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REVIEW

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Apple Valsa canker: insights into pathogenesis and disease control

Hao Feng[1], Chengli Wang[1], Yanting He[1], Lin Tang[1], Pengliang Han[1], Jiahao Liang[1] and Lili Huang[1*]

Abstract

Apple Valsa canker (AVC) has caused significant losses worldwide, especially in East Asia. Various fungal species from the genus *Cytospora/Valsa* can infect tree bark and cause tissue rot, and *Valsa mali* (*Vm*) is responsible for the most severe tree branch deaths and yield losses. Since AVC was first reported in Japan in 1903, the pathogen species, biological characteristics, infection and pathogenesis, spore dissemination, and disease cycle have been intensively investigated. Based on the new cognition of the disease dynamics, the disease control strategy has shifted from scraping diseased tissue to protecting the bark from infection. In this review, we summarize new knowledge of the *Vm* infection process mediated by various kinds of virulence factors, including cell wall degrading enzymes, toxins, effectors, microRNA-like RNAs, and pathogenic signaling regulators. We also introduce progress in evaluating germplasm resources and identifying disease response-related genes in apples. In addition, we elaborate current understanding of spore dissemination and disease cycles in orchards and disease prevention techniques. Finally, we provide recommendations for developing more cost-effective strategies for controlling AVC by applying genetic resistance and biological fungicides.

Keywords Disease resistance, *Malus domestica*, Virulence factors, *Valsa mali*

Background

Apple (*Malus domestica* Borkh) is one of the world's most widely planted fruit crops (Cornille et al. 2014; Daccache et al. 2020). China is the largest producer and consumer of apples globally. According to the most recent statistical data, the total production was 47.57 million tons in 2022 (http://www.stats.gov.cn/), accounting for almost half of the world's apple production. However, fruit quality and yield per unit area in China are much lower than in other agriculturally developed countries. The main reason for this is thought to be the fungal disease, apple Valsa canker (AVC), which occurs in almost every apple-growing region each year, usually with an incidence of more than 50% (sometimes even 100%), significantly limiting the production (Chen et al. 1982a; Vasilyeva and Kim 2000; Wang et al. 2005, 2020; Cao et al. 2009; Zhang et al. 2014; Xu et al.2020a).

AVC was first reported in Japan in 1903, where it caused severe damage and economic losses in orchards (Ideta 1909; Tanaka 1918; Togashi 1925). Later, AVC was reported in many countries, including the United States, Korea, Iran, Canada, England and South Africa (Stevens 1919; Leonian 1921; Nakata and Takimoto 1928; Fisher and Reeves 1931; Ogilvie 1933; Leyendecker 1952; Proffer and Jones 1989; Brown-Rytlewski and McManus 2000; Adams et al. 2006; Fotouhifar et al. 2010). In China, AVC was first discovered in Liaoning province in 1916, and large numbers of fruit trees were destroyed, resulting in huge economic losses (Liu et al. 1979; Chen et al. 1982a).

*Correspondence:
Lili Huang
huanglili@nwsuaf.edu.cn
[1] State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, China

A variety of fungal species from the genus *Cytospora*/*Valsa* have been associated with AVC, but *V. mali* (*Vm*) is believed to be the most devastating in China (Lu 1992; Wang et al. 2011, 2014a; Gui et al. 2015; Ma et al. 2018; Liu et al. 2020; Pan et al. 2020; Li et al. 2022). AVC caused by *Vm* mainly occurs in the stems and large branches of trees. The bark surface is often wet and becomes slightly uplifted; the bark tissues become putrefied and easily ruptured. The infected tissues then gradually dry out, collapse slightly, and finally form localized cankers. Under high levels of disease, branches and trees may wither or die (Fig. 1). This review summarizes the current knowledge of AVC, especially regarding the pathogenic mechanism of *Vm* and disease control techniques.

The colonization of *Vm* can occur not only in the bark tissues but also in the xylem

Early evidence indicated that ascospores and conidia of *Vm* could invade the tissues only through macroscopic wounds, such as pruning wounds, and dead tissues, such as rhytidomes (Tamura et al. 1973; Sakuma 1978; Chen et al. 1981). Subsequently, it was thought that pruning wounds, especially when fresh, were the main invasion points (Wang et al. 2016b). However, histocytological analysis showed that the conidial germination tubes or hyphae from germinated conidia could invade through tiny wounds, natural openings, and microscopic ostioles on the bark surface (Fig. 2). The hyphae further colonized the cortical parenchyma cells and phloem tissues by both inter- and intracellular hyphal growth, and even invaded the xylem (Ke et al. 2013). These new findings revealed a need for more detailed analysis of the infection process and development of new prevention strategies.

