damage after herbivory; the subsequent accumulation of aliphatic isothiocyanates is a major determinant of plant-herbivore interactions (*20*, *22*, *23*). However, bacterial infections do not normally cause similar tissue damage. We examined whether infection released isothiocyanates into the apoplastic space that hemibiotrophic bacteria colonize (*10*). Relative to mock treatments, sulforaphane levels were reduced in diffusate and residue samples treated with wild-type *Pst* DC3000, whereas infection with the *Pst*Δ*saxAB* strain increased sulforaphane levels in diffusate (Fig. 1B). Thus, *Pst* DC3000 infection triggers glucosinolate degradation and release of isothiocyanates into the apoplast, and this *Arabidopsis* response is countered by *Pst* DC3000 in a *sax*-dependent manner.

To determine whether the isothiocyanate resistance of *Pst* DC3000 plays an important role in pathogenesis on *Arabidopsis*, we monitored bacterial growth after infiltration of *Pst* DC3000 or *saxAB/F/D/G*. Consistent with a previous report (*24*), we found that sulforaphane levels in apoplastic diffusates of young leaf discs were higher by a factor of ~100 than in old leaf discs infected with the *Pst*Δ*saxAB* strain (Fig. 3A), which suggests differences in their glucosinolate-mediated

Table 1. Inhibitory activity of *Arabidopsis* extracts on bacterial growth in vitro. Bacterial growth was determined 24 hours after inoculation, or as indicated otherwise. Data are means ± SD. *Pst* strains are from this study; other strain sources are as indicated. Strains ES4326, M1, M2, M3, M5, M6, and DC3000 grow on crucifers. "With ITC" denotes *myb* mutant plant extract supplemented with authentic sulforaphane (4-MSB ITC) at a concentration of 200 μg ml^{-1}. Statistical significance of growth inhibition (rightmost column): **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (two-tailed *t* test; other values are not significant).

