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The secretome of *Verticillium dahliae* in collusion with plant defence responses modulates Verticillium wilt symptoms

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ABSTRACT

*Verticillium dahliae* is a notorious soil-borne pathogen that enters hosts through the roots and proliferates in the plant water-conducting elements to cause Verticillium wilt. Historically, Verticillium wilt symptoms have been explained by vascular occlusion, due to the accumulation of mycelia and plant biomacromolecule aggregation, and also by phytotoxicity caused by pathogen-secreted toxins. Beyond the direct cytotoxicity of some members of the secretome, this review systematically discusses the roles of the *V. dahliae* secretome in vascular occlusion, including the deposition of polysaccharides as an outcome of plant cell wall destruction, the accumulation of fungal mycelia, and modulation of plant defence responses. By modulating plant defences and hormone levels, the secretome manipulates the vascular environment to induce Verticillium wilt. Thus, the secretome of *V. dahliae* colludes with plant defence responses to modulate Verticillium wilt symptoms, and thereby bridges the historical concepts of both toxin production by the pathogen and vascular occlusion as the cause of wilting symptoms.

**Key words:** vascular pathogen, *Verticillium dahliae*, secretome, toxins, vascular occlusion, Verticillium wilt.

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I. INTRODUCTION

*Verticillium dahliae* is a well-known hemi-biotrophic soil-borne pathogen that infects over 200 hosts and causes billions of dollars in losses annually (Inderbitzin & Subbarao, 2014). The pathogen attacks plants through the roots and colonizes the unique ecological niche of the plant xylem, resulting in Verticillium wilt, the symptoms of which include foliar wilting, chlorosis, stunting, necrosis and vein clearing (Fradin & Thomma, 2006; Chen et al., 2021). Over the past 60 years, two main mechanisms that may explain the induced wilt symptoms, i.e. vascular occlusion and through the involvement of toxins have been advanced in the literature (Fradin & Thomma, 2006; Báidez et al., 2007).

Following successful infection of the plant, *V. dahliae* reaches the xylem and increases its biomass concurrent with plant defence responses that aim to restrict the pathogen, including the production of tyloses and accumulation of a colloidal matrix (Robb, Powell & Street, 1989; Báidez et al., 2007; Yadeta & Thomma, 2013). This eventually leads to vascular occlusion and plant wilting. An alternative view argues that the pathogen delivers toxins, which in this case refers broadly to a wide-array of molecules and complex compounds such as large polysaccharides, protein-lipopolysaccharides, glycoproteins, and enzymes including some that act as effectors that induce plant wilting (Fradin & Thomma, 2006; de Sain & Rep, 2015). Both hypotheses are equally credible (Pegg & Brady, 2002; Fradin & Thomma, 2006; Báidez et al., 2007; de Sain & Rep, 2015), and in fact, the two are tightly interlinked as the secretome, including these toxins, colludes with plant defence responses to cause Verticillium wilt symptoms. Here, we review recent findings and the mechanistic actions of the secretome of *V. dahliae*, which produces conidia, which germinate, ramify and penetrate adjacent vascular cells to continue colonization (Fradin & Thomma, 2006; Yadeta & Thomma, 2013).

Since *V. dahliae* enters the xylem tissue, it can be trapped in the pit cavities at the xylem perforation plates. Because the nutritional value of the xylem fluid is limited, the pathogen draws its nutrition by degrading the vascular cell wall using an abundance of diverse extracellular enzymes encoded within its genome (Klosterman et al., 2011; Chen et al., 2016). The insufficient degradation of the xylem walls and pit membranes causes the deposition of polysaccharides (Fradin & Thomma, 2006; Kubiczek, Starr & Glass, 2014). In addition, this triggers plant defences to produce tyloses and other biomacromolecules (resins, gums, gels, etc.) from neighbouring parenchyma cells, which act as a defensive barricade to restrict pathogen spread (Benhamou, 1995; Yadeta & Thomma, 2013). Analyses of the plant vascular system using electron microscopy during *Verticillium* wilt of tomato (*Lycopersicon esculentum* L.) indicated that terminal occlusion leading to sealing of entire veins is a limiting factor preceding the irreversible appearance of vascular wilt (Robb, Powell & Street, 1989). Thus, the pathogen rapidly proliferates, accompanied by continuous deposition of polysaccharides and plant defence-driven structures and biomacromolecules (tyloses, suberin, etc.) that interfere with the flow of water and nutrition, ultimately leading to plant wilting (Pegg & Brady, 2002; Báidez et al., 2007; Yadeta & Thomma, 2013).

