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Review article

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Extracellular niche establishment by plant pathogens

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Abstract

The plant extracellular space, referred to as the apoplast, is inhabited by a variety of microorganisms. Reflecting the crucial nature of this compartment, both plants and microorganisms seek to control, exploit and respond to its composition. Upon sensing the apoplastic environment, pathogens activate virulence programmes, including the delivery of effectors with well-established roles in suppressing plant immunity. We posit that another key and foundational role of effectors is niche establishment — specifically, the manipulation of plant physiological processes to enrich the apoplast in water and nutritive metabolites. Facets of plant immunity counteract niche establishment by restricting water, nutrients and signals for virulence activation. The complex competition to control and, in the case of pathogens, exploit the apoplast provides remarkable insights into the nature of virulence, host susceptibility, host defence and, ultimately, the origin of phytopathogenesis. This novel framework focuses on the ecology of a microbial niche and highlights areas of future research on plant–microorganism interactions.

Sections

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Introduction

Plants are colonized by microorganisms from various kingdoms of life that have beneficial, neutral or detrimental effects on plant productivity. Microorganisms residing on the leaf surface, referred to as epiphytes, are largely commensal but also include potentially pathogenic microorganisms that are restricted by their lack of access to the apoplast, which is the extracellular space in the interior of plant tissues (Box 1). Endophytes, including bacteria, fungi and oomycetes, colonize the apoplast after gaining access via wounds, insect vectors, or natural openings such as stomata or hydathodes. The apoplast provides respite from ultraviolet radiation and desiccation but can also be unwelcoming owing to limited water and nutrient availability as well as constitutive and induced antimicrobial defences. Most endophytes engage in symbiotic or neutral interactions with the host, but pathogens parasitize the host tissue to support their typically high level of proliferation. Endophytes secrete effector molecules (generally toxins or proteins) that manipulate host processes to enable their survival. Effectors are either secreted and active in the extracellular space, for example, in the apoplast, or are translocated from the microorganism into the interior of host cells (Box 1).

Plants recognize microbial invasion of the apoplast and respond by producing barriers and antimicrobial compounds. However, pathogens can counteract these responses through the action of cytoplasm-delivered effectors, many of which have well-established functions in suppressing host defences[1–3]. More recent findings indicate that pathogenesis also depends on effectors that promote niche establishment, defined here as the enrichment of water and nutrients in the apoplast, through manipulation of plant metabolic and physiological processes[4,5]. To counteract pathogen niche establishment, plant immune responses reduce the quality of the apoplastic niche by restricting nutrient and water availability as well as altering the apoplast composition to prevent activation of microbial virulence programmes.

Pathogenic microorganisms support their proliferation in the apoplast through two modes. During biotrophy, resources are obtained from living host cells. During necrotrophy, host cells are killed to liberate resources. Many (perhaps most) pathogens are hemibiotrophic, exhibiting both lifestyles over the course of an infection. This Review focuses on the biotrophic lifestyle, including the biotrophic phase of hemi-biotrophy. We first introduce the concept of effector-driven extracellular niche establishment (EDEN), which contributes foundationally to pathogen virulence through the perturbation of metabolic and physiological processes that are fundamental to plant growth and development. We then consider EDEN in the context of the plant immune system, including the distinct contributions of EDEN and effector-driven immune suppression (EDIS). We propose that antagonism between niche establishment and defence-induced niche restriction drives physiological mechanisms, leading to either susceptibility or disease resistance.

This Review predominantly draws on examples from bacterial pathogens, although examples from fungal and oomycete pathogens are also considered, where such studies exist. We have limited our discussions to the apoplast when discussing fungal and oomycete pathogens and have excluded discussion of the specialized feeding structures, called haustoria, formed by many of these filamentous pathogens. Nonetheless, we propose that many of the principles discussed herein are pertinent to filamentous pathogens and that this will be a fruitful area for further research.

Niche establishment

Recent reports indicate that bacterial infections rapidly convert the apoplast into a water-rich and nutrient-rich environment[6–8]. To account for this phenomenon, we propose that niche establishment is based on a feedforward relationship between water and metabolite accumulation in the apoplast (Fig. 1). Based on this model, we posit that the outputs of diverse effectors converge to synergistically facilitate EDEN. In this section, we discuss mechanisms by which effectors drive apoplast hydration, consider implications of increased apoplast hydration for metabolite trafficking, and discuss mechanisms by which effectors drive metabolite accumulation in the apoplast.

Apoplast accumulation of water

Stomata are switchable gates between the apoplast and leaf exterior. One key function of stomata is the regulation of gas exchange, including evaporative water loss. For example, drought stimulates the synthesis of the phytohormone abscisic acid (ABA), which induces stomatal closure to promote water retention by the plant[9]. Microorganism-associated molecular patterns (MAMPs) also elicit ABA-dependent stomatal closure, which restricts entry of epiphytic, potentially pathogenic, microorganisms into the apoplast[10]. This so-called 'stomatal defence', along with how pathogen effectors reopen stomata to facilitate apoplast invasion, has been reviewed extensively elsewhere[11,12].