Bacterial strain and source	log(CFU ml^{-1})				
	Col-0	35S::*saxA*	*myb28-1/myb29-1*		
			Without ITC	With ITC	Inhibition
P. syringae pathovars					
pv. *maculicola* ES4326*	8.9 ± 0.05	9.1 ± 0.10	9.2 ± 0.03	7.6 ± 0.08	1.6**
pv. *maculicola* M1†	9.3 ± 0.10	9.2 ± 0.05	9.3 ± 0.10	9.1 ± 0.18	0.2
pv. *maculicola* M2†	8.5 ± 0.06	8.9 ± 0.16	8.8 ± 0.07	8.6 ± 0.12	0.2
pv. *maculicola* M3†	8.8 ± 0.09	9.2 ± 0.13	9.0 ± 0.01	7.8 ± 0.09	1.2**
pv. *maculicola* M5†	8.1 ± 0.07	7.4 ± 0.02	7.0 ± 0.03	7.0 ± 0.10	0
pv. *maculicola* M6†	9.2 ± 0.11	9.4 ± 0.19	9.2 ± 0.27	9.3 ± 0.03	−0.1
pv. *tomato* DC3000*	8.9 ± 0.03	9.0 ± 0.07	8.6 ± 0.10	7.9 ± 0.05	0.7*
pv. *tomato* T1‡	4.3 ± 0.07	8.9 ± 0.06	8.7 ± 0.05	3.5 ± 0.33	5.2**
pv. *apii* ATCC9654*	7.2 ± 0.08	9.3 ± 0.24	9.1 ± 0.17	4.5 ± 0.05	4.6***
pv. *coronafaciens* KN221‡	8.5 ± 0.13	9.5 ± 0.10	9.4 ± 0.00	7.2 ± 0.34	2.2*
pv. *glycinea* race 4*	5.5 ± 0.20	7.7 ± 0.00	7.5 ± 0.11	4.3 ± 0.03	3.2***
pv. *glycinea* race 5*	6.4 ± 0.16	7.5 ± 0.12	7.4 ± 0.16	5.4 ± 0.07	2.0**
pv. *lachrymans* ATCC 7386*	6.9 ± 0.01	8.6 ± 0.07	8.3 ± 0.03	<3.3§	5.0***
pv. *phaseolicola* 1448A‡	8.8 ± 0.08	9.5 ± 0.03	9.3 ± 0.12	7.7 ± 0.09	1.6**
pv. *syringae* B728A†	7.7 ± 0.07	9.5 ± 0.03	9.5 ± 0.02	6.3 ± 0.17	3.2**
pv. *syringae* PSC1B‡	3.3 ± 0.10	7.3 ± 0.11	7.0 ± 0.10	4.5 ± 0.08	2.5**
pv. *tabaci* 6605†	5.4 ± 0.53	8.8 ± 0.03	8.5 ± 0.30	5.7 ± 0.11	2.8**
Xanthomonas spp.					
X. perforans race 4†	8.0 ± 0.30	9.2 ± 0.09	8.9 ± 0.01	<3.3§	5.6***
X. campestris pv. *vesicatoria* 69-1†	6.0 ± 0.06	8.7 ± 0.13	8.4 ± 0.03	<3.3§	5.1***
E. coli					
DH5α*	4.6 ± 0.08	8.9 ± 0.21	9.2 ± 0.14	4.7 ± 0.10	4.5***
Pst-derived mutants and transformants‖					
ΔsaxAB	8.7 ± 0.07	9.0 ± 0.04	8.8 ± 0.05	7.5 ± 0.15	1.3**
ΔsaxF/D/G	<3.3§	9.0 ± 0.10	8.8 ± 0.04	<3.3§	5.5***
ΔsaxF/D/G (*saxF*)	8.8 ± 0.15	8.9 ± 0.08	8.7 ± 0.12	8.1±.0.2	0.6*
ΔsaxAB/F/D/G	<3.3§	9.0 ± 0.12	9.0 ± 0.00	<3.3§	5.4***
ΔsaxAB/F/D/G (*saxF+saxCAB*)	8.9 ± 0.11	8.8 ± 0.16	8.8 ± 0.31	8.9 ± 0.03	−0.1
T1	6.0 ± 0.07	8.8 ± 0.12	8.2 ± 0.27	3.5 ± 0.21	4.7**
T1 (*saxCAB*)	8.7 ± 0.22	8.8 ± 0.11	8.0 ± 0.21	8.7 ± 0.12	−0.7

*Strain from Lamb lab stock. †Strain from C. Zipfel. ‡Strain from K. Sohn. §No colony was detected from 1/10 dilution of the culture. ‖Bacterial growth of these strains was determined at 48 hours, except for *ΔsaxAB*.

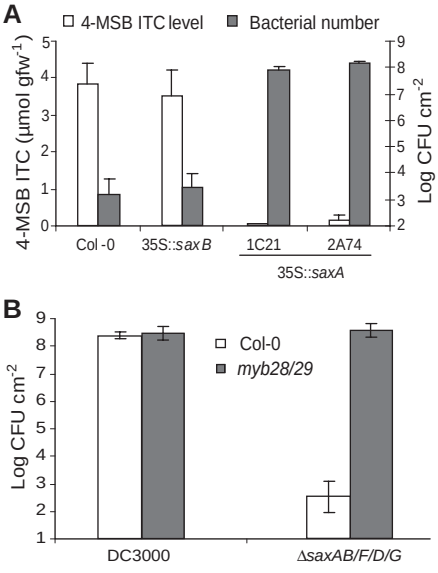

Fig. 2. Accumulation of aliphatic glucosinolates and isothiocyanates is necessary for suppressing the quintuple *sax* mutant in young *Arabidopsis* leaf tissue. (**A**) Representative *Arabidopsis* transgenic plants expressing the bacterial *saxA* gene fail to produce high levels of sulforaphane (4-MSB ITC) in crude extracts and are fully susceptible to the quintuple *sax* mutant. (**B**) Disruption of aliphatic glucosinolate biosynthesis abolished resistance to the quintuple *sax* mutant in young *Arabidopsis* leaf tissue. In (A) and (B), discs from young rosette leaves were treated as described (Fig. 1B) and bacterial titer analyzed 3 days after inoculation. Data are means ± SD.