Various 'weapons' of *Vm* help it invade and colonize the stem tissue

Pathogens have evolved many 'weapons' to overcome host immune systems and permit successful infection. Based on genome and transcriptome analyses, many virulence determinants have been predicted to be associated with *Vm* infection and colonization; these include cell wall degrading enzymes, toxins and secondary metabolic synthesis-related enzymes, and various effectors (Ke et al. 2014; Yin et al. 2015, 2016b; Sun et al. 2022). Traditionally, *V. mali* is considered saprophytic fungi or weakly parasitic fungi, and its infection mainly depends on the pectinases and toxins. As the research continues, more and more evidence show that the pathogen can secrete effectors to regulate host immunity with the characteristics of biotrophic fungi. Thus, we speculate that there may be a parasitic stage in the early stage of infection.

Effector proteins: pathogenic factors that attack the host immune system

Effectors are a class of proteins or small molecules secreted by pathogens to facilitate infection and/or trigger defense responses by altering the cell structures or metabolic pathways of host plants (Jones and Dangl 2006; Kamoun 2007; Vleeshouwers and Oliver 2014). Yin et al. (2015) identified 193 candidate effector genes in the *Vm* genome using bioinformatic methods. *VmEP1* was the first effector gene found to be associated with virulence of *Vm* (Li et al. 2015). Since then, several more have been functionally analyzed, and deletion of *VmPOD3*,

Fig. 1 Field symptoms of AVC. AVC caused by *Vm* occurs mainly on the trunks and large branches of trees. The bark surface is often wet and slightly raised; the tissues become putrid and easily split. The infected tissues gradually dry out, collapse slightly, and finally form localised cankers. If the disease is severe, branches wither and die, and trees may die