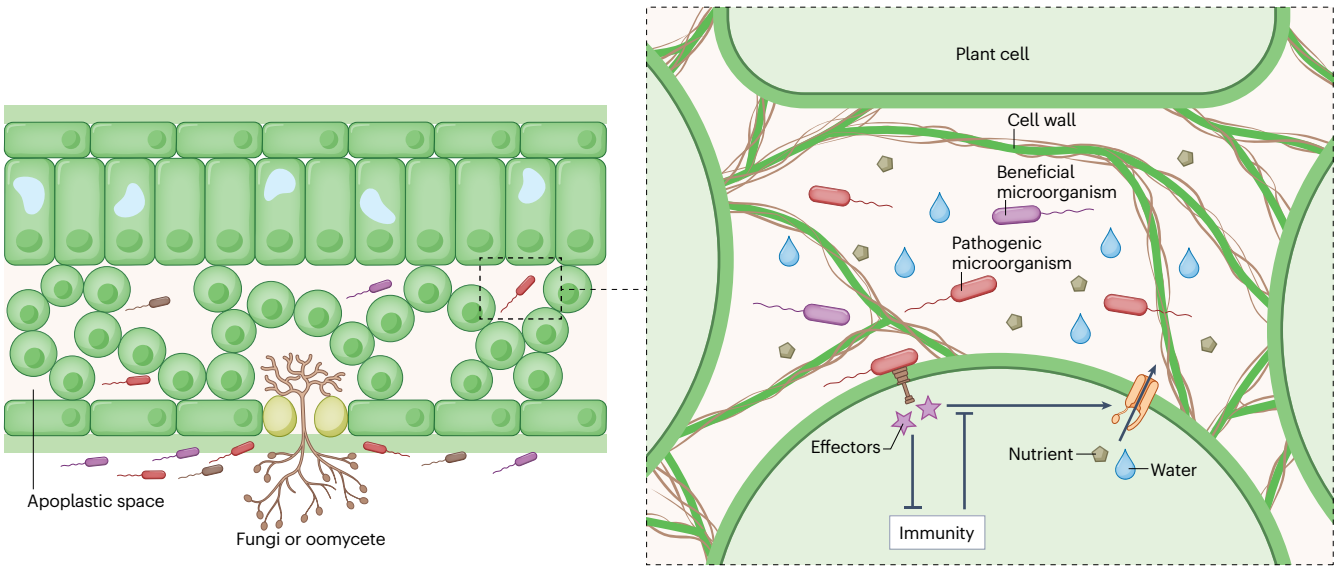
Remarkably, in addition to initially 'opening' stomata to enable invasion, pathogens later 'close' them to prevent evaporation and thus promote apoplast hydration (Fig. 1). This dynamic control of stomatal aperture is exemplified by the interaction between *Arabidopsis thaliana* and *Pseudomonas syringae* pv. tomato. Epiphytic *P. syringae* pv. tomato deploys the phytotoxin coronatine to reopen stomata that closed upon perception of bacterial MAMPs[10]. However, a pioneering study found that *P. syringae* pv. tomato could also reduce stomatal conductance in an effector delivery-dependent manner once inside the apoplast[13]. Following invasion, either of two unrelated type III secreted effector (T3E) proteins, HopM1 or AvrE1, induce the apoplast hydration that is necessary to support high-level growth of *P. syringae* pv. tomato[14]. These T3Es reverse the initial, coronatine-dependent opening of stomata to later prevent evaporation from the apoplast[6,7]. Highlighting the significance of targeting ABA for EDEN, HopM1 and AvrE1 activate ABA accumulation and signalling by multiple, distinct mechanisms. HopM1 increases ABA transport into stomatal guard cells, at least in part, through exploitation of the ABA transporter ABCG40, thus potentiating ABA function in stomata[6]. Concomitantly, interaction of AvrE1 with type I protein phosphatases derepresses SnRK2-dependent transcription of ABA-responsive genes[7]. Furthermore, AvrE1 as well as WtsE, an AvrE-homologue from the maize pathogen *Pantoea stewartii* subsp. *stewartii*, interact with subunits of the heterotrimeric phosphatase PP2A, which negatively regulates ABA signalling[15,16]. The targeted PP2A subunits are required for the virulence contribution of both AvrE1 and HopM1 to *P. syringae* pv. tomato[15]. Consistent with these results, *A. thaliana* mutants deficient in ABA biosynthesis or perception show reduced susceptibility to *P. syringae* pv. tomato, which correlates with a lack of bacterial-induced stomatal closure and apoplast hydration[6,7,17,18].

In a broader perspective, ABA signalling is a susceptibility node that is targeted in a variety of ways by effectors from diverse, agronomically important, (hemi)biotrophic phytopathogens[19]. Pathogenic varieties of *Xanthomonas oryzae*, *Xanthomonas translucens* and *Xanthomonas campestris* promote stomatal closure and water-soaking in rice, wheat and *A. thaliana*, respectively, dependent on induction of plant ABA biosynthesis and/or signalling[20–22]. XopD, a T3E from *X. campestris*, induces transcriptional reprogramming that promotes ABA signalling, and Tal8, a transcriptional activator-like effector (TALE) from *X. translucens*,

Box 1

The apoplastic landscape during pathogen colonization

The apoplast refers to the extracellular spaces in the interior of plant tissues, consisting of air, liquid and cell wall material. This space can be colonized by bacteria, filamentous pathogens and other microorganisms (see the figure) through openings such as stomata, which mediate gas exchange between the inside and outside of plant tissues. The apoplast of healthy plants contains a paucity of water and nutrients. Many pathogens respond to metabolic cues present in the apoplast by activating virulence programmes, which leads to the production of effector molecules. Effectors are typically delivered to the host cytoplasm, for example, via the depicted type III secretion system apparatus of a bacterium. Cytoplasm-delivered effectors have diverse functions, including inhibition of host immune defences, manipulation of plant metabolism, and induction of or function as transporters, which increase the efflux of nutrients and water into the apoplast, creating a resource-rich environment. In some cases, activation of plant immunity in the absence of effectors (that is, microorganism-associated molecular pattern detection) can prevent modification of the apoplastic space.



directly activates transcription of the gene encoding the rate-limiting enzyme for ABA biosynthesis[21,22]. Other examples of T3Es targeting ABA include HrpN from *Erwinia amylovora*, which induces ABI2-dependent ABA signalling, and AvrPtoB from *P. syringae* pv. tomato, which induces degradation of a cytochrome P450 monooxygenase, CYP707A1, that normally de-activates ABA through hydroxylation[23,24]. In addition to activation of ABA signalling by T3Es, plant pathogenic fungi from the genera *Botrytis*, *Magnaporthe*, *Ceratocystis*, *Fusarium* and *Cercospora* biosynthesize ABA, which in many cases is crucial for their virulence[19,25–27]. Notably, these fungi induce water-soaking during their early, likely biotrophic, phases of infection. Furthermore, by retaining apoplast hydration as mesophyll cells lose their integrity, ABA-induced stomatal closure may facilitate sustained growth of bacterial and fungal pathogens alike as they transition to necrotrophy. Collectively, these examples indicate that phytopathogens convergently, repeatedly and diversely target ABA signalling during EDEN.

The EDEN concept can also be applied to microenvironments generated at the leaf surface, such as the watery niche generated by the virulence factor syringafactin from *P. syringae* strain B728a[28]. This hygroscopic biosurfactant notably functions at high humidity. It is also required for virulence once inside the apoplast, but whether it contributes to apoplastic water-soaking is unknown. However, this study expands our understanding of niche establishment by demonstrating an ABA-independent mechanism of water acquisition at the leaf surface and perhaps also in the apoplast.

Apoplast hydration occurs during biotrophy

While ABA-dependent stomatal closure promotes apoplast hydration by preventing evaporation, it is not clear in all cases whether the water accumulates in the apoplast outside of intact plant cells or, as has been speculated, 'leaks' from plant cells upon the onset of necrosis. The latter may be the underlying cause of water-soaking during necrotrophy such as that caused by *Pectobacteria* spp.[29]. Indeed, closure of stomata would likely prove beneficial by preventing desiccation during necrotrophy as well as biotrophy. However, it has been recently demonstrated that two hemibiotrophic bacterial pathogens, *P. syringae* pv. tomato and *P. stewartii* subsp. *stewartii*, cause apoplast hydration during the biotrophic phase of infection, that is, while the bacteria proliferate and the apoplast remains a physically separate compartment from the interior of plant cells[8,30].

Where does the water come from when apoplast hydration occurs during biotrophy? One source is liquid water from the plant surface as has been demonstrated for water-soaking of tomato leaves induced by AvrHah1, a TALE of *Xanthomonas gardneri*[31]. AvrHah1 induces expression of a bHLH transcription factor that, in turn, induces expression of pectin-modifying genes. One of those genes, which encodes a pectate lyase enzyme, is sufficient to induce water-soaking through uptake of water from the surface of wet leaves[31]. However, apoplast hydration commonly occurs independently of moisture on the surface of plant tissues. High humidity increases the efficiency with which pathogens induce apoplast hydration by reducing evaporation, similar to, and likely synergizing with, stomatal closure[14]. The water that fills the apoplast may unload directly from mesophyll cells, for example, via aquaporins or the pore formed by AvrE-family effectors[32], supported by symplastic transport from the vasculature. Alternatively, water could be unloaded into the apoplast directly from the phloem or, perhaps more likely, from the xylem. Consistent with the significance of vascular flow for pathogenesis, numerous T3Es from *P. syringae*, including HopM1 and AvrE1, prevent its defence-induced inhibition[33]. Regardless of the route, reduced water potential in the apoplast, resulting from increased solute content or hygroscopic virulence factors, would facilitate the movement of water into the apoplast.

