How plants defend themselves

David Guest

Professor of Plant Pathology
University of Sydney, NSW
david.guest@sydney.edu.au

Unlike animals, plants cannot flee, fight or hide from predators. Plants lack mobile defender cells or an adaptive immune system and have instead evolved defences based on pre-formed barriers and inducible cellular responses regulated by local and systemic signals. The interaction between pathogen effectors and these defences sets up an intriguing molecular arms race between plants and pathogenic fungi, bacteria, viruses, viroids and nematodes.

Plant surfaces present formidable physical and chemical barriers as the first line of defences against pathogens. Waxy cuticles, cellulosic or lignified cell walls and antimicrobial phytoanticipins and defensins exclude most microbes. In a classic experiment, JC Walker and colleagues showed that brown onions resist smudge disease, caused by the fungus *Colletotrichum circinans,* because of the antifungal phenolic protocatechuic acid in their dry outer leaves[1]. White onions, lacking these compounds, are susceptible. Following damage or infection, plant metabolites present in inactive forms may be converted to more inhibitory substances, including glucosinolates, cyanides and toxic quinones[2]. Avenacins, triterpenoid saponins found in the outer cortex of oat roots, confer resistance to take-all caused by non-specific races of *Gauemannomyces graminis*[3]. The virulent oat-specific pathogen *G. graminis* var. *avenae* produces the detoxifying enzyme avenacinase, and races of the pathogen from other cereals acquired virulence on oats when transformed with the *G. graminis* var. *avenae* avenacinase gene[4], while oat mutants defective in avenacin biosynthesis became susceptible[5].

Plant surfaces are also equipped with arrays of cell surface pattern recognition receptors (PRRs) that detect pathogen-associated molecular patterns (PAMPs). Recognition activates mitogen-activated protein kinase (MAPK) cascades that both positively and negatively regulate PAMP-triggered immunity (PTI) within the cell[6-8]. PTI includes responses that disrupt parasitism, activate defensive cell death programs, defence-related gene expression and antibiotic accumulation, and reinforce plant cell walls[7,9].

Successful plant pathogens attempt to establish parasitic nutrition by stealth –*biotrophy*, or lethal force – *necrotrophy,* using effector molecules that suppress and overcome PTI. For example, Oomycete pathogens such as *Phytophthora* release RXLR effectors, similar to those used by the malarial pathogen *Plasmodium,* to disrupt MAPK signalling and thus suppress defences[10]. To counter this, plants have evolved another level of defence by deploying variable proteins encoded by specific resistance genes that recognise these effectors. Most are membrane-associated NB-LRR proteins with a Leucine Rich Repeat pattern receptor domain, and a regulatory Nucelotide Binding domain that elicits effector-triggered immunity (ETI)[6].

ETI is characterised by an amplified, more rapid and intense PTI response that includes the hypersensitive response (HR), a defensively-deployed form of programmed cell death[6,11,12]. The HR is a central component of effective defence against biotrophs, but its role in defence against necrotrophs is ambiguous as there is striking evidence that these pathogens exploit the oxidative burst and HR to promote their own necrotrophic requirements[13,14]. A recent report suggests that autophagy restricts necrotroph-induced cell death and lesion development by removing ROS-induced "pro-death" signals[15].

The sequence of cellular responses is similar in both PTI and ETI. Within seconds of recognition the plant plasma membrane depolarises and specific ion channels open, causing the rapid uptake of Ca^{2+} and H^+ into, and efflux of K^+ and Cl^- from, the cytosol[7]. Ca^{2+} is a key second messenger in plant cells that regulates calcium-dependent protein kinases and the regulatory protein calmodulin, and activates membrane-bound NADPH oxidase to release the superoxide anion (O_2^-), triggering an oxidative burst[16,17]. While basal resistance elicits a weak transient oxidative burst, PTI and ETI elicit a second, sustained and amplified burst that intensifies downstream resistance responses[12].

Protective antioxidant mechanisms in plant cells dismutate superoxide to hydrogen peroxide (H_2O_2)[16]. H_2O_2 is directly antimicrobial, orchestrates the HR, and initiates cell wall reinforcement at the point of pathogen penetration by cross-linking cell wall structural proteins and the deposition of the β1,3 glucan, callose[18]. The oxidative burst damages cellular macromolecules, releasing oxidation products that can activate both local and systemic acquired resistance (SAR)[12,16].

The oxidative burst also releases nitric oxide (NO), which interacts with the other ROS to either amplify or suppress the response[16]. The different components of the oxidative burst cause profound changes in metabolism through disturbances to cytosolic pH and redox homeostasis[19,20]. The ROS-scavenging enzymes superoxide dismutase and catalase, and the ascorbate-glutathione-NADPH cycle normally protect cells against damage

caused by ROS, but changes in their levels or activities modulate ROS-dependent signalling[12,19]. Down-regulation of antioxidant enzymes, such as the salicylic acid (SA)-induced suppression of catalase, or disturbance of the cellular redox balance following the ROS-induced oxidation of glutathione, activates SA and jasmonic acid (JA)/ethylene signalling pathways, the HR, and defence gene expression[12,19].