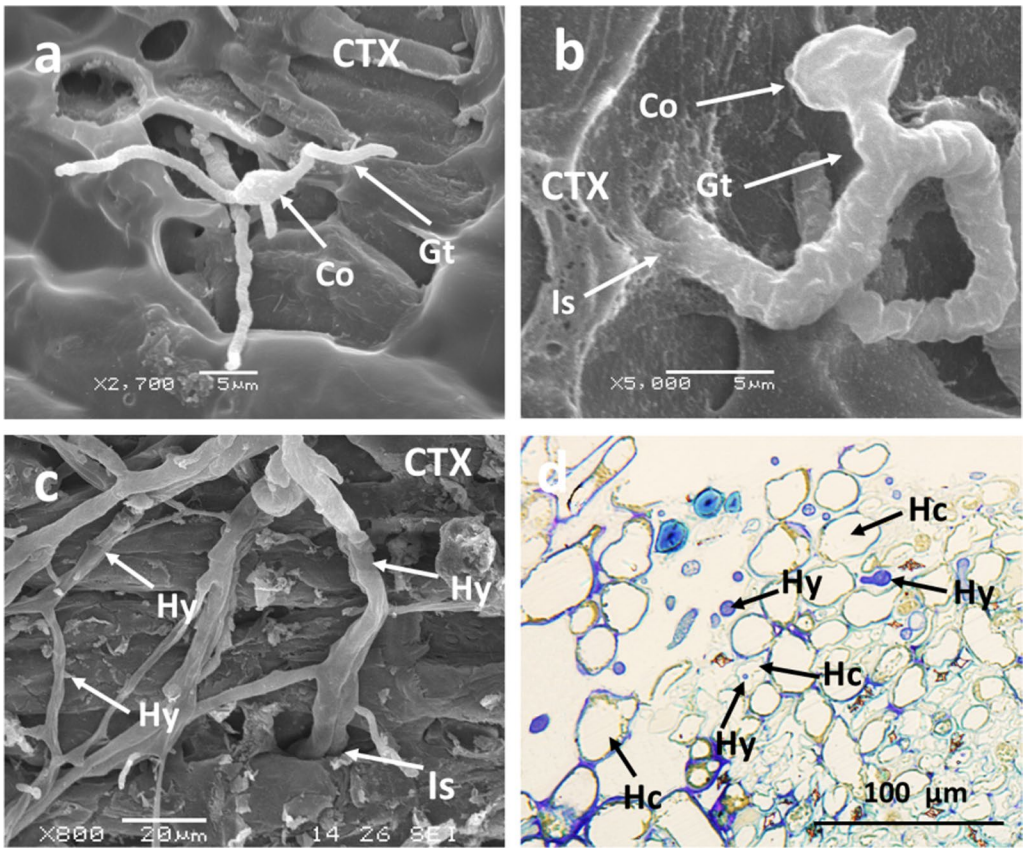

Fig. 2 Conidial germination of *Vm* and infection to apple bark tissues. **a** Conidium (Co) germinates to form germ tubes (Gt) that penetrate the cortex (CTX) through ostioles. **b** Germ tubes (Gt) invading dead host tissue at an infection site (Is). **c** Spread of pathogenic hyphae (Hy) in the cortex (CTX) and the production of new invasion site (Is). **d** Hyphae (Hy) invade the bark tissues and spread in host cells (Hc) and intercellular spaces, causing tissue decay

VmNLP2, *VmPR1a*, or *VmPR1c* significantly reduces the virulence of *Vm* (Feng et al. 2018; Liu et al. 2021a; Wang et al. 2021a). Further studies show that VmPxE1 and VmHEP1 promote infection of apples by targeting the apple L-ascorbate peroxidase MdAPX1 and leucine-rich repeat structure receptor kinase MdLRRP1, respectively (Zhang et al. 2018a, 2019a). It has been demonstrated that VmEP1 contributes to virulence by targeting pathogenesis-related protein 10 and K homology (KH) domain-containing proteins (MdKRBP4) in apple to inhibit the accumulation of reactive oxygen species and reduce callus development, and Vm1G-1794 competes with MdEF-Tu to target MdATG8i and prevent MdEF-Tu degradation, in turn, promoting susceptibility of apple to *Vm* (Wang et al. 2021b; Che et al. 2022). In addition, the small cysteine-rich protein VmE02 has been discovered, and the receptor-like protein RE02 in *N. benthamiana* was shown to be necessary for VmE02-induced necrosis and immune responses (Nie et al. 2019, 2021). The research also found that VmNIS1 is an immunity elicitor with no obvious influence on *Vm* virulence; however, a homolog, VmNIS2, was confirmed to be an immunity suppressor and a contributor to pathogen virulence (Nie et al. 2022). Although the functions of some effector proteins have been elucidated, the molecular mechanisms are still poorly understood, especially the interrelationships between many effector proteins during infection are still unclear (Fig. 3).

MicroRNA-like RNAs: virulence modulators that regulate pathogenic factor expression or confer cross-kingdom interference with host immunity

RNA interference (RNAi) is an ancient and conserved mechanism that affects many biological processes in most eukaryotes (Carthew and Sontheimer 2009; Jin and Zhu 2010; Li et al. 2017). The main components of the RNAi pathway are Dicers, Argonautes (AGOs), and RNA-dependent RNA polymerases (RdRPs), which are responsible for small RNA generation, target gene repression, and silencing signal amplification, respectively (Cerutti and Casas-Mollano 2006;