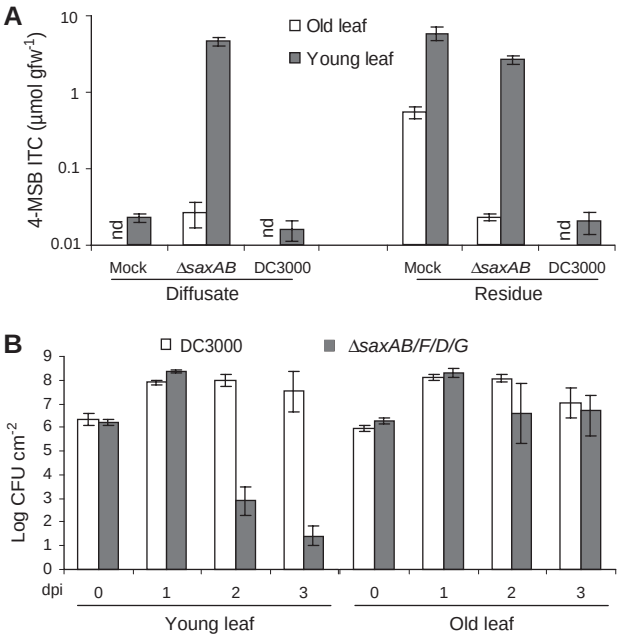

Fig. 3. *sax* genes are required for *Pst* DC3000 survival during infection of young *Arabidopsis* leaf tissue releasing high levels of sulforaphane (4-MSB ITC). (**A**) Levels of 4-MSB ITC differed significantly between young and old *Arabidopsis* leaves. (**B**) The quintuple *sax* mutant is unable to maintain infection on young *Arabidopsis* leaf tissue. The conditions for these bacterial infection experiments were the same as described in Fig. 1B. Data are means ± SD; dpi, days post-inoculation.

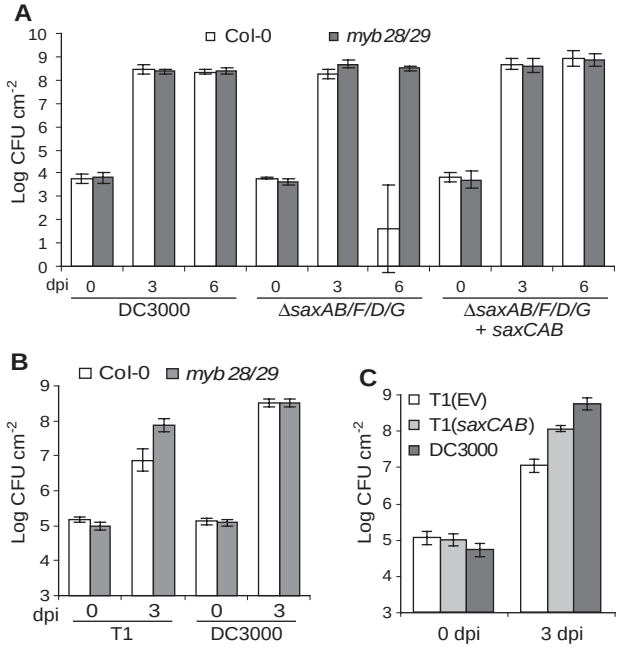

Fig. 4. Aliphatic isothiocyanates restrict bacterial growth in planta. (**A**) Young leaves of Col-0 and *myb28/29* plants were syringe-infiltrated with bacteria (OD_{600} = 0.001). (**B**) Aliphatic glucosinolate biosynthesis is required for growth suppression of the non-host *Pst* T1 strain. (**C**) The *saxCAB* operon promoted growth of *Pst* T1 on Col-0 plants. In (B) and (C), young leaves of 5- to 6-week-old plants were infiltrated with bacteria (OD_{600} = 0.02). Data are means ± SD; EV, empty vector control.

defense capacity. Relative to wild-type *Pst* DC3000, bacterial titer was reduced by six orders of magnitude in the Δ*saxAB/F/D/G* strain in the young but not the old leaf discs (Fig. 3B). In planta assays with much lower inoculum density (Fig. 4A) or when inoculated by spraying (fig. S7) produced similarly reduced levels of growth of the Δ*saxAB/F/D/G* strain. Thus, *sax* genes are important for *Pst* DC3000 to penetrate host barriers and maximize its population in the young leaves.