Apoplast accumulation of metabolites

Increased apoplast hydration contributes to the accumulation of metabolites in the apoplast. Infiltration–centrifugation methods, when tailored to particular plant species, are useful for determining the gas and liquid content of the leaf apoplast as well as for isolating the apoplast fluid for subsequent analyses[30,34–36]. Flooding the apoplast of maize seedling leaves by vacuum infiltration causes apoplast accumulation of several classes of metabolites, including amino acids, sugars and organic acids, relative to non-infiltrated leaves[8]. This so-called 'buffer effect' occurred without any change in the overall abundance of metabolites in the leaf, indicating that, consistent with the niche establishment cycle (Fig. 1), apoplast hydration shifts the partitioning of metabolites towards the apoplast. In addition to a cautionary note regarding infiltration-based assays, this finding highlights the role of water in promoting apoplast metabolite accumulation. Conversely, AvrHah1 induces apoplast solute which, in addition to a potential source of nutritive metabolites for the bacteria, facilitates water uptake from the leaf surface[31]. Thus, rather than a cause–effect relationship between water and solute, a mutually reinforcing feedforward relationship supports the apoplast accumulation of these two essential components for niche establishment (Fig. 1).

Sources of carbon (C) and nitrogen (N) utilized by pathogens are often present in the apoplast of uninfected plants[37]. However, whether this baseline nutrient supply is sufficient to support pathogen outgrowth is largely unknown. During *P. stewartii* subsp. *stewartii* infection of maize leaves, WtsE causes metabolite accumulation in the apoplast that exceeds the 'buffer effect' and is heightened for metabolites that are utilizable by *P. stewartii* subsp. *stewartii* as C and N sources. Furthermore, measurement of cellular and apoplast metabolites and the

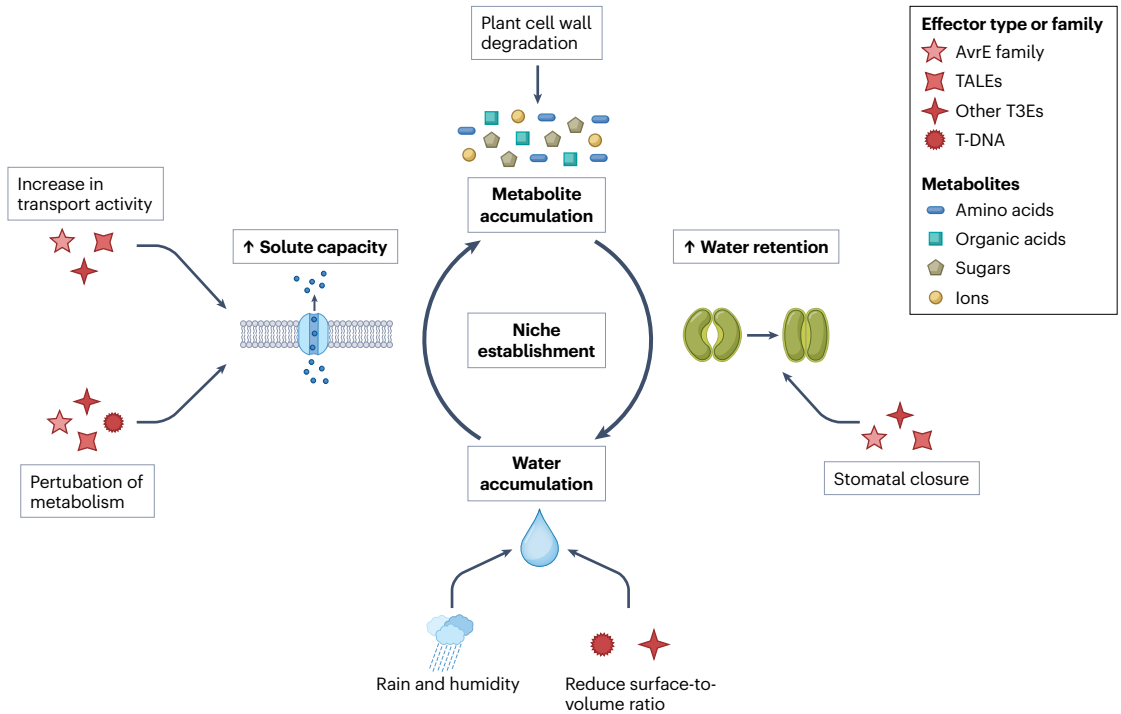
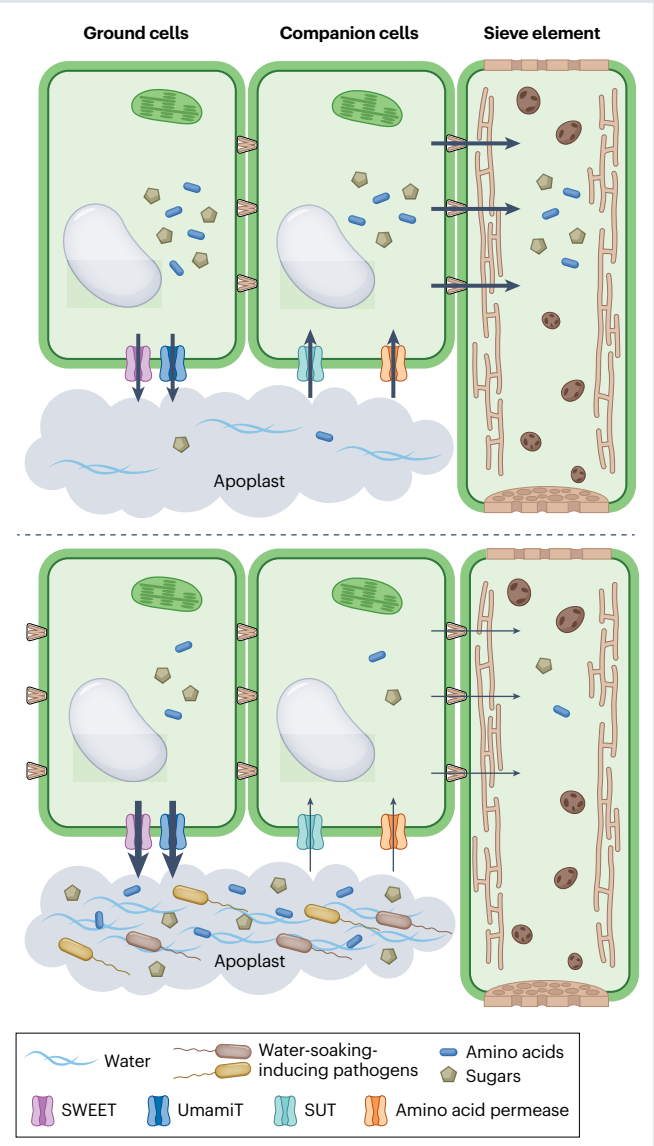


Fig. 1 | A model for a feedforward loop supporting pathogenic niche establishment. Different effector types manipulate various physiological and metabolic aspects of plant biology to favour water accumulation and retention in the apoplast. Water accumulation is further exacerbated by environmental conditions such as precipitation and high relative atmospheric humidity. Water accumulation increases solute capacity and thus facilitates the accumulation of metabolites in the apoplast. Diverse effectors increase apoplast metabolite accumulation by manipulating host metabolism, increasing transporter expression or degrading plant cell wall components. Increased solute accumulation reduces water potential, which further promotes water accumulation in the apoplast. T3E, type III secreted effector; TALE, transcriptional activator-like effector; T-DNA, transfer DNA.