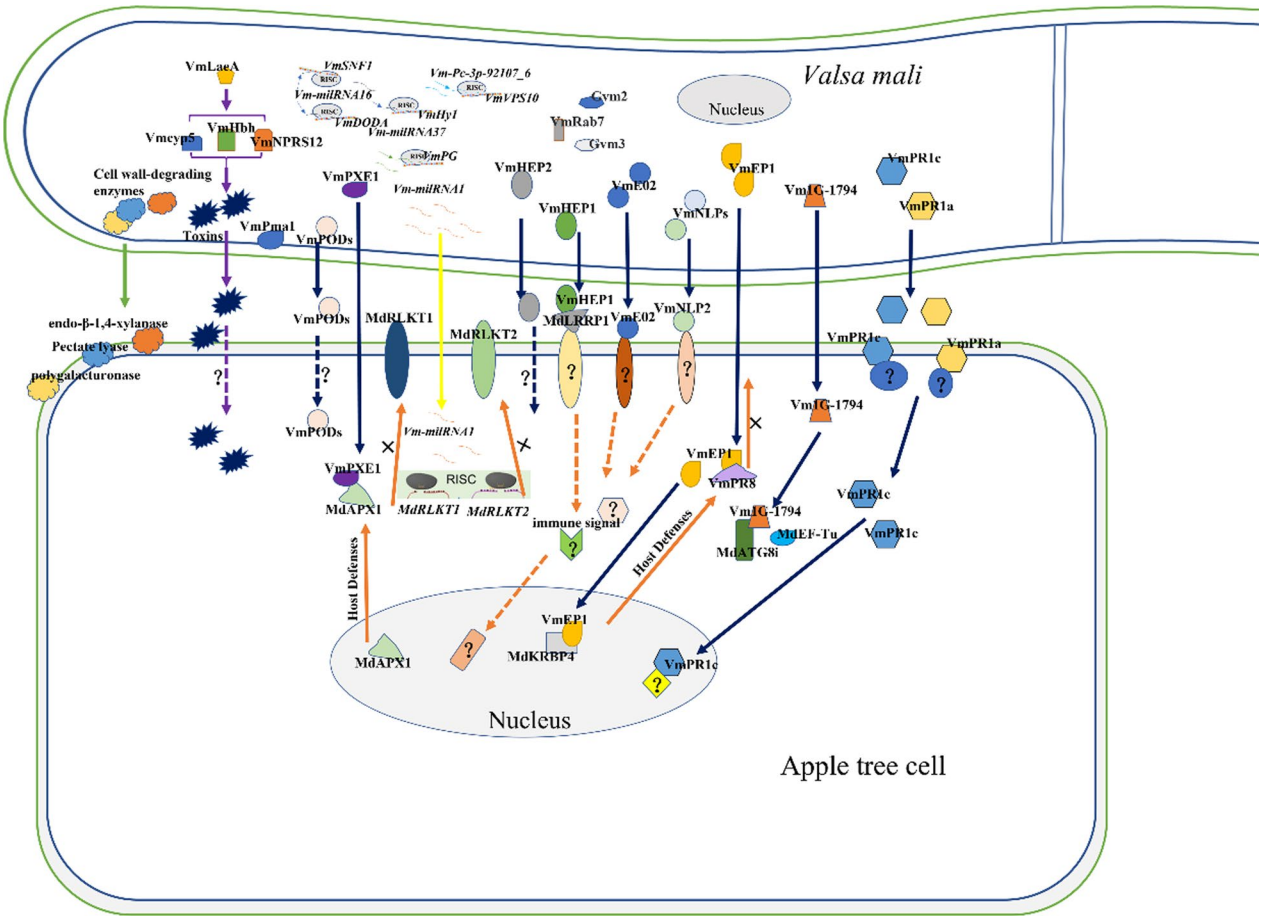

Fig. 3 Pathogenicity mechanisms of *Vm*. The action pathways of cell wall degrading enzymes, such as endo-β-1, 4-xylanase and pectate lyase, are shown using green arrows. Regulatory, synthetic, and secretory pathways of toxins are shown with purple arrows. Functional mechanisms of milRNAs that suppress endogenous genes in *Vm* and inhibit the apple resistance-related genes in a cross-kingdom manner are shown using yellow arrows. The action of the secreted proteins, including effectors and elicitors, are shown using blue arrows. Regulators, such as *Gvm2*, *Gvm3*, *VmVeA,* and *VmVelB*, are labeled using different shapes and colors. Immunity signal molecules and immunity-related proteins are also labeled using different shapes and colors. The transmission of host immunity signals is indicated using orange dashed lines

Ronemus et al. 2006; Shabalina and Koonin 2008). Previous studies have shown that there are two Dicer-like genes (*VmDCL1* and *VmDCL2*) and three AGO genes (*VmAGO1*, *VmAGO2*, and *VmAGO3*) in *Vm*, which play important roles in growth, virulence, and small RNA (sRNA) generation (Feng et al. 2017a, b). Based on this, the VmDCL2-dependent microRNA-like RNA (milRNA), *Vm*-Pc-3p-92107_6, was found to participate in infection by interfering with the expression of virulence factor VmVPS10 (Guo et al. 2021). Meanwhile, various milRNAs differentially expressed during pathogen vegetative growth and infection have been identified. Among them, *Vm*-milR37 has a role in virulence by regulating the expression of glutathione peroxidase gene *VmGP*, which contributes to the oxidative stress response during infection (Feng et al. 2021). A core milRNA *Vm*-milR16 increases the expression levels of several virulence factors (such as *VmSNF1*, *VmDODA*, and *VmHy1*) by reducing its expression during the infection process to improve virulence (Xu et al. 2020b). Further, the effector VmSP1 has also been found to be regulated by *Vm*-milR16 to facilitate the infection by targeting an apple defense-related gene *MdbHLH189* (Xu et al. 2023). In addition, milRNAs regulate the expression of host immunity-related genes to promote disease. Currently, the milR1 is the only known milRNA promoting virulence in *Vm,* and it acts by inhibiting host resistance-related genes *MdRLKT1* and *MdRLKT2* in a cross-kingdom regulatory manner (Xu et al. 2022b). The milRNAs and their functions have been identified in tree stem disease systems (Fig. 3). However, a diagram of the detailed regulatory

network needs to be illustrated, and the relationship between small RNAs and other pathogenic factors needs to be elucidated.