In another classic set of experiments, Müller and Borger demonstrated that potato tuber slices undergoing a HR to an incompatible race of *Phytophthora infestans* synthesise low molecular weight antibiotics, collectively named phytoalexins[21]. Phytoalexins are widespread throughout the plant kingdom and are chemically diverse, including phenylpropanoid, terpenoid and aliphatic molecules as well as inorganic sulphur[22]. Individual phytoalexins are taxonomically restricted, but one plant species may accumulate several phytoalexins.

While the biosynthesis and accumulation of phytoalexins following pathogen challenge correlates with resistance, direct evidence for a causal role is elusive. The cotton terpenoid phytoalexin dehydrogossypol accumulates to toxic levels in xylem tissues of a resistant cultivar in advance of hyphae of *Fusarium oxysporum* f.sp. *vasinfectum*, the cause of vascular wilt[23]. In a susceptible cultivar the phytoalexin only accumulates behind the invading hyphae and thus fails to restrict infection. Recently, intense accumulation of the oat phytoalexin avenanthramide A in mesophyll cells responding hypersensitively to an incompatible race of the rust pathogen, *Puccinia coronata*, was observed at the time of attempted haustorial development, while it was absent in a compatible interaction[24]. Given the critical role of haustoria in effector release and parasitism, this observation directly confirms many previous reports that phytoalexins accumulate to toxic concentrations at the right time and place to arrest pathogen development. While the phytoalexin-deficient *A. thaliana* pad-3 mutant acquires susceptibility to *Alternaria brassicicola*, its response to a range of other bacterial, oomycete or fungal pathogens remains unchanged[25]. Thus, while phytoalexins are decisive in some plant-pathogen interactions, in many interactions they play a less decisive antiseptic "mopping up" role, and in others they appear to be absent[22].

Plants that deploy the hypersensitive response and survive the initial pathogen attack develop systemic acquired resistance (SAR) and their defences become primed against further attack. In SAR, priming involves the systemic accumulation of salicylic acid (SA) and the presence of the regulatory protein Non-expressor of Pathogenesis-Related protein 1 (NPR1). Chemically-induced SAR involves the accumulation of inactive MAPKs[26] that become activated upon pathogen challenge,. Independent and antagonistic JA/ethylene-mediated systemic responses follow priming by rhizosphere microbes (ISR) and insects[27].

A key feature of SAR is the systemic accumulation of multiple families of pathogenesis-related (PR) proteins[28]. Many have antimicrobial activity, including β-1, 3-glucanase (PR-2) and chitinase (PR-3, PR-8, PR-11) that catalyse the degradation of microbial cell wall polysaccharides. PR-5 are thaumatin-like proteins, PR-6 is a protease inhibitor, PR-9 has peroxidase activity and is associated with the cell wall lignification response and PR-13 are thionins. In addition, plants produce ribosome-inhibiting proteins (RIPs) and polygalacturonase-inhibiting proteins (PGIPs) that disrupt pathogenesis[29].

Priming can be induced by some natural and synthetic compounds and wounding. Functional analogues of salicylic acid such as benzothiadiazole or 2,6 dichloroisonicotinic acid, and the non-protein amino acid β-amino butyric acid (BABA), directly elicit defence responses and prime SA-dependent resistance against subsequent challenges[30,31]. Even more interesting are the recent reports that the phosphite anion, a competitive antagonist of phosphate metabolism widely used to manage diseases caused by *Phytophthora* spp. and other Oomycetes, primes the SA signalling pathway (Massoud and Saindrenan, personnal communication) by suppressing MAP kinase 4, a negative regulator of SA-dependent defences in *A. thaliana*. With these findings defence priming has emerged as a promising means for sustainable disease management in the field.

Plants and pathogenic microbes are engaged in a complex evolutionary arms race. The similarity of innate immunity across eukaryote Kingdoms, involving receptor complexes and signalling networks using Ca^{2+}, ROS and MAPK cascades, suggests an early evolutionary origin. To survive and flourish in potentially septic and stressful environments plants have acquired sophisticated and complex defence mechanisms regulated with an exquisite level of control. Modern plant breeding has sometimes sacrificed plant defences for yield, with disastrous and unforseen consequences. Understanding how plants orchestrate their defences is vital to sustaining food, fibre and biofuel production and to managing our environment.

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References

1. Walker, J.C. Link K.P. and Angell, H.R. (1929) Chemical aspects of disease resistance in the onion. *Proc. Natl. Acad. Sci. USA* 15, 845-850.
2. Nicholson, R.L. Hammerschmidt R. (1992) Phenolic compounds and their role in disease resistance. *Annu Rev. Phytopathol.* 30, 369-389.
3. Osbourn, A. (2001) Plant mechanisms that give defence against soilborne pathogens. *Australas. Plant Pathol.* 30, 99-102.
4. Bowyer, P. *et al.* (1995) Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science* 267, 371-374.
5. Papadopoulou, K. *et al.* (1999) Compromised disease resistance in saponin-deficient plants. *Proc. Natl. Acad. Sci. USA*, 96, 12923-12928.
6. Jones, J.D. and Dangl, J.L. (2006) The plant immune system. *Nature* 444, 323-329.
7. Nürnberger, T. and Scheel, D. (2001) Signal transmission in the plant immune response. *Trends Plant Sci.* 6, 372-379.