Box 2

Effect of hydration on apoplastic nutrient trafficking

In a source tissue, sugars and amino acids move passively along the concentration gradient from ground cells into the apoplast via SWEET and UmamiT uniporters, respectively[39]. Thus, increased apoplast hydration favours mobilization of metabolites into the apoplast by decreasing their apoplast concentration. Conversely, uptake of sugars and amino acids by phloem companion cells depends on sucrose uptake transporters (SUTs) and amino acid permeases, which are H^+/substrate symporters, respectively[39]. Increased apoplast hydration will disfavour uptake from the apoplast both by steepening the concentration gradient of the substrate and reducing the H^+ concentration. Furthermore, the energy-dependent uptake process may be disfavoured owing to the broader energy demands of an ongoing defence response by the plant. Notably, these favourable conditions for pathogen exploitation also apply during apoplastic phloem unloading into sink tissues owing to the inverted relationship between uniporters and symporters for transport into and from the apoplast, respectively[39]. See the figure for a model of apoplastic phloem loading in a healthy source tissue (top) and how pathogen-induced apoplast hydration dysregulates the process, as indicated by arrow weights to indicate metabolite movement through transporters, to favour metabolite accumulation in the apoplast (bottom).



amount of C and N assimilated by *P. stewartii* subsp. *stewartii* indicates that a dynamic flux of metabolites from cells of infected maize leaves into the apoplast and then into *P. stewartii* subsp. *stewartii* cells supports a level of nutrient availability vastly exceeding that in uninfected leaves[8]. Determining the generalizability of this observation will require investigation of the C and N budget between hosts and microorganisms in additional pathosystems. However, given that WtsE is part of the widely conserved AvrE family of T3Es, it is likely that the growth of bacterial pathogens from diverse genera in susceptible plant tissues requires dynamic mobilization of nutritive metabolites into the apoplast beyond just offsetting defence-induced nutrient restriction (discussed below).

We posit that increased solute capacity of an excessively hydrated apoplast facilitates metabolite accumulation by dysregulating normal plant metabolite trafficking (Box 2). Phloem (un)loading occurs via symplastic and apoplastic pathways. The apoplastic loading pathway is based on metabolites flowing (1) passively along their concentration gradient, via uniporters, from ground cells into the apoplast; (2) via diffusion through the apoplastic space; (3) actively against their concentration gradient, via H^+/substrate symporters, from the apoplast into phloem

companion cells; and (4) via a symplastic route into sieve elements[38]. Increased apoplast hydration will favour transport into the apoplast and disfavour uptake from the apoplast by decreasing the concentration of metabolites in the apoplast as well as diluting or facilitating the diffusion of H^+ needed for symporter function. Notably, phloem unloading in sink tissues is based on the inverse use of uniporters in companion cells and H^+/substrate symporters in ground cells. Thus, regardless of whether a tissue is a source or a sink, increased apoplast hydration will enhance apoplast metabolite accumulation by dysregulating apoplastic trafficking to or from the phloem, respectively.

Just as diverse mechanisms promote apoplast hydration, EDEN modulates the metabolite and ion content of the apoplast both by controlling the partitioning of metabolites between the interior of plant cells and the apoplast and by influencing plant metabolism (Fig. 2). We first consider altered partitioning. Sugar transport is a process widely targeted by pathogens. A variety of TALEs from *Xanthomonas* spp. have evolved convergently to target the promoters of genes encoding SWEET sugar uniporters, specifically the clade 3 sucrose transporters[39,40]. WtsE induces accumulation of sucrose in the apoplast of maize leaves, indicating that AvrE-family T3Es may also target clade 3 SWEETs or form a sucrose-permeable pore[8,32]. Additionally, the transcription of SWEET transporter-encoding genes is induced by various other plant pathogens, including *P. syringae* pv. tomato and the fungal pathogens *Golovinomyces cichoracearum*, *Botrytis cinerea* and *Rhizoctonia solani*[41,42]. Some genes encoding SWEET transporters are downregulated during infection. Whether this is a consequence of plant immune

