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A war over water when bacteria invade leaves

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Abstract
A bacterial leaf pathogen actively targets plant cell processes to create an aqueous environment favorable for growth, revealing that control of water is a fundamental element of bacterial virulence.

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Comments
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Plant microbiology

Water at the nexus of the leaf-pathogen battle

A bacterial leaf pathogen actively targets plant cell processes to create an aqueous environment favorable for growth, revealing that control of water is a fundamental element of bacterial virulence.

Bacteria require water for growth, but water can be in short supply for bacteria invading aerial plant leaves. Leaves minimize water in their intercellular spaces, collectively termed the apoplast, to maximize gas exchange for photosynthesis, but the absence of water in the apoplast may also function in deterring the growth of bacterial invaders. A critical step in the pathogenesis of foliar bacterial pathogens is disarming the plant's defenses. Pathogens manage this disarmament by injecting proteins, called effectors, directly into plant cells through a special secretion system and suppressing bacterial-activated immune responses. On page XX of this issue, Xin *et al.*[1] identify another critical step in the pathogenesis of foliar pathogens, namely the injection of effector proteins that help promote hydration of the apoplast. The work elegantly demonstrates that the foliar pathogen *Pseudomonas syringae* actively targets plant cell processes to create an environment favorable for bacterial growth, and that plant immunity and active maintenance of a water-limited apoplast are the primary, or only, host processes that must be modulated for this pathogen to cause disease after leaf invasion.

High humidity strongly favors the development of foliar bacterial diseases, particularly leaf spot diseases. An early symptom of leaf spot diseases is water soaking, which is a localized abundance of water in the apoplast. Extensive water soaking is associated with epidemics and high disease severity. These observations are consistent with a role for water soaking in promoting bacterial growth. Xin and colleagues[1] provide leaf imaging data that document not only the transient nature of water soaking during leaf spot development, suggestive of a window of opportunity for bacterial growth, but also co-localization of sites exhibiting transient water soaking, early aggressive bacterial multiplication, and the eventual appearance of disease lesions. These data are the most direct evidence, to date, linking water soaking to bacterial growth and lesion development.

Previous studies highlighted the ability of members of the AvrE/WtsE effector protein family to promote water soaking in some plant species[2]. Xin *et al.*[1] expressed effector proteins one-by-one in a *P. syringae* strain lacking the majority of its 36 effectors. They demonstrated that AvrE and an additional effector, HopM1, each promote water soaking, and that this water soaking occurs whether the effectors are expressed by the bacteria or transgenically within the plant. Their subsequent discovery that HopM1 induces water soaking via a pathway involving degradation of the MIN7 protein in the host *Arabidopsis thaliana* provides critical evidence that pathogens can actively modulate host targets to promote hydration of the apoplast.

The data of Xin *et al.*[1] support an emergent model in which plants maintain a water-limited apoplast as a defense barrier against bacterial growth, and pathogen injection of specific effectors disarm this barrier leading to water soaking. This model is illustrated by HopM1-targeted degradation of MIN7, a protein contributing to the active prevention of fluid loss (Figure 1). The mechanisms by which pathogens influence plant cell fluid loss are not known, but could involve changes in plasma membrane function and stability, aquaporin function, and/or ion transport activity. The plasma

membrane is increasingly implicated as a target for pathogen-mediated impacts on water movement, as supported by the role of the HopM1 target, MIN7, in maintaining a normal plasma membrane[3], the targeting of AvrE to the plasma membrane[1], the influence of HopM1 and AvrE on the expression of genes for plasma membrane proteins[1], and the role of a protein central to *P. syringae-Arabidopsis* interactions, NDR1, in helping to prevent fluid loss from cells[4].

The strong growth of an otherwise nonpathogenic *P. syringae* strain in plant mutants lacking both MIN7 and pattern-triggered immunity (PTI) pathways indicate that water limitation in the apoplast and PTI comprise a minimal set of host functions that must be modulated for pathogenesis in the phyllosphere. In a fascinating extension of these studies, the *Arabidopsis* mutants that lacked both functions supported abundant growth of endogenous bacterial communities in the apoplast, whereas mutants defective in only one of these functions did not, highlighting the need for both defense mechanisms in minimizing colonization by the resident microbiota. Interestingly, bacterial community growth in the apoplast correlated with the appearance of necrosis and chlorosis in the leaves, revealing that bacterial proliferation within leaves, even of organisms not known to be pathogens, can exact a detectable toll on plant health.

The ambient air forms a continuous channel through stomata to the leaf apoplast – this enables the ambient humidity to dramatically influence a plant's ability to maintain a water-limited apoplast. This connectivity has important implications about the role of the environment in leaf pathogenesis. Low ambient humidity should help bolster water limitation in the apoplast as a defense strategy; this is consistent with the low leaf spot disease pressure common in arid conditions. In contrast, continuous water soaking should nullify this defense strategy and would explain the abundant growth observed for nonpathogens in the apoplast of persistently water-soaked leaves, such as occur in a driving rain or intense dew[5]. Xin and colleagues[1] provide a compelling case that the ambient humidity must be relatively high to enable pathogens to induce water soaking, suggesting a limited power of *P. syringae* to promote plant cell fluid loss. This strong humidity requirement for pathogenesis illustrates both the effectiveness of water-limitation as a plant defense barrier against pathogens, and a fundamental tenet of plant pathology – that disease depends on the host, the pathogen, and the environment.