(2) The role of toxins in *Verticillium* wilt

The role of toxins could underlie *Verticillium*-induced foliar wilting was first proposed from results obtained using components from culture filtrates that induced wilt symptoms (Stoddart & Carr, 1966; Meyer & Dubery, 1993). Since then, efforts have been made to elucidate the role of toxins in the mechanisms of foliar wilting (Pegg & Brady, 2002). While some evidence linking toxins to foliar wilting is undeniable, other investigations have yielded ambiguous results (Fradin & Thomma, 2006). For instance, a low molecular weight phytotoxic polypeptide that exhibited toxicity to potato (*Solanum tuberosum* L.) leaves was detected using immunofluorescent antibodies in the xylem of infected potato stems (Nachmias, Buchner & Burstain, 1983). A similar toxin induced the typical foliar wilting when injected into *Arabidopsis thaliana* seedlings (Jing, Fan & Wu, 2005). Incremental progress in understanding the toxins involved has occurred with advances in genomics and metabolomics technologies, and some toxin components have been verified, including a range of proteins with enzymatic or effector activity (Klimes et al., 2015), metabolites (Zhang et al., 2016; Zhang et al., 2019a), and volatile compounds.

(1) The role of vascular occlusion in *Verticillium* wilt

Following successful breaching of the endodermis, *V. dahliae* generally is confined to the fluid environment of the xylem cells and exerts its effects on host physiology indirectly (Yadeta & Thomma, 2013). Unlike prokaryotic vascular pathogens that break out of the vascular system and spread indiscriminately in the root and shoot parenchyma, *V. dahliae* seldom leaves the confines of the xylem until the death of the surrounding tissue or the host (Pegg & Brady, 2002). Key steps in the pathogenesis of *V. dahliae* include germination of microsclerotia, hyphal penetration of the root epidermis, and growth in the cortex that crosses the endodermis finally breaching the highly structured and rigid secondary xylem walls to enter vessels of the root (Zhao et al., 2014). Once in the xylem, the pathogen
**V. dahliae secretome contributes to Verticillium wilt**

(Li & Kang, 2018). Proteins secreted from *V. dahliae* that serve as toxins have been shown to play critical roles in its pathogenesis resulting in foliar wilting through plant cell wall destruction, manipulation of host responses, or cytotoxicity (de Snij & Rep, 2013; Klimes et al., 2015; Chen et al., 2016). The secreted proteins have been suggested to target various sites, including the cell wall, plasma membrane, and other intracellular components (Meyer & Djabery, 1993), and also to induce changes in the microfilaments and microtubules in plant cells (Zhao et al., 2020), leading to rapid cell lesions.

II. FUNCTIONS OF THE *VERTICILLIUM DAHLIAE* SECRETOME

Fungal pathogens secrete numerous proteins (i.e. their secretome) to modulate plant defences by altering host cellular structure and physiology, and to facilitate colonization (Hogenhout et al., 2009). Since *V. dahliae* mainly resides in the vascular tissue during its disease cycle, understanding the role of the secretome in contributing to vascular occlusion or producing toxins could offer a better understanding of the mechanisms that underlie foliar wilting. Genomics-driven research has shown that *V. dahliae* encodes more than 700 potential secreted proteins (Klosterman et al., 2011; Chen et al., 2018), and hundreds are delivered into the extracellular space with generalist functions (Chen et al., 2016). Currently, many secreted proteins have been shown to contribute to pathogenesis, resulting in Verticillium wilt via diverse modes of action (Table 1). Thus, *V. dahliae* employs its secretome to degrade plant cell walls, manipulate host immunity, exert cytotoxicity, neutralize host oxidative stress, compete with its host for nutrients, and even to affect the host microbiome to facilitate Verticillium wilt development (Fig. 1).

*Verticillium dahliae* employs its secretome to proliferate in the low-nutrient confines of the xylem. Its secretome encodes a large arsenal of enzymes that allow the complete degradation of many plant polysaccharides (Klosterman et al., 2011). These processes inevitably induce xylem collapse and the deposition of polysaccharides. The host plants respond by producing biocatalytic molecules (gums, gels, and other resins) and tyloses as defensive structures which protrude into the vascular tissue to block progress of the pathogen. Together, the massive propagation of hyphae, the deposition of polysaccharides and the production of biocatalytic molecules by the plant cause xylem occlusion and plant wilting (Fig. 2).

1) **Proliferation in the plant xylem**

(a) Cell wall degradation

During the infection process, *V. dahliae* must break through plant root cell wall barriers to reach the xylem. During this process, *V. dahliae* produces an array of cell wall degrading enzymes (CWDEs) (Kubicek, Starr & Glass, 2014). Some of these CWDEs destroy the highly structured and rigid secondary xylem walls, and also enable breaches of the pectin-enriched pit cavities or the vascular end walls (Zhao et al., 2014). Additional secreted enzymes include two members of the glycoside hydrolase 12 family, the endoglucanases VdEG1 and VdEG3, which participate in cellulose/hemicellulose degradation (Gui et al., 2017) similar to the CWDEs involved in degradation of the plant cuticle and xylan (Gui et al., 2018; Yin et al., 2021; Wang et al., 2021a). Furthermore, transcription regulators of CWDEs such as the sucrose non-fermenting protein kinase VdSNF1 (Tzima et al., 2021) and fungal-specific transcription factor VdFTF1 (Zhang et al., 2018) regulate CWDEs expression or secretion, and significantly contribute to pathogenesis. The superabundance of secreted carbohydrate-active enzymes suggests an extraordinary capacity of *V. dahliae* to degrade plant cell wall barriers during early infection stages and to degrade plant xylem walls during proliferation in these vessels.