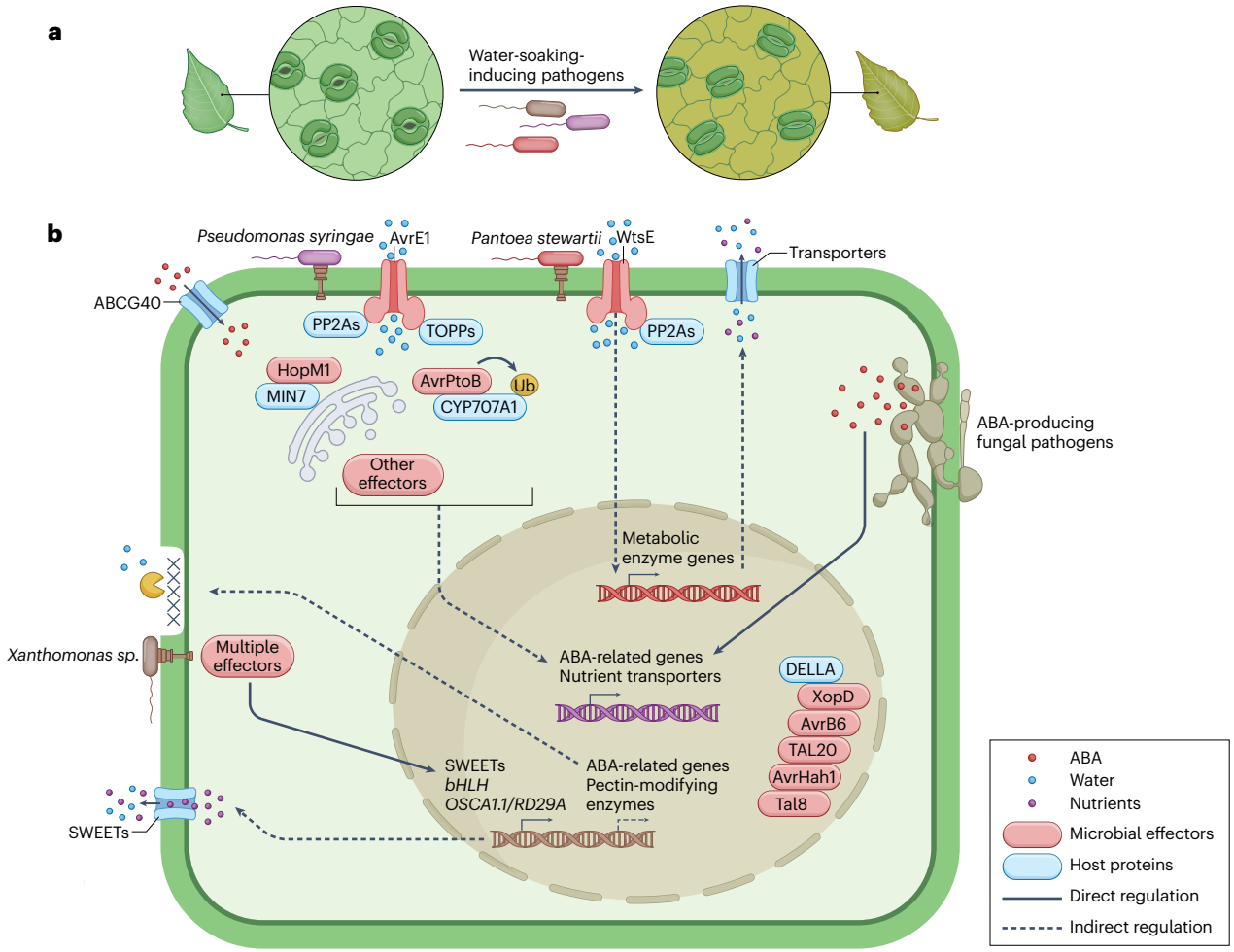


Fig. 2 | Microbial strategies leading to niche establishment. a, Water-soaking-inducing pathogens commonly induce stomatal closure to promote apoplast hydration by reducing the rate of evaporation. **b**, Pathogens have evolved diverse strategies to induce effector-driven extracellular niche establishment (EDEN) through the actions of effector proteins. Common strategies of niche establishment include the manipulation of abscisic acid (ABA)-related pathways such as the induction of ABA synthesis by the plant or the pathogen, inhibition of ABA negative regulators (PP2A, type I protein phosphatases (TOPPs), CYP707A1), induction of ABA transporters (ABCG40) and induction of positive regulators of stomatal closure (OSCA1.1, RD29A). Other processes include inducing the production of sugar transporters (SWEETs), other nutrient transporters and metabolic enzymes as well as modification of host cell wall properties (for example, pectin-modifying enzymes regulated by the bHLH family of transcription factors). HopM1 induces EDEN through an increase in *ABCG40* transcripts, leading to increased ABA accumulation in guard cells, and through manipulation of the *trans*-Golgi network–early endosome-localized protein MIN7. EDEN by members of the AvrE family of effectors, represented by AvrE1 and WtsE, results from multiple activities, including functioning as a water-permeable and solute-permeable channel that likely enriches the extracellular space in water and nutrients, perturbing host metabolism and promoting apoplast hydration by stimulating ABA pathways.

activation or a result of effector virulence activity is unclear. Alongside sugar transporters, the expression levels of various amino acid transporters changes following challenge with a variety of pathogens[43]. Some of these changes may promote apoplast accumulation of amino acids, whereas others may participate in nutrient restriction (discussed below). Similarly, transporters of organic acids and ions represent additional potential targets through which pathogens may promote the export of metabolites into the apoplast. Although organic acid transport has been mostly associated with the recruitment and establishment of symbiotic interactions[44–46], its enhancement represents an additional facet of EDEN[47]. Pathogens also deploy effectors that activate the transport of mineral nutrients such as sulfate and phosphate[48,49]. The induced accumulation of organic acids in the apoplast, along with pathogen production of siderophores, promotes the partitioning of iron away from plant cells and cell wall association and into the apoplast fluid[50]. Thus, EDEN relies on diverse mechanisms to mobilize nutritive compounds into the apoplast.

A second mechanism for apoplast metabolite accumulation during EDEN is the activation of plant metabolism. A key implication of the dysregulation of phloem (un)loading caused by increased apoplast hydration (Box 2) is that the abundance of metabolites inside plant cells is linked directly, via available transporters, to their accumulation in the apoplast. It is well established that pathogens influence metabolism in infected plants, though the distinction between defence-promoting and virulence-promoting shifts is not always clear, and the two are likely intertwined[5]. However, pathogens infecting susceptible host plants frequently alter plant metabolism to enhance the production of their favoured or essential nutrients. In a classic example, *Agrobacterium tumefaciens* uses its type IV secretion system to deliver transfer DNA encoding enzymes that drive the biosynthesis of opines, which are a primary source of nutrition for the bacteria, within plant cells[51]. As another example, *Ustilago maydis*, which lacks cell wall-degrading enzymes, perturbs maize metabolism and source–sink relationships to promote the production of nutritive metabolites and their mobilization to the infection site[52–54]. The systemic manipulation of plant metabolism may be a common strategy as several types of pathogens manipulate host biology to create nutrient sinks[55]. Other filamentous pathogens of maize, barley and tomato also induce plant production of nutritive metabolites[56,57]. Among the elegant mechanisms involved in microbial nutrition, the oomycete pathogen *Phytophthora sojae* secretes an effector, AEP1, that facilitates sugar uptake through aldose mutarotation in the apoplast[58]. Similarly, bacterial pathogens induce metabolic shifts in host plants consistent with fulfilment of their nutritional requirements. The T3E RipI from *Ralstonia solanacearum* induces tomato or *A. thaliana* cells to produce γ-aminobutyric acid, which is an essential nutrient for full bacterial virulence in planta[59,60]. The suite of T3Es from *P. syringae* pv. tomato induces transcriptional and corresponding metabolic shifts in infected *A. thaliana*[61]. WtsE from *P. stewartii* subsp. *stewartii* induces transcriptional changes in infected maize leaves indicative of enhanced C and N metabolism, consistent with the observed increases in apoplast metabolite abundance during *P. stewartii* subsp. *stewartii* infection[8,62]. Notably, in addition to closing stomata to promote apoplast hydration, pathogen perturbation of plant ABA signalling may also influence plant metabolism[63]. For a comprehensive description of mechanisms by which pathogens alter plant metabolism and enhance metabolite transport, we refer readers to a recent review[5].

The classic plant pathology disease triangle invokes a role for environmental factors, along with pathogen and plant varieties, in determining infection outcomes. Accordingly, EDEN is expected to be sensitive to environmental factors. For example, soil nutrient availability affects plant metabolism and thus likely influences the efficiency with which pathogens induce the accumulation of nutrients and/or hydration-promoting metabolites in the apoplast. Similarly, precipitation or humidity will produce wet soils that support increased vascular pressure, reduce water vapour loss via evaporation and/or enable water uptake from plant surfaces[14,64]. The niche establishment model predicts synergy between EDEN and environmental moisture and thus conforms to the longstanding correlation between precipitation and plant disease appearance in the field (Fig. 1).

We propose that manipulation of host metabolism and physiology to promote niche establishment is foundational to the virulence of plant pathogens. Consistent with this supposition, effectors engaged in EDEN are ancient. The AvrE-family T3Es are broadly distributed across numerous genera of phytopathogenic bacteria and are deeply rooted with the encoding genes located in the conserved effector locus alongside the regulatory and structural genes of the type III secretion system (T3SS). HopM1 also resides in the conserved effector locus of *Pseudomonas* spp. and displays functional overlap with AvrE1 in promoting virulence of *P. syringae* pv. tomato. The most agriculturally important genera of plant pathogenic bacteria lacking AvrE-family T3Es, *Xanthomonas* spp., instead possess a diverse collection of TALEs that have convergently evolved to perturb host metabolic and physiological processes. Notably, AvrE-family T3Es and TALEs converge on mechanisms that promote the niche establishment cycle (Fig. 2).