We then examined transgenic *Arabidopsis* plants overexpressing *sax* genes to examine whether *sax*-dependent reactions are sufficient to negate bacterial restriction by the host. The levels of major glucosinolates were unchanged in the *saxA*-expressing transgenic plants (table S3), suggesting that SaxA targets steps after glucosinolate breakdown. Sulforaphane levels in extracts of young leaves from *saxA* transgenic plants were lower than those from wild-type Col-0 and *saxB* transgenic plants by a factor of >20 (Fig. 2A). Growth inhibition of the *ΔsaxAB/F/D/G* mutant observed in leaves from Col-0 plants was lost in *saxA* transgenic plants (Fig. 2A) and in the *myb28/29* background (Fig. 2B, Fig. 4A, and fig. S7). These observations indicate that aliphatic isothiocyanates are potent host defense effectors that restrict bacterial populations. However, we also observed that the titer of the quintuple *sax* mutant did not fall until necrotic symptoms had developed, implying that the isothiocyanate-mediated defense might be potentiated by cell death in the host.

To examine the broader role of aliphatic isothiocyanates in restricting non-host pathogens, we tested an expanded panel of crucifer and non-crucifer pathogens (Table 1) with *Arabidopsis* extracts. In extracts prepared from wild-type Col-0 plants, all the crucifer pathogens tested were able to grow well, whereas the titer of the noncrucifer strains varied between $10^{3.3}$ and $10^{8.8}$ CFU/ml and showed a clear trend of growth inhibition. However, when grown in extracts from *myb28/29* or *35S::saxA* plants, growth inhibition was attenuated in the noncrucifer pathogens. After supplementing extracts of *myb28/29* plants with sulforaphane (Table 1) or iberin (3-methylsulfinylpropyl isothiocyanate) (table S4), restriction of noncrucifer pathogens was restored, whereas we observed only small growth differences of the crucifer pathogens between different treatments. Hence, aliphatic isothiocyanates are essential for *Arabidopsis* extracts to restrict the growth of many noncrucifer bacterial pathogens.

We examined whether *saxCAB* was sufficient to allow non-host pathogens to overcome *Arabidopsis* aliphatic isothiocyanate–dependent defenses. A tomato isolate of *P. syringae*, *Pst* T1, which is not pathogenic in *Arabidopsis* (*25*, *26*), does not grow on *Arabidopsis* extracts (Table 1). *saxA* is absent from a homologous region of the *Pst* T1 genome (fig. S6A), whereas its *sax*-related efflux systems are ≥99% identical to those in *Pst* DC3000 (fig. S6B). When challenged with *Pst* T1, young leaves of *myb28/29* plants supported ~10 times as much bacterial growth as did Col-0 (Fig. 4B). Transformation of *Pst* T1 with *Psm* ES4326 *saxCAB* rescued *Pst* T1 growth in *Arabidopsis* extracts containing sulforaphane (fig. S8) and enhanced growth in young Col-0 leaves by a factor of ~10 (Fig. 4C). Similar results were obtained with the non-host pathovar *Ps apii* (ATCC 9654) (Table 1 and fig. S9). We conclude that isothiocyanate-mediated defense effectively limits infection by non-host pathogenic *Pseudomonas* bacteria and that *sax* genes strongly enhance *Pseudomonas* virulence in *Arabidopsis*.

Indolic glucosinolates are required for *Arabidopsis* to prevent penetration of non-adapted powdery mildew strains (*27*) and to activate callose deposition as an innate immune response (*28*). Our findings show that *Pseudomonas* pathogens require *sax* gene–dependent mechanisms to overwhelm aliphatic isothiocyanate–mediated non-host resistance in *Arabidopsis*. This and other work (*27*, *28*) shows how non-host resistance, unlike many examples of major *R* gene resistance to specific pathogen genotypes, can be resilient, durable, and broadly effective against many potential pathogens, thereby revealing emergent principles for engineering sustainable field resistance for major crop diseases.