(b) Scavenging host reactive oxygen species

Plants respond to pathogen attack with a transient burst of reactive oxygen species (ROS) that play a central role in plant immune responses (Qi et al., 2017). Pathogens can counteract this by secreting ROS-scavenging proteins to neutralize or degrade ROS, such as superoxide dismutases (SODs), catalases, and peroxidases (Broxton & Culotta, 2016; Khademian & Inlay, 2021). Comparative analyses of the *V. dahliae* secretome have revealed that many secreted proteins with ROS-scavenging and oxidative stress response functions are activated when the pathogen is cultured on host tissue or under nutrient-starvation conditions (El-Bebany, Rampitsch & Daayf, 2010; Chu et al., 2015; Chen et al., 2016). *V. dahliae* secretes SODs to detoxify host-generated extracellular ROS, contributing significantly to virulence during plant infection (Tian et al., 2020, 2021a,b). *V. dahliae* ROS production facilitates penetration fungal and extracellular ROS, contributing significantly to virulence during plant infection (Tian et al., 2020, 2021a,b). *V. dahliae* ROS production facilitates penetration peg formation during the initial colonization of cotton (*Gossypium hirsutum* L.) roots (Zhao, Zhou & Guo, 2016). Thus, the deployment of ROS during infection combined with counter-provisions for neutralization of host-generated oxidative stress is key to successful infection and proliferation in the vascular tissue of host plants.

(c) Suppressing host immunity

The ability to secrete effector proteins that can enter plant cells and manipulate host immunity is a key determinant of a successful plant pathogen (He et al., 2020). In *V. dahliae*, the role of effectors in manipulating host immunity has been studied in detail (Fig. 3). To enable successful infection and proliferation in host plants, *V. dahliae* has evolved a range of effector proteins that inhibit host immune responses (Ding & Redkar, 2018), including inactivation of the salicylic acid (SA) pathway by isochorismate synthase VdIs1 (Liu et al., 2014), cellulose-binding module family 1-member (VdCBM1) (Gu et al., 2017, 2018; Wang et al., 2021b), small cysteine-rich protein VdSCP41 (Qin et al., 2018), lysose acid.  

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Table 1. Description of some secreted proteins involved in various biological processes of *Verticillium dahliae*.