Intriguingly, gall, tumour and canker-forming pathogens such as *Agrobacterium* spp., *U. maydis* and *Xanthomonas citri* may employ a variant of EDEN that couples metabolic reprogramming with the induction of plant cell hyperplasia and/or hypertrophy that likely expands the niche volume and/or promotes water retention by reducing the surface-to-volume ratio. In the case of *Agrobacterium* spp., transgenes that promote both host metabolic shifts and plant cell proliferation are contained within the transfer DNA delivered by the evolutionarily ancient type IV secretion system. The mechanisms of niche establishment deployed by filamentous pathogens, aside from ABA biosynthesis, are less well understood. Remarkably, HaRxL23, an effector protein from an oomycete pathogen of the genus *Hyaloperonospora* that shares structural similarity to AvrE, can complement the ability of *P. syringae* pv. tomato strains lacking AvrE1 to produce water-soaked lesions[65]. This example of trans-kingdom complementation of AvrE1 indicates that niche establishment is also likely foundational for filamentous pathogens.

The AvrE superfamily of effectors is highly conserved in microbial phytopathogens, including bacteria and oomycetes. For example, induction of water-soaking in apple and pear by *Erwinia amylovora* is dependent on DspA (also known as DspE), another AvrE-family T3E that targets PP2A[66]. It has not been reported whether WtsE and DspA/E targeting of PP2A promotes water-soaking by manipulating ABA signalling as observed with the AvrE1 effector of *P. syringae* pv. tomato. Similarly, it has not been reported whether AvrE orthologues from other bacterial pathogens that induce water-soaking, such as *Pantoea* spp., *Dickeya dadantii*, *Dickeya solani* and *Pectobacterium* spp., do so by targeting ABA signalling. However, ABA has been shown to positively impact *D. dadantii*, *Dickeya solani* and *E. amylovora* growth in plants[23,67], further supporting a role for ABA as a susceptibility factor in multiple plant–bacteria interactions. Given that oomycete pathogens from the genus *Hyaloperonospora* promote the appearance of water-soaked lesions during early biotrophic phases and possess effector proteins with structural and functional similarity to AvrE1 (ref. 65), we speculate

that the distribution of AvrE-like effector proteins across kingdoms is due to their ability to induce stomatal closure by manipulating ABA action. This strategy serves to enhance host susceptibility to infection by providing an aqueous microenvironment, which is to the benefit of the pathogen.

Niche establishment in the context of host immunity

The plant immune system has been conceptualized as consisting of two main branches[1]. MAMPs elicit pattern-triggered immunity (PTI) upon their direct and typically extracellular recognition by pattern-recognition receptors, whereas effectors elicit effector-triggered immunity (ETI) upon their typically indirect and intracellular recognition by resistance proteins[68]. Many outputs of PTI and ETI are independent of niche restriction, for example, the production of barriers, reactive compounds, and bacteriostatic and bacteriocidal phytochemicals. However, consistent with the foundational role of niche establishment for pathogenesis, other outputs of plant immunity limit water and nutrient availability in the apoplast.

Immune restriction of apoplast water

EDEN promotes apoplast hydration by increasing apoplast solute levels, reducing surface-to-volume ratio and/or promoting stomatal closure to limit evaporative water loss (Fig. 1). One mechanism by which plant immune activation combats apoplast hydration is to promote stomatal opening. PTI induces phytocytokines (small phytocytokines regulating defence and water loss (SCREWs)) that counter-regulate ABA signalling to promote stomatal opening[69]. Similarly, PTI and ETI induce the plant defence hormone salicylic acid, which, in combination with light, counteracts effector-induced stomatal closure and apoplast hydration[17]. Thus, the inhibition of pathogen growth by SCREWs and salicylic acid likely results, at least in part, from stomatal opening to promote evaporative drying of the apoplast. A second mechanism limiting water availability in the apoplast is the cessation of vascular flow to infected tissues following plant defence activation[13]. Thus, defence-induced modulation of plant physiological processes limits apoplast water by reducing availability from the vasculature as well as by promoting evaporation through stomata.

A third mechanism that plants use to combat apoplast hydration is the hypersensitive response, during which pathogen-engaged cells die and the infected tissue desiccates. A seminal study linked ETI and water availability during *P. syringae* pv. tomato infection of *A. thaliana*[70]. Wild-type *P. syringae* pv. tomato experienced a decrease in apoplast water potential, relative to a non-virulent T3SS-deficient mutant strain, that was non-growth-restrictive, consistent with T3E-induced apoplast hydration during EDEN. However, strains of *P. syringae* pv. tomato carrying T3Es that elicit a hypersensitive response experienced growth-restrictive reductions in water potential. Similarly, water-soaking induced by the *Xanthomonas* spp. TALEs AvrBs3 and AvrHah1 is prevented by elicitation of ETI[71]. More generally, the importance of water loss for an effective hypersensitive response is supported by observations that high humidity prevents tissue collapse, desiccation and restriction of pathogen growth[14,72,73]. Thus, the effectiveness of hypersensitive response-based plant defence may hinge, at least in part, on the rate of desiccation at the site of infection. Notably, defence responses coincident with the hypersensitive response, such as stomatal opening resulting from PTI-induced or ETI-induced production of SCREWs and/or salicylic acid, may enhance the rate of hypersensitive response-induced desiccation. The resulting reduction in apoplast hydration may enhance the efficiency of PTI, for example, by concentrating elicitors or defence signalling molecules. Thus, restriction of apoplast hydration via distinct mechanisms may contribute to the mutual potentiation between PTI and ETI during plant defence[74–77].

Immune control of apoplast metabolites

Plant immune activation can indirectly inhibit EDEN by modifying the metabolite content of the apoplast to reduce microbial virulence expression. PTI in *A. thaliana*, dependent on MAPK phosphatase 1, restricts apoplast levels of amino acids and organic acids that induce expression of the T3SS of *P. syringae* pv. tomato[78,79]. Similarly, during PTI in *A. thaliana*, phosphorylation-based activation of a H^+ symporter, STP13, promotes the uptake of monosaccharides from the apoplast and thus reduces expression of the T3SS of *P. syringae* pv. tomato[80]. Mirroring the restriction of virulence-inducing molecules, recent reports indicate that PTI also induces apoplast accumulation of distinct amino acids that restrict bacterial virulence expression[81–83]. Thus, regulation of the metabolite composition of the apoplast to limit pathogen virulence expression is key to plant defence, including restriction of EDEN.