Whereas PTI responses and a water-limited apoplast provide barriers to pathogen invasion and growth, plant defense pathways associated with recognition of specific effectors, termed effector-triggered immunity (ETI), invokes a more drastic defense response involving localized plant cell death; this generally confers complete resistance to a pathogen. Xin *et al.*[1] found that activation of ETI pathways stabilizes MIN7 and completely blocks water soaking, indicating that ETI prevents bacterial-induced changes in water availability. The water potential in the apoplast has been found to be dramatically lower during ETI than during pathogenesis of *P. syringae* in *Arabidopsis*[6], consistent with ETI preventing bacterial-induced hydration of the apoplast.

Plants and foliar pathogens clearly engage in a battle for control of the flood gates to the apoplast. For pathogens, a virulence strategy of creating an aqueous living space may unwittingly invite other bacteria to share the space, as shown in the discovery that pathogen-induced water soaking promotes growth of the human pathogen *Salmonella enterica* in leaves[7]. For plants, these defense strategies of activating PTI and preventing plant cell fluid loss may prevent bacterial growth after apoplast invasion, but a more lethal strategy of actively downregulating fluid movement into the apoplast may be employed during ETI, to effectively starve invaders for water[8]. A critical next step in understanding this fascinating plant-pathogen battle for control of water will be to identify the nature of the "flood gates" and the molecular mechanisms by which pathogens help them open.

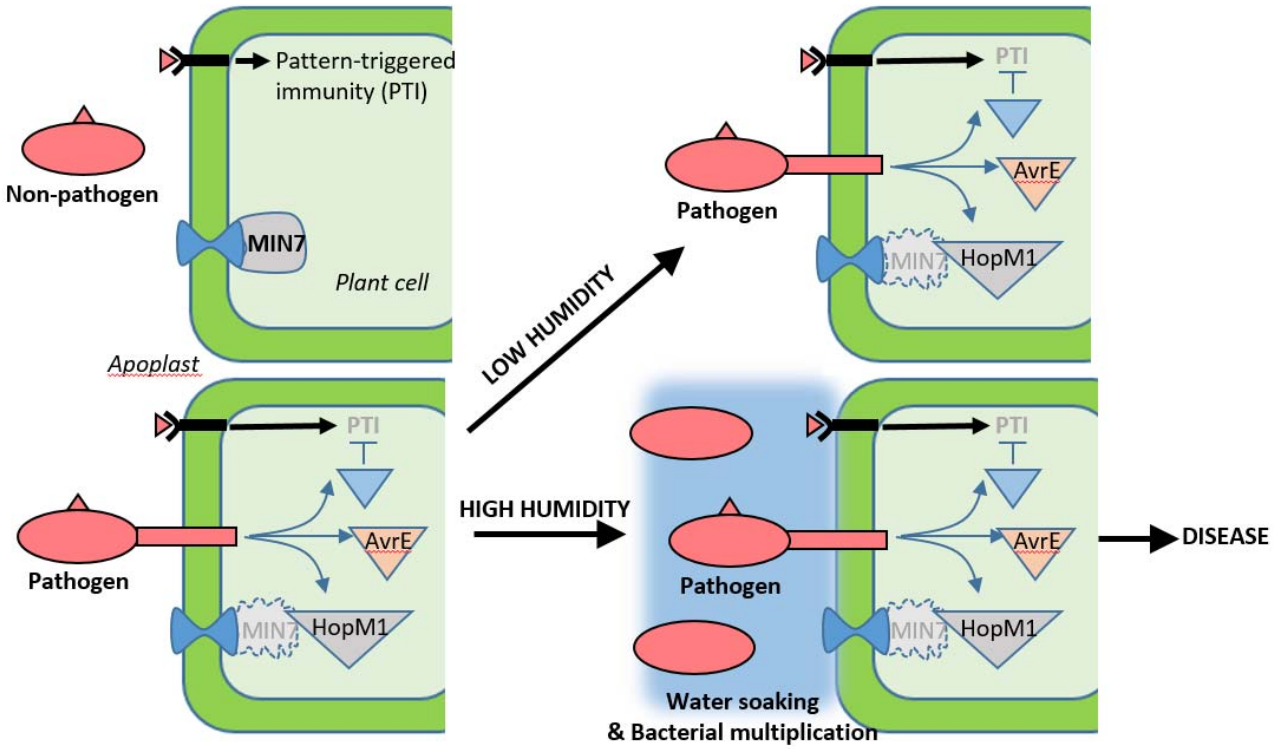

Figure 1. Foliar bacterial pathogens disarm two major plant barriers to bacterial growth. Plants minimize the growth of bacteria in leaves by limiting water movement into the intercellular spaces, or apoplast, and activating defenses in response to bacterial invasion. Bacterial pathogens such as *Pseudomonas syringae* inject a variety of effector proteins (triangles) directly into plant cells, some of which suppress bacterial pattern-triggered immunity (PTI). Xin *et al.*[1] report that two of these effector proteins, HopM1 and AvrE, can each induce hydration of the apoplast, and demonstrate that this induction involves HopM1-targeted degradation of MIN7, a protein that appears to be a key contributor to limiting plant cell fluid loss. The development of an aqueous living space in the apoplast, which can be visualized as localized regions of water soaking, requires a high ambient humidity, with higher humidity levels supporting more extensive water soaking, bacterial multiplication, and disease, and low levels limiting the accumulation of sufficient water to support pathogen multiplication.

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