| Secreted protein | Function description | Cell wall degradation | Host immunity manipulation | Hormone homoeostasis | Interference | Cytotoxicity | Oxidative stress neutralization | Fungal nutrition | Morphological development | Microbiome manipulation | Host molecular target | Reference |
|-----------------|----------------------|-----------------------|---------------------------|---------------------|-------------|-------------|-----------------------------|----------------|--------------------------|----------------------|---------------------|----------|
| VD18.5          | Phytotoxic protein   | √                     |                           |                     |             |             |                             |                |                         |                      | Palmer, Saleeba & Lyon (2005) |
| VDH1            | Class II hydrophobin gene |                   |                           |                     |             |             |                             |                |                         |                      | Klimes & Dobinson (2006)    |
| VdEg-1          | Endoglucanase 1      | √                     |                           |                     |             |             |                             |                |                         |                      | Maruthachalam et al. (2011) |
| VdAve1          | Avulence gene of race 1 |                      |                           |                     |             |             |                             |                |                         |                      | de Jonge et al. (2012); Snelders et al. (2020) |
| VdNLP1          | Necrosis- and ethylene-inducing-like protein |                   |                           |                     |             |             |                             |                |                         |                      | Zhou et al. (2012); Santhanam et al. (2013) |
| VdNLP2          | Necrosis- and ethylene-inducing-like protein |                   |                           |                     |             |             |                             |                |                         |                      | Zhou et al. (2012); Santhanam et al. (2013) |
| VdSSP1          | Cell wall degradation related protein |     |                           |                     |             |             |                             |                |                         |                      | Liu et al. (2013)            |
| VdSGC1          | Isorhizomutase       | √                     |                           |                     |             |             |                             |                |                         |                      | Isorhizomutase Liu et al. (2014) |
| VdPLA1          | Pectin lyase         | √                     |                           |                     |             |             |                             |                |                         |                      | Chen et al. (2016)           |
| VdPLA3.1        | Pectin lyase         | √                     |                           |                     |             |             |                             |                |                         |                      | Chen et al. (2016)           |
| VdLysM          | Chitin-binding lysin motif |               |                           |                     |             |             |                             |                |                         |                      | Plant chitinases Kombink et al. (2017) |
| VdASP F2        | Allergen Asp F2-like protein |          |                           |                     |             |             |                             |                |                         |                      | Xie, Li & Yang (2017)        |
| VdCBM1          | Cellulose binding module |                   |                           |                     |             |             |                             |                |                         |                      | Gui et al. (2017, 2018); Wang et al. (2020a) |
| VdCP1           | Cerealo-platanin protein 1 |              |                           |                     |             |             |                             |                |                         |                      | Plant chitinases Zhang et al. (2017a) |
| VdEG1           | GH12 domain-containing protein |               |                           |                     |             |             |                             |                |                         |                      | Gui et al. (2017)           |
| VdEG3           | GH12 domain-containing protein |               |                           |                     |             |             |                             |                |                         |                      | Gui et al. (2017)           |
| VdSCP7          | Small cysteine-rich protein |               |                           |                     |             |             |                             |                |                         |                      | Zhang et al. (2017b)        |
| VdPEL1          | Pectin lyase         | √                     |                           |                     |             |             |                             |                |                         |                      | Yang et al. (2018)          |
| VdPL1           | Polysaccharide lyase | √                     |                           |                     |             |             |                             |                |                         |                      | Zhang et al. (2018)         |
| VdCUT11         | Cattinase            | √                     |                           |                     |             |             |                             |                |                         |                      | Gui et al. (2018)           |
| PevD1           | Alt a 1 family protein |               |                           |                     |             |             |                             |                |                         |                      | Gu et al. (2019)            |
| VdSCP41         | Small cysteine-rich protein |           |                           |                     |             |             |                             |                |                         |                      | CRP60g and SARD1 Qi et al. (2018) |
| VdOCH1          | Alpha-1, 6-mannosyltransferase |               |                           |                     |             |             |                             |                |                         |                      | Zhang et al. (2019b)        |
| VdPDA1          | Polysaccharide deacetylase |           |                           |                     |             |             |                             |                |                         |                      | Chitin oligomer Gao et al. (2019) |
| VdSEP1          | Secretary Ser protease 1 |               |                           |                     |             |             |                             |                |                         |                      | Plant chitinases Han et al. (2019) |
| VdSCP27         | Small cysteine-rich protein |           |                           |                     |             |             |                             |                |                         |                      | Wang et al. (2020a)         |
| VdSCP113        | Small cysteine-rich protein |           |                           |                     |             |             |                             |                |                         |                      | Wang et al. (2020a)         |
| VdSCP126        | Small cysteine-rich protein |           |                           |                     |             |             |                             |                |                         |                      | Wang et al. (2020a)         |
| VdSOD6          | Superoxide dismutase | √                     |                           |                     |             |             |                             |                |                         |                      | Tian et al. (2020)          |
| VdAMP2          | Antimicrobial effector |           |                           |                     |             |             |                             |                |                         |                      | Snelders et al. (2020)      |
| VdSOD5          | Superoxide dismutase | √                     |                           |                     |             |             |                             |                |                         |                      | Tian et al. (2021a)         |
| VdSOD1          | Cu/Zn superoxide dismutase |         |                           |                     |             |             |                             |                |                         |                      | Tian et al. (2021b)         |

(Continues)
(LysM) protein Vd2LysM (Kombrink et al., 2017), secretory serine protease 1 (VdSSEP1) (Han et al., 2019) and polysaccharide deacetylase (VdPDA1) (Gao et al., 2019) (Fig. 3; Table 1). For instance, VdPDA1 deacetylates chitin oligomers; since the N-acetyl group contributes to host LysM-containing receptor recognition for ligand-triggered immunity, the action of VdPDA1 renders the host susceptible to V. dahliae by evading host surveillance through inactivation of a chitin-triggered host immune response (Gao et al., 2019). Together, these effectors mainly function in the early stages of infection and employ a variety of strategies to overcome immunity to facilitate pathogen colonization in roots.

(d) Fungal nutrition

Fungi secrete many plant CWDEs such as cellulase, hemicellulase, and pectin-degrading enzymes that act on plant tissues to produce monomeric and/or small polymeric sugars that are subsequently transported into the fungal cell via membrane-bound transporters (Glass et al., 2013). In this way, V. dahliae obtains nutrition for proliferation in the vascular tissue. Many secreted carbohydrate-active enzymes from V. dahliae catalyse the release of nutrients as the degradation products of cell walls (Klosterman et al., 2011; Xiong, Wang & Tian, 2015; Chen et al., 2016) (Table 1, Fig. 3), some of which function in the generation of energy and acetyl-CoA during nutrient starvation (Chu et al., 2015; Xiong, Wang & Tian, 2015). The V. dahliae alpha-1,6-mannosyltransferase (VdOCH) plays a crucial role in N-linked oligosaccharide glycoprotein metabolism (Zhang et al., 2019b). The abundant pectate lyases secreted by V. dahliae are thought to have key roles in its adaptation to its vascular niche (Klosterman et al., 2011).