Plant defences can also directly inhibit EDEN by modifying the composition of the apoplast. Stress-induced changes in apoplast pH and levels of reactive oxygen species, including those observed during plant–pathogen interactions, alter apoplast water and metabolite composition through complex regulation of stomatal movements, ABA signalling and transporters[84,85]. Defence-induced shifts in apoplast composition that limit pathogen virulence expression may also limit the nutritional quality of the apoplast, for example, activation of STP13 during PTI may also restrict *P. syringae* pv. tomato growth by reducing the abundance of nutritive sugars in the apoplast[80]. *P. sojae* engages in a complex struggle with the soybean immune system to control nutrient availability in the apoplast. Secretion of PsXEG1, an apoplast-localized endoglucanase with broadly distributed alleles among *P. sojae* isolates, generates monosaccharide and polysaccharide cell wall breakdown products required for the growth of *P. sojae* and possibly also apoplast hydration by *P. sojae*[86]. Plants use multiple defensive measures to restrict EDEN by PsXEG1: (1) PsXEG1 induces a hypersensitive response-like cell death in a variety of plants[86]; (2) soybean secretes GmGIP1, a high-affinity inhibitor of the cell wall-degrading activity of PsXEG1, into the apoplast[87]; and (3) soybean secretes a protease, GmAP5, that targets PsXEG1 (ref. 88). Further highlighting the deeply rooted evolution of the struggle to control PsXEG1 activity, *P. sojae* glycosylates PsXEG1 to reduce its targeting by GmGIP1 and secretes an inactive paralog of PsXEG1, PsXLP1, which has a higher affinity for and thus depletes GmGIP1 (refs. 87,88). For deeper insights into the role of effector proteins secreted in the apoplast by fungal and oomycete pathogens, we refer the reader to a recent review on this subject[89].

Mirroring the struggle to control the availability of sugars in the apoplast, restriction of nutritive amino acids also constitutes a plant defence response. For example, long-term activation of PTI induces amino acid uptake from the apoplast, at least in part through increased expression of LHT1 and additional H^+/amino acid symporters[82,83]. Beyond the primary metabolites, PTI and ETI also restrict apoplast sulfate and iron[90,91]. This competition for nutrients has been presented as a tug-of-war between plants and pathogens for nutrition-related susceptibility versus resistance[48]. A major open question is whether EDEN simply offsets defence-induced nutrient restriction or promotes a net increase in nutrient availability above the levels in unchallenged

plants. Consistent with the latter, WtsE mobilizes nutrients into the apoplast to levels that are well above baseline and correspond with N and C assimilation during pathogenic growth of *P. stewartii*[8]. The generality of this finding awaits further examination.

Refining the definition of ETS and the associated implications for ETI

The original conception of effector-triggered susceptibility (ETS) focused primarily on suppression of PTI and ETI to limit the amplitude of plant defence[1]. Rather than targeting immune function, EDEN is based on the perturbation of primary plant physiological processes, that is, processes crucial for the growth and development of non-infected plants. Thus, we propose that EDEN is conceptually distinct from EDIS, such that EDEN + EDIS = ETS. In this view, niche establishment results from both EDEN and the subset of EDIS directed at niche-restrictive immune responses, that is, those discussed in the previous section.

On this basis, we propose a model for the relationship between plant immunity, EDIS and EDEN in determining niche quality (Fig. 3). PTI and ETI limit the availability of water and virulence-eliciting and nutritive metabolites. Niche restriction may be the evolutionarily preferred mode of defence actuation for regulating low-abundance or non-aggressive endophytes owing to lower energy costs and reduced autotoxicity relative to more aggressive antimicrobial outputs. Niche-restrictive outputs of ETI may be triggered by effectors engaged in either EDEN or EDIS. Pathogens overcome defensive niche restriction through the collective action of EDEN and a subset of EDIS. This subset of EDIS, which inhibits niche-restricting defence responses, can, at best, restore niche quality to that found in healthy plants but alone is insufficient to drive niche quality beyond the threshold required for pathogenicity. EDEN tips the balance to levels of apoplast hydration and metabolite content sufficient to support high-level pathogen growth.

This conception agrees with a study of the relationship between defence suppression and niche establishment on endophyte colonization of *A. thaliana* plants defective in PTI and/or lacking AtMIN7. EDEN by HopM1 depends on proteasome-mediated elimination of AtMIN7, and *Atmin7*-mutant plants support spontaneous partial water-soaking that facilitates niche establishment. Endophyte levels remained at the same low level in the leaves of wild-type, PTI-deficient or *Atmin7*-mutant plants but increased ~100-fold when PTI deficiency was combined with the *Atmin7* mutation, indicating that both defence suppression and niche establishment are required for endophyte outgrowth[14,92]. Thus, EDEN could potentially lead to niche competition between pathogens and endophytes. Pathogens may overcome this competition through metabolic specialization or the direct antagonism of competitors.

The niche establishment model points to a distinction between types of plant cell death observed during plant–microorganism interactions. Cell death during a hypersensitive response, such as often occurs during ETI, reduces niche quality. Paradoxically, cell death and/or loss of cell membrane integrity also occur during many compatible interactions. We posit that runaway niche establishment creates conditions, such as hypoxia resulting from a hydrated apoplast, imbalanced metabolism resulting from effector perturbations, and/or the depletion of energy and metabolites resulting from the mobilization of metabolites into the apoplast, that lead to necrosis. Thus, plant cell death during interactions with pathogenic microorganisms ranges along a continuum from a hypersensitive response, during which effective resistance is characterized by rapid desiccation and release of antimicrobial compounds that precedes EDEN, to

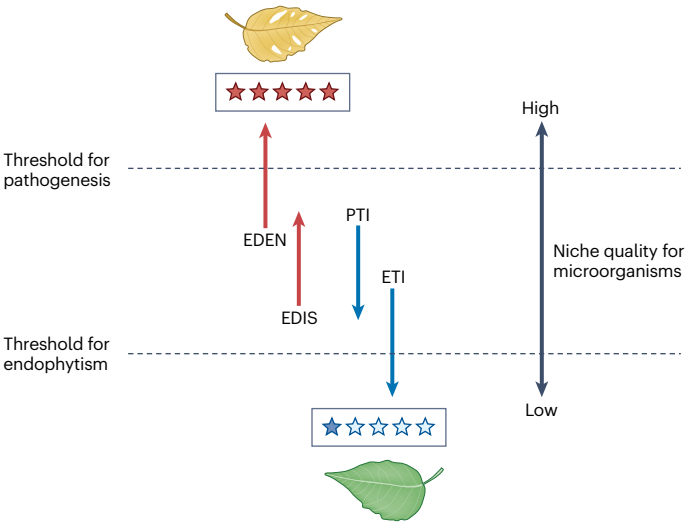


Fig. 3 | Opposing forces of effector-triggered susceptibility and plant immunity determine niche quality. Effector-driven extracellular niche establishment (EDEN) and aspects of plant immune defence compete for control of primary physiological processes that dictate niche quality. EDEN creates a niche capable of supporting pathogen growth by enriching the apoplast in water and nutritive metabolites. At the same time, effector-driven immune suppression (EDIS) reinforces EDEN by suppressing facets of immunity that restrict niche quality. Defence mechanisms (pattern-triggered immunity (PTI) and effector-triggered immunity (ETI)) reduce niche quality by reducing water content and nutrient availability to limit the proliferation of potential pathogens and endophytes. In the case of ETI, this often causes a hypersensitive response that couples antimicrobial responses and desiccation to render the tissue inhospitable. The varying strengths of these opposing forces determine whether the apoplastic niche can support pathogenesis, endophytism or neither. Stars represent the quality of the niche supporting microbial proliferation (five-star being highly suitable for growth and one-star being low and unsuitable for growth).