References and Notes

1. M. C. Heath, *Curr. Opin. Plant Biol.* **3**, 315 (2000).
2. K. S. Mysore, C. M. Ryu, *Trends Plant Sci.* **9**, 97 (2004).
3. T. Nürnberger, V. Lipka, *Mol. Plant Pathol.* **6**, 335 (2005).
4. P. Bednarek, A. Osbourn, *Science* **324**, 746 (2009).

5. K. Papadopoulou *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12923 (1999).
6. J. D. Hipskind, N. L. Paiva, *Mol. Plant Microbe Interact.* **13**, 551 (2000).
7. R. W. Sandrock, H. D. Vanetten, *Physiol. Mol. Plant Pathol.* **58**, 159 (2001).
8. R. N. Bennett, R. M. Wallsgrove, *New Phytol.* **127**, 617 (1994).
9. J. Ellis, *Plant Cell* **18**, 523 (2006).
10. See supporting material on *Science* Online.
11. J. D. Bendtsen, H. Nielsen, G. von Heijne, S. Brunak, *J. Mol. Biol.* **340**, 783 (2004).
12. J. Y. Shi, T. L. Blundell, K. Mizuguchi, *J. Mol. Biol.* **310**, 243 (2001).
13. H. Daiyasu, K. Osaka, Y. Ishino, H. Toh, *FEBS Lett.* **503**, 1 (2001).
14. M. T. Gallegos *et al*., *Microbiol. Mol. Biol. Rev.* **61**, 393 (1997).
15. C. R. Buell *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **100**, 10181 (2003).
16. J. M. Blair, L. J. Piddock, *Curr. Opin. Microbiol.* **12**, 512 (2009).
17. U. Wittstock, B. A. Halkier, *Trends Plant Sci.* **7**, 263 (2002).
18. B. L. Petersen, S. X. Chen, C. H. Hansen, C. E. Olsen, B. A. Halkier, *Planta* **214**, 562 (2002).
19. I. E. Sønderby *et al*., *PLoS ONE* **2**, e1322 (2007).
20. J. Beekwilder *et al*., *PLoS ONE* **3**, e2068 (2008).
21. K. F. M. J. Tierens *et al*., *Plant Physiol.* **125**, 1688 (2001).
22. A. A. Agrawal, N. S. Kurashige, *J. Chem. Ecol.* **29**, 1403 (2003).
23. U. Wittstock *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4859 (2004).
24. P. D. Brown, J. G. Tokuhisa, M. Reichelt, J. Gershenzon, *Phytochemistry* **62**, 471 (2003).
25. M. C. Whalen, R. W. Innes, A. F. Bent, B. J. Staskawicz, *Plant Cell* **3**, 49 (1991).
26. N. F. Almeida *et al*., *Mol. Plant Microbe Interact.* **22**, 52 (2009).
27. P. Bednarek *et al*., *Science* **323**, 101 (2009); 10.1126/science.1163732.
28. N. K. Clay, A. M. Adio, C. Denoux, G. Jander, F. M. Ausubel, *Science* **323**, 95 (2009); 10.1126/science.1164627.

29. We thank D. Haas, C. Zipfel, K. Sohn, and D. J. Kliebenstein for providing the pME vectors, bacterial strains, and *Arabidopsis myb* mutants used in this study; B. Lee and S. Kopriva for help with the glucosinolate assay; and C. Zipfel, C. Dean, A. Maule, and V. Vitart for critical reading of earlier versions of the manuscript. Supported by a grant from the UK Biotechnology and Biological Sciences Research Council (C.L.). J.F., C.C., G.C., L.H., S.F., and P.D. dedicate this paper to the memory of Chris Lamb.

Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6021/1185/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 to S6
References

28 October 2010; accepted 24 January 2011
10.1126/science.1199707