(e) Microbiome manipulation

Although pathogen effectors are typically considered to act exclusively through direct host immunity manipulation, plant pathogens also utilize effectors to target the host microbiota in order to facilitate niche colonization (Rovenich, Boshoven & Thomma, 2014; Snelders et al., 2018). Recent work (Snelders et al., 2020, 2021) suggests that V. dahliae exploits secreted proteins to manipulate the host microbiome through its antibacterial or antifungal properties, to promote infection. The V. dahliae avirulence effector (VdAv1) displays antimicrobial activity and facilitates colonization of tomato and cotton through the selective manipulation of the host microbiota in the roots, as well as in the xylem by suppressing antagonistic bacteria. The antimicrobial protein VdAMP2, is exclusively expressed by V. dahliae when in the soil and exerts antibacterial activity that contributes to niche establishment (Snelders et al., 2020). Thus, VdAMP2 and VdAv1 likely have complementary functions for optimal soil and host colonization. Another ancient antimicrobial protein (VdAMP3) is specifically expressed to ward off fungal niche competitors during resting structure formation in senescing host mesophyll tissues (Snelders et al., 2021), allowing V. dahliae to tailor the expression of microbiome-manipulating effectors based on the
microbiota that it encounters during different stages of the disease cycle. This promotes *V. dahliae* colonization and proliferation in host plants to accelerate symptom development.

(2) **Xylem wall collapse and deposition of polysaccharides**

Compared to many fungi, the genome of *V. dahliae* encodes a significant expansion of gene families involved in plant cell wall degradation, and specific members of this repertoire may be essential for adaptation to its vascular niche (Klosterman *et al.*, 2011). For example, among plant cell wall polysaccharides, pectin has the highest structural and functional complexity (Mohnen, 2008; Harholt, 2010), with four major structural classes: homogalacturonan (HG), rhamnogalacturonan I (RG-I), xylogalacturonan (XG), and rhamnogalacturonan II (RG-II). Enzymes required for efficient degradation of pectin (Martens-Uzunova & Schaap, 2009) include polygalacturonases and rhamnogalacturonases (glycoside hydrolases family 28, GH28), polysaccharide lyases (pectin and pectate lyases, PL1, PL2, and PL3), and rhamnogalacturonan lyases (PL4), as well as pectin methylesterases (carbohydrate esterase family 8, CE8), pectin acetyesterases (CE12 and CE13), and rhamnogalacturonan acetyesterases (CE12). Among all sequenced fungal genomes, *V. dahliae* has one of the most complete repertoires of polysaccharide lyases and associated glycoside hydrolases for the breakdown of complex pectin (Klosterman *et al.*, 2011). Experimental evidence indicates that the *V. dahliae* polysaccharide lyase VdPL1 (Zhang *et al.*, 2018), pectate lyase VdPEL1 (Yang *et al.*, 2018), pectin lyases VdPL3.1 and VdPL3.3

*Fig. 1. Versatile biological functions of the secretome in *Verticillium dahliae*. Acetyl-coA, acetyl co-enzyme A; ET, ethylene; JA, jasmonic acid; ROS, reactive oxygen species; SA, salicylic acid.*

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V. dahliae secretome contributes to Verticillium wilt

(Chen et al., 2016), and the pathotype-specific secretory protein VdSSP1 (Liu et al., 2013) are all involved in virulence and pectin degradation. Since pectin is enmeshed with other polysaccharides to form plant cell walls, the result is that incompletely degraded and intermediate polysaccharides contribute directly to vascular occlusion (Kubicek, Starr & Glass, 2014). In addition, in response to infections, pectin gels are usually released into the xylem (Rioux et al., 1998; Clérievet et al., 2000). Similarly, starch hydrolysis also contributes to vessel occlusion related to wilt symptoms in olive (Olea europaea L.) stems of susceptible cultivars infected by V. dahliae (Trapero et al., 2018). Thus, while degradation of the xylem walls and pit membranes by V. dahliae CWDEs supplies nutrients for pathogen proliferation in the low-nutrient confines of the xylem, it also causes xylem collapse and blockage by deposition of insufficiently degraded polysaccharides.