Cell wall degrading enzymes: virulence factors that disrupt host resistance by degrading cell walls

Plant pathogens produce an array of cell wall degrading enzymes (CWDEs) that enable them to penetrate host tissues by degrading wax, cuticular tissue, and cell walls. Pectinase was the earliest virulence factor identified in *Vm* (Fig. 3), and early work mainly focused on enzyme isolation and activity studies (Liu et al. 1980). More work based on genome and transcriptome analyses indicated that a large number of CWDE genes are significantly upregulated during *Vm* infection, especially pectinase genes (Ke et al. 2014; Yin et al. 2015). Histological and cytological investigations also indicated that pectinases play a vital role in *Vm* infection and colonization (Ke et al. 2013). Further research showed that deletion of pectate lyase gene *Vmpl4,* polygalacturonase genes *Vmpg7* and *Vmpg8*, and endo-β-1,4-xylanase gene *VmXyl1* result in reduced virulence of *Vm* (Xu et al. 2016, 2017; Yu et al. 2018).

Toxins: powerful molecules that kill host cells

Toxins, mainly secondary metabolites produced by some plant pathogenic fungi, can mediate pathogen infection by changing cell membrane permeability and disrupting host mitochondria, chloroplasts, and other ultra-structures (Tsuge et al. 2013). Previous studies on *Vm* toxins mainly focused on the isolation and identification of toxin compounds. Various toxins have been isolated and identified, including *p*-hydroxybenzoic acid, *p*-hydroxyacetophenone, phloroglucinol, 3-(*p*-hydroxyphenyl) propionic acid, protocatechuic acid, 1-(3′-vinyl-phenyl)-1,2-ethylene glycol, and isocoumarin derivatives (Koganezawa and Sakuma 1982; Natsume et al. 1982; Wang et al. 2014b; Zhen et al. 2017). Four volatile substances (isoamyl alcohol, 4-ethyl-2-methoxyphenol, 2-phenylethanol, and 4-ethylphenol) from *Vm*, were also toxic to apples (Li et al. 2018). Two further compounds, ethyl *p*-hydroxyphenylpropionate and ethyl *p*-hydroxycinnamate, recently isolated from fermentation broths of *Vm*, exhibited toxicity against both host and non-host plants (Zhang et al. 2022). However, the target sites and mechanisms of toxicity remain unclear (Fig. 3).

Some research has focused on the synthesis pathways of toxins. Fungal secondary metabolites can be divided into four categories: non-ribosomal peptides (NRPs), polyketides (PKs), terpenes, and alkaloids (Brakhage 2013). Whole genome sequencing and transcriptome analysis have shown that *Vm* contains abundant gene clusters for the biosynthesis of PKs, NRPS, and other secondary metabolites, and most of them are significantly upregulated during *Vm* infection (Ke et al. 2014; Yin et al. 2015). More importantly, the virulence of *Vm* decreased significantly when the non-ribosomal peptide synthase gene *VmNRPS12* or CYP450 gene *Vmcyp5*, *VmHbh1*, and *VmHbh4* were individually knocked out (Ma et al. 2016; Gao et al. 2018; Meng et al. 2021). *VmLaeA* regulates more than half of the secondary metabolite gene clusters and is essential to virulence (Feng et al. 2020); however, it is unclear whether the deletion of secondary metabolite genes affects the production of toxins and thus reduces virulence.

In addition to the main pathogenic factors mentioned above, important signal transduction and regulatory factors may also be involved in the virulence of *Vm* (Fig. 3). For example, G protein α subunit genes *Gvm2* and *Gvm3*, mitogen-activated protein kinase gene *VmPmk1*, and velvet protein family genes *VeA* and *VelB*, affect virulence by altering the expression of several CWDE genes, especially pectinase genes (Song et al. 2017; Wu et al. 2017, 2018a). Transcription factor *VmSeb1* affects the virulence of *Vm* by regulating the expression of melanin genes (Wu et al. 2018b). In addition, *VmPacC* and *VmPma1* participate in virulence by acidification (Wu et al. 2018c; Zhang et al. 2023), and *VmRab7*, *VmMon1,* and *VmCcz1* affect virulence through vacuolar fusion and autophagy (Zhang et al. 2021; Xu et al. 2022a).