8. Yang, K. Liu, Y. and Zhang, S. (2001) Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc. Natl. Acad. Sci. USA* 98, 741-746.
9. Thatcher, L.F. Anderson, J.P. and Singh, K.B. (2005) Plant defence responses: what have we learnt from *Arabidopsis? Funct. Plant Biol.* 32, 1-19.
10. Cheng, B. *et al.* (2012) Phytophthora sojae effector Avh331 suppresses the plant defence response by disturbing the MAPK signalling pathway. *Physiol Molec. Plant Pathol.* 77, 1-9.
11. Greenberg, J.T. and Yao, N. (2004) The role and regulation of programmed cell death in plant–pathogen interactions. *Cellular Microbiol.* 6, 201-211.
12. Torres, M.A. Jones, J.D.G. and Dangl, J.L. (2006) Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* 141, 373-378.
13. Alkan, N. *et al.* (2009) Ammonium secretion by *Colletotrichum coccoides* activates host NADPH oxidase activity enhancing host cell death and fungal virulence in tomato fruits. *Molec. Plant-Microbe Interactions* 22, 1484-1491 .
14. Glazebrook, J. (2005) Contrasting mechanisms of defence against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–27.
15. Lenz, H.D. *et al.* (2011) Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. *Plant J.* 66, 818-830.
16. Bolwell, G.P. and Daudi, A. (2009) Reactive oxygen species in plant-pathogen interactions. *In* LA del Rio and A. Puppo (eds) *Reactive Oxygen Species in Plant Signaling*. Springer-Verlag Berlin Heidelberg.
17. Grant, J.J. and Loakem, G.J. (2000) Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. *Plant Physiol.* 124, 21-29.
18. Schulze-Lefert, P. (2004) Knocking on Heaven's wall: pathogenesis of and resistance to biotrophic fungi at the cell wall. *Curr Op. Plant Biol.* 7, 377-383.
19. Foyer, C.H. and Noctor, G. (2011) Ascorbate and glutathione: the heart of the redox hub. *Plant Physiol.* 155, 2-18.
20. Shetty, N.P. *et al.* (2008) Roles of reactive oxygen species in interactions between plants and pathogens. *Eur. J. Plant Pathol.* 121, 267-280.
21. Müller, K.O. and Borger, H. (1940) Experimentelle Untersuchungen über die *Phytophthora infestans*-Resistenz der Kartoffel. *Arb. Biol. Reichsanst. Land Forstwirtsch*. 23, 189–231.
22. Hammerschmidt, R. (1999) Phytoalexins: what have we learned after 60 years? *Annu. Rev. Phytopathol.* 37, 285-306.
23. Hall, C. Heath, R. and Guest, D.I. (2011) Rapid and intense accumulation of terpenoid phytoalexins in infected xylem tissues of cotton (*Gossypium hirsutum*) resistant to *Fusarium oxysporum* f.sp. *vasinfectum*. *Physiol Molec. Plant Pathol.* 76, 182-188.
24. Uchihashi, K. *et al.* (2011) *In situ* localization of avenanthramide A and its biosynthetic enzyme in oat leaves infected with the crown rust fungus, *Puccinia coronata* f. sp.*avenae*. *Physiol. Molec. Plant Pathol.* 76, 173–181.
25. Thomma, B.P. *et al.* (1999) Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. *Plant J.* 19, 163-171.
26. Beckers, G.J. *et al.* (2009) Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *Plant Cell* 21, 944-953.
27. Conrath, U. Pieterse, C.M.J. and Mauch-Mani, B. (2002) Priming in plant-pathogen interactions. *Trends Plant Sci.* 7, 210-216.
28. Van Loon, L.C. and Van Strien, E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.*, 55, 85-97.
29. Fritig, B. Heitz, T. and Legrand, M. (1998) Antimicrobial proteins in induced plant defense. *Curr Op. Immunol.* 10, 16-22.
30. Kauss, H. *et al.* (1992) Dichloroisonicotinic acid and salicylic acid, inducers of systemic acquired resistance, enhance fungal elicitor responses in parsley. *Plant J.* 2, 655-660.
31. Zimmerli, L. *et al.* (2000) Potentiation of pathogen-specific defence mechanisms in Arabidopsis by β-aminobutyric acid. *Proc. Natl. Acad. Sci. USA* 97, 12920-12925.

Biography

David Guest is the Professor of Plant Pathology and Associate Dean (Development) in The Faculty of Agriculture, Food and Natural Resources at The University of Sydney. His current research interests include understanding plant disease resistance mechanisms, and using this knowledge to manage *Phytophthora* diseases in tropical horticulture and in Australian ecosystems. He teaches undergraduate courses at all levels and has supervised over 20 PhD and Research Masters students. His extensive fieldwork activities involve partnerships with research institutes and farming communities around the Asia-Pacific region. He is President of the Asian Association of Societies of Plant Pathology and a Past-President of the Australasian Plant Pathology Society.