(3) Induction of host response that aggravate vascular occlusion

While secretion of an array of CWDEs is required for breaching cell wall barriers and nutrient acquisition, V. dahliae also must overcome host defence responses. It achieves this with a combination of secreted effectors and interference with host hormone signalling, both of which contribute to vascular occlusion and wilt symptoms. Among plant hormones, SA, jasmonic acid (JA) and ethylene (ET) are all significant in the hormonal regulation of plant immune responses (Shigenaga & Argueso, 2016). SA has been implicated in the protection of plant tissues during the initial biotrophic phase of V. dahliae infection, while JA is more prominent following the establishment of V. dahliae in the xylem and its switch to a necrotrophic lifestyle (Dhar et al., 2020). ET activates defence responses to limit spread of the pathogen during the early stages of V. dahliae infection; but during subsequent colonization and the switch to the necrotrophic phase, ET can facilitate establishment of the pathogen and accelerate the progression of Verticillium wilt (Dhar et al., 2020). The V. dahliae secretome interferes with the intricate and delicate balance of phytohormones during V. dahliae–plant interactions, regulating the immune responses in a synergistic or antagonistic manner that contributes to Verticillium wilt progression (Dhar et al., 2020). Manipulation of SA-JA signalling has been demonstrated for several known effectors in V. dahliae, including four VdSCPs (Wang et al., 2012; Wang et al., 2020a), the cutinase VdCUT11 (Gui et al., 2018), the necrosis- and ethylene-inducing-like protein VdNLP (Wang et al., 2004), the endoglucanases VdEG1 and VdEG3 (Gui et al., 2017), VdSCP7 (Zhang et al., 2017a,b) and the xylanase VdXyn4 (Wang et al., 2021a,b) (Fig. 3). Additionally, VdNLP involved in ET signalling aids in the establishment of the pathogen and accelerates the progression of Verticillium wilt and death of the host (Wang et al., 2004; Dhar et al., 2020), and PevD1 induces ET biosynthesis by directly binding to the senescence-associated NAC transcription factor ORE1 (Zhang et al., 2021).

In response to V. dahliae infection and colonization, the plant may mount a rapid defence including deposition of coating materials such as suberin on vascular cell walls, forming a barrier to fungal penetration and horizontal spread (Robb, Powell & Street, 1989). At this stage, infection can result in vessel occlusion by gums, gels, tyloses and other deposited resins secreted by the neighbouring parenchyma cells (Robb et al., 1979; Benhamou, 1995). In addition, plant hormone levels can be manipulated by effectors secreted by pathogens, influencing plant responses to the pathogen, including vascular occlusion. During plant–pathogen interactions, many phytohormones regulate the formation of coating materials on vascular cell walls, including ET, which is involved in lignin and suberin biosynthesis, and tyloses formation, while abscisic acid coordinates suberin deposition. JA contributes to lignin biosynthesis and tyloses formation, and SA can abolish the induction of tyloses by JA (Sun et al., 2007; Lesniewska et al., 2017; Hu et al., 2018; Liu et al., 2020; Wang et al., 2020b). Some of these regulatory mechanisms have been inferred in V. dahliae–plant interactions (Hu et al., 2019; Dhar et al., 2020). In V. longisporum infections, Arabidopsis thaliana plants can generate xylem de novo by transdifferentiation of the chloroplast-containing bundle sheath cells into functional xylem elements, enhancing water transport, storage capacity, and drought tolerance (Reusche et al., 2012). Whether a similar mechanism exists during V. dahliae–plant interactions with a role in relieving plant wilt symptoms requires further investigation. Together, the secreted effectors from V. dahliae cause host immune responses and disturb the phytohormone balance contributing to vascular occlusion and the induction of Verticillium wilt symptoms (Fig. 2).

(4) Toxicity

A cytotoxic function for all identified secreted proteins is difficult to demonstrate explicitly because a similar plant necrosis phenotype accompanies immunity- and cytotoxin-induced necrosis. While some studies have shown that crude Verticillium culture extracts cause microfilaments and microtubules to collapse in plant cells (Zhao et al., 2020), it remains unclear whether it is toxic activity that directly causes necrosis. A prominent example of a fungal protein with cytotoxic activity is NLP purified from the culture filtrates of Fusarium oxysporum (Bailey, James & James, 1997). A NLP member identified from the mycelia of a V. dahliae strain pathogenic to cotton displayed the ability to cause foliar necrosis in various plant species (Wang et al., 2004). With the advent of genomics, seven additional members of the NLP family have been identified from the V. dahliae genome, two of which cause cellular necrosis (Zhou et al., 2012; Santhanam et al., 2013). The proposed mechanism of NLP-mediated cell lysis is that glycosylsinositol phosphorylceramide (GIPC) sphingolipids act as NLP receptors to form complexes with terminal monomeric hexose moieties of GIPCs that insert into the plant plasma membrane (Ottmann et al., 2009; Lenarčič et al., 2017) (Fig. 3). Additionally, a member of the xylanase family, VdXyn4 induces vein necrosis and plant wilting during advanced stages of infection, but not immunity-triggered cell death, suggesting that it is a
root epidermis and cortical cells, that acts as a functional fungus-host penetration interface for the delivery of secreted fungal proteins (Zhou, Zhao & Guo, 2017). Subsequently, the secretome overcomes host defence responses by manipulating host immunity, interfering in hormone homeostasis, neutralization of oxidative stress, and cytotoxicity. The secreted CWDEs breach the plant cell walls to allow further penetration and proliferation (Fradin & Thomma, 2006). Even with these specialized tools, only a few invading hyphae successfully traverse into the vessel cells (Zhao et al., 2014). In addition, several CWDEs are involved in manipulating host immunity and altering hormone homeostasis (Table 1). Prior to penetration of plant roots, the rhizosphere environment is also crucial for V. dahliae survival and its eventual invasion. Here, effector proteins can suppress the growth of antagonistic bacteria (Snelders et al., 2020, 2021). Thus, “toxic” activities of the V. dahliae secretome are critical for successful penetration, infection, and xylem colonization (Fig. 4).