'disease-associated' cell death that results from EDEN. A rapid hypersensitive response can severely limit pathogen proliferation. A slower hypersensitive response, such as that observed during trailing necrosis in response to filamentous pathogens, more modestly limits pathogen proliferation[93,94]. As plant–pathogen interactions move further along the continuum towards 'disease-associated' cell death resulting from EDEN, a shift from desiccation and the release of antimicrobial compounds towards sustained hydration and the additional release of cytoplasmic nutrients creates conditions favourable for both biotrophic and subsequent necrotrophic growth.

Effectors engaged in either EDEN or EDIS have the potential to elicit ETI, with outputs that are both niche restrictive, for example, reduced water availability, and antimicrobial, for example, phytoalexin production. We suggest that differences in the nature of host perturbations associated with EDEN or EDIS constrain the outputs deployed during ETI. During EDIS, effectors target the plant immune signalling machinery and frequently do so in ways that are not typical in a healthy plant, for example, ADP-ribosylation or proteolytic cleavage of AtRIN4 by AvrRpm1 or AvrRpt2, respectively[95,96]. Detection of such modifications justifies a 'hair-trigger' for extreme responses, such as the rapid hypersensitive response induced by the RPM1 or RPS2 nucleotide oligomerization domain-like receptors in response

to these perturbations of AtRIN4, despite the balanced fitness cost of maintaining these receptors[97–100]. Contrary to EDIS, effectors engaged in EDEN perturb basic plant physiological processes such as ABA signalling, activation of metabolic enzymes or the expression of nutrient transporters. As these regulatory events occur during normal plant growth and development, the negative consequence of rapid and extreme responses to their perturbation would likely cause detrimentally outsized fitness costs.

The mechanisms by which plants combat EDEN align with this prediction. For example, resistance to TALEs that induce transcription of genes regulating host metabolism and physiology occurs by one of two mechanisms. In the first case, loss of effector-binding elements in the promoter of the susceptibility gene, for example, a SWEET transporter, provides recessive resistance based on preventing EDEN[101]. In the second case, evolution of an effector-binding element in the promoter of a novel group of executor genes provides dominant resistance[102–104]. Note that both cases rely on changes to the plant genome and in neither case does the molecular or physiological perturbation underlying EDEN serve as an elicitor of immune activation. The recessive resistance conferred by mutations in TALE targets is essentially the result of incompatibility between host and pathogen. We predict that similar examples may contribute to many cases of non-host or quantitative resistance. That is, these types of resistance may result from pathogen effectors failing to engage with EDEN-related targets that vary between host plants. In fact, engineering plants to be EDEN resistant represents a potentially effective means of preventing disease. Indeed, gene editing of TALE-binding sites in *SWEET* gene promoters has been used to engineer rice resistant to *X. oryzae*[105]. However, translating this approach to other pathosystems will require further research to identify targets of EDEN.

How clear is the distinction between EDEN and EDIS? Some effector outputs specifically promote niche establishment, for example, RipI promoting γ-aminobutyric acid synthesis to support nutrition of *Ralstonia solanacearum* or TALEs targeting the promoters of SWEETs[41,60]. Other effectors specifically suppress plant immune function, for example, AvrPtoB or HopAI1 disrupt PTI signal transduction through the degradation of pattern-recognition receptors or the irreversible dephosphorylation of MPK3 and MPK6, respectively[106,107]. Some effectors may simultaneously engage in both EDEN and EDIS, for example, modulation of host metabolism may facilitate both nutrient acquisition and defence suppression, or the activation of ABA biosynthesis may promote niche establishment through stomatal closure and simultaneously inhibit plant defence through other outputs of ABA such as interaction with the plant stress hormone network[108]. Similarly, several effectors that drive water-soaking also suppress plant immune responses[92,109–112]. For example, in addition to promoting niche establishment through ABA-dependent stomatal closure and apoplast metabolite accumulation, AvrE-family effectors also inhibit outputs of PTI, including salicylic acid signalling and cell wall reinforcement[110,111,113]. The unusually large size of AvrE-family effectors, relative to other T3Es, raises the possibility that these distinct outputs result from effector multifunctionality. Indeed, AvrE-family effectors interact with multiple classes of kinases and phosphatases[7,15]. On the other hand, niche establishment may indirectly suppress some aspects of plant defence. For example, a hydrated apoplast with altered metabolite composition may disrupt cell wall reinforcement or dilute MAMPs, phytoalexins or water-soluble defence signalling molecules such as superoxide or salicylic acid. If so, the longstanding observation that the majority of T3Es, when overexpressed, are defence suppressive[107,114,115] may be explained by the participation of many effectors in EDEN. This supposition, which is plausible given the foundational importance of niche establishment to pathogenesis, points to a functional relationship between niche establishment and suppression of plant immunity.

Conclusion and perspectives

Recent insights into how EDEN modulates the apoplastic landscape have refined our understanding of susceptibility to infection in plants. However, we hypothesize that the concept of EDEN may not apply solely to biotrophic pathogens. Indeed, EDEN could also be extended to the nutritive niche established by necrotrophic pathogens upon induction of cell death in their hosts as it leads to intense microbial growth[116–118]. Considering that necrotrophs could induce EDEN by killing host cells, it would be of great interest to understand how plant immune responses associated with defence against necrotrophy may prevent or modulate cell death to reduce niche establishment. Regardless of pathogen lifestyle, much of our knowledge is based on experimental systems that enable temporally controlled tissue scale analyses but omit certain aspects of natural infections. For example, examining spatiotemporal aspects of infection biology has led to insights regarding the balance between susceptibility and defence[119]. Such approaches, under conditions mimicking natural infections, will also be of interest to understanding EDEN. Likewise, it is interesting to consider parallels between EDEN and mechanisms involved in symbiotic plant–microorganism relationships. Indeed, it is well established that such interactions often involve the establishment of niche structures (for example, nodules and arbuscules) as well as the exchange of nutrients, including the induction of nutrient transporters[120].

Exploring how the concept of EDEN applies to pathogens that colonize the apoplast of compartments other than the mesophyll (that is, the root and vascular apoplasts) will further our understanding of global concepts in pathogenesis. Furthermore, exploring how EDEN influences non-pathogenic microorganisms inhabiting the apoplast and how it may contribute to dysbiosis and exacerbation of disease will be of great interest[121]. The variety of mechanisms through which pathogens are already known to regulate the composition of water and metabolites in the apoplast indicates deep complexity in the conflict to control this environment. As such, we expect that the roles of many more pathogen effectors and host defence mechanisms that regulate niche quality are yet to be characterized. Additionally, multi-omic approaches to assess the pathogen transcriptional and metabolic responses to the apoplast environment and mutational approaches to determine pathogen genes required for survival and growth in this environment will provide insight into how pathogens respond to and exploit the environment created during EDEN[122,123].

Our concept of EDEN as being critical for rendering a host susceptible to infection may also apply to studies exploring pathogen–host interactions in non-plant systems. Animal–pathogen interactions are also characterized by tugs-of-war for nutrients, and animals have developed strategies similar to plants based on restricting nutrient acquisition by pathogens, known as nutritional immunity[124]. The latter has been largely characterized concerning the withholding of metals, particularly iron. Although some animal pathogens have been reported to manipulate host metabolism and nutrient transport to their benefit[125,126], it will be of interest to determine whether they use mechanisms similar to plant pathogens to acquire non-metal nutrients.

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