(2) The vascular battlefield

Once V. dahliae crosses the endodermis and reaches the xylem (generally 2–4 days), the hyphae begin to branch, develop terminal conidia and proliferate in the xylem (Fradin & Thomma, 2006). At this stage, the pathogen channels energy into vegetative growth resulting in hyphal extension and conidia formation in the xylem (Zhao et al., 2014). During this stage, V. dahliae uses its extensive range of CWDEs to survive in the barren environment of the xylem (Klosterman et al., 2011), and also secretes effectors with toxicity functions, for example VdXyn4 acts as a toxin during the colonization of vessels by destroying the host vascular system (Wang et al., 2021a). Concurrently, the host plants respond to the extensive sporulation and proliferation in the xylem by initiating rapid defence responses (Benhamou, 1995; Heinz et al., 1996; Pegg & Brady, 2002). In resistant hosts, colonization and proliferation of the pathogen are hindered by its compartmentalization in the vascular tissue. In susceptible hosts, the pathogen escapes these host traps by counteracting host defences via secreted effectors and hyphal growth that progresses through the occluded xylem (Fradin & Thomma, 2006). In this process, V. dahliae effectors also disturb the xylem microbiome (Snelders et al., 2020, 2021), further accelerating fungal proliferation and initiating a resistance response, that in turn leads to further xylem occlusion. Therefore, in the battle over control of the plant xylem, V. dahliae deploys its secretome to aid mycelial proliferation, resulting in release of incompletely degraded polysaccharides from the plant cell walls and induction of biomacromolecules from a host response, all of which contribute to vascular occlusion and the appearance of wilt symptoms (Fig. 4).

(3) Final victory in the shoot

The successful proliferation of V. dahliae in the xylem results in two damaging effects: vascular occlusion blocking water and nutrient transport; and rapid pathogen transmission to
V. dahliae secretome contributes to Verticillium wilt

Fig. 3. V. dahliae employs its secretome to manipulate host immunity and interfere with hormone homeostasis. See Table 1 for V. dahliae protein names. VdAve1 is recognized as an avirulence determinant by tomato plants that carry the corresponding Ve1 immune receptor. VdEG1 and VdEG3 are glycoside hydrolase 12 proteins that trigger immunity dependent on the LRR-RLPs/SOBIR1/BAK1 and LRR-RLKs/BAK1 complexes, respectively. Vd2LysM binds long- or short-chain chitin oligomers and prevents degradation of chitin by plant chitinase. VdPDAl directs deacetylation of chitin oligomers and inhibits perception by host LysM-containing receptors, thus avoiding ligand-triggered immunity. VdSSEP1 hydrolyses cotton Chi28 directly, inhibiting the production of chitin oligomers. VdEIX3 exhibits immunity-inducing activity in Nicotiana benthamiana, recognized by the leucine-rich repeat receptor-like protein NbEIX2. VdCP1 protects the V. dahliae cell wall from chitinase degradation. VdSCP7 targets the host nucleus to modulate plant immunity. VdSCP11 targets the plant-specific transcription factors CBP60g and SARD1 to modulate immunity. VdISC1 disrupts the plant salicylic metabolism pathway by suppressing the transformation from isochorismate to salicylic acid. VdPL1 plays a virulence function during infection of cotton. VdCBM1 suppresses VdEG1-, VdEG3-, VdSCP27-, VdSCP113-, VdSCP126-, VdCUT11- and VdXyn1-induced cell death and some PAMP-triggered immunity in N. benthamiana. VdCUT11 induces plant defence responses in N. benthamiana in a BAK1- and SOBIR1-dependent manner. VdNLP1 and VdNLP2 are GIPC sphingolipids that act as NLP toxin receptors; NLPs form complexes with terminal monomeric hexose moieties of GIPCs and insert into the plant plasma membrane, causing cell lysis. VdSCP27, VdSCP113 and VdSCP126 induce defence responses in N. benthamiana in a BAK1- and SOBIR1-dependent manner. VdXyn4 plays a cytotoxic function and induces a necrosis phenotype in N. benthamiana, depending on simultaneous localization to the nuclei and chloroplasts in a BAK1- and SOBIR1-dependent manner. VdPEL1 exhibits pectin hydrolytic activity and induces cell death in plants. VdPL3.1/3 have virulence functions during infection of cotton. PevD1 induces ethylene biosynthesis by directly binding to ORE1. VDAL protects transcription factor MYB6 from degradation by interacting with the E3 ligases PUB25 and PUB26 to enhance Verticillium wilt resistance. BAK1, LRR-RLK BRI1-associated kinase-1; CBP60g, calmodulin binding protein 60 family member g; CEBiP, chitin-elicitor binding protein; CERK, receptor chitin elicitor receptor kinase; Chi28, chitinase 28; EIX2, leucine-rich repeat receptor-like protein; ET, ethylene; GIPC, glycosylsphingosine phospholipid; JA, jasmonic acid; LRR, leucine-rich repeat; LysM, lysin motif; MYB6, MYB domain protein 6; NLP, necrosis- and ethylene-inducing-like protein; ORE1, A senescence-associated NAC transcription factor; PAMP, pathogen-associated molecular pattern; PR5, pathogenesis-related protein 5 (PR5)-like protein; PUB25/26, plant U-box 25/26; RLK, receptor-like kinase; RLP, receptor-like protein; SA, salicylic acid; SARD1, systemic acquired resistance deficient 1; SOBIR1, LRR-RLK suppressor of BIR1-1; Ve1, leucine-rich repeat receptor-like protein.
secreting the alt a1 family protein PevD1 to regulate the ORE1–ACS6 (1-aminoacyclopseudoape-1-carboxylate synthase) cascade and enhance ET production (Zhang et al., 2021). In addition, cytotoxicity also plays a critical role in inducing cell necrosis, as VdNLPs are constitutively expressed during later stages of infection (Santhanam et al., 2013), accelerating disease symptoms and the production of microsclerotia to begin a new disease cycle.

V. dahliae effectors contribute to microsclerotia formation in senescing mesophyll tissue by warding off other fungal niche competitors (Snelders et al., 2020, 2021).

IV. CONCLUSIONS

(1) Verticillium wilt caused by V. dahliae is a notorious vascular wilt disease that leads to chlorosis, stunting and wilting. Two notable hypotheses exist regarding the mechanisms underlying Verticillium wilt symptom development: vessel occlusion and direct toxicity through secreted toxins (Fradin & Thomma, 2006; Bádez et al., 2007). Vascular occlusion occurs as a result of the biomass of the pathogen present in the plant xylem, in addition to defensive tylosose or colloid production by the plant (Bádez et al., 2007). The toxin hypothesis suggests that the pathogen delivers complex compounds into its host, including large polymers of polysaccharides, protein-lipopolysaccharides, glycoproteins, enzymes, etc., that induce foliar wilting via cytotoxicity (Fradin & Thomma, 2006; de Sain & Rep, 2015).

(2) Similar to other plant pathogens, the secretome of V. dahliae has evolved to alter host structure and physiology, facilitating colonization. Specifically, components of the secretome of V. dahliae degrade plant cell walls, manipulate host immunity, exert cytotoxicity, neutralize host oxidative stresses, compete with its host for nutrients, and alter host endophytic associations and the rhizosphere microbiome, finally contributing to Verticillium wilt.

(3) Except for some V. dahliae secretome members with direct cytotoxicity, V. dahliae mainly employs its secretome to support proliferation in the plant xylem. These activities contribute to xylem wall collapse and the deposition of polysaccharides, and induction of host plant responses that promote vascular occlusion, finally compromising the host by causing total vascular occlusion. Thus, the V. dahliae secretome manipulates plant
V. dahliae secretome contributes to Verticillium wilt
decides to contribute to vascular occlusion simultaneously with its toxicity effects to cause wilt symptoms.
(4) There are several key phases in the V. dahliae—plant interaction in which the weapons available in the secretome play critical roles in the induction of Verticillium wilt symptoms. In the initial infection zone in the roots, these effector activities specifically counter plant defences, neutralize oxidative stress, manipulate host immunity, and interfere with hormone homeostasis. Subsequently, in the vascular battlefield, the secretome aids mycelial proliferation, the release of incompletely degraded polysaccharides from plant cell walls and the induction of biomacromolecules from an active host defence response, all of which contribute to vascular occlusion. Final victory in shoot occurs as the toxic functions of defence response, all of which contribute to vascular occlusion.

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