Various apple germplasms and genes are associated with resistance to AVC

The cultivation of disease-resistant varieties is the most effective and economical way to control AVC. Although many apple varieties have been evaluated for resistance to AVC by different methods (Bessho et al. 1994; Wei et al. 2010), no immune cultivar (or rootstock) has been found; however, there are significant differences in disease responses, and some germplasms show good levels of resistance, including *Malus sikkimimensis*, *M. hupehensis*, *M. sieboldii*, *M. hupehensis*, *M. baccata* cv. 'Kelegou Baccata LF', *M. domestica* cv. 'Aomori Early', *M. domestica* cv. 'Tsugalu', and others (Abe et al. 2007; Li et al. 1991; Liu et al. 1990, 2011; Zhang et al. 2019b).

Identification of disease resistance genes is key to creating *Vm*-resistant varieties. However, our knowledge of the genetics of apple resistance to *Vm* is scant. Transcriptome analyses have suggested a large number of potential resistance-related genes involved in the regulation of resistance to AVC (Yin et al. 2016b; Wang et al. 2022a). The transcription factors MdMYB88 and MdMYB124, pathogenesis-related MdPR10, receptor-like kinase MdSRLK3, MdRLKT1 and MdRLKT2, and K

homology domain-containing protein MdKRBP4 function as positive regulatory factors in AVC response in transient overexpression analysis (Geng et al. 2020; Wang et al. 2021b; Xu et al. 2022; Han et al. 2022). In addition, apple receptor-like kinase gene *MdMRLK2* and the BR-signaling kinase gene *MdBSK1*, UDP-GLUCOSE: PHLORETIN 2'-O-glucosyltransferase gene *MdUGT88F1*, and cyclic nucleotide-gated ion channel genes *MdCN11* and *MdCN19* were found to negatively regulate the resistance of apple to AVC (Zhou et al. 2019; Mao et al. 2021; Jing et al. 2022; Wang et al. 2022a). However, none of these genes has been used in developing an AVC-resistant apple variety.

Prevention of AVC based on new information

A detailed understanding of the disease dynamics is essential for the development of disease prevention and control technologies. In addition to diseased plant tissues, branches and twigs pruned from trees are the main sources of overwintering of *Vm* in orchards. Conidia and ascospores released under rainfall or high humidity are dispersed by raindrops, wind, and insects (Wang et al. 1988). Moreover, conidia can be produced in enormous numbers and disseminated year-round, especially during the flowering period (i.e., April in Shaanxi Province) (Du et al. 2013). Infection occurs most likely between the petal fall and the young fruit stage. Since infection mainly occurs through small cracks and ostiole, it is essential to protect the bark surface (Ke et al. 2013). Further spread of the pathogen within the tree following initial infection depends largely on the vigor of the tree (Chen et al. 1982b; Tamura and Saito 1982; Du et al. 2013). AVC has latent infection features. More than 50% of infections can be asymptomatic, which is an important reason for the continued high incidence of the disease (Liu et al. 1979; Chen et al. 1981; Zang et al. 2012; Zhang et al. 2018b; Meng et al. 2019; Xu et al. 2021). Thus, slowing disease development by maintaining tree vigor is also an important measure in reducing AVC.

Previous control methods for AVC mainly focus on scraping to remove diseased tissues and applying various chemical agents to the scraped wounds (Chen 1980; Chen et al. 1981; Liu et al. 1988; Liu et al. 1992; Wang et al. 2009; Jiao et al. 2015; Yuan et al. 2017). However, this does not solve the problem because *Vm* can infect the xylem. Thus, more active prevention is required. First, it is essential to reduce the pathogen source by removing dead trees or branches and pruning residues from orchards. These should be collected in the early spring and taken away from orchards to prevent further reproduction and spread of the pathogen. Next, it is critical to protect the bark surface with fungicides such as tebuconazole, difenoconazole, and pyraclostrobin after the peak period of pathogen dissemination and infection during the young fruit development stage (generally June–August) (Fig. 4) (Feng et al. 2020; Jiao et al. 2015). If the disease is severe, the fungicide concentrations should be appropriately increased and used for two years, with applications carried out 2–3 times at intervals of 10–15 days (Jiao et al. 2015; Wang et al. 2019). These high concentrations should not be applied to the leaves and fruits to avoid fungicide injury. Bio-control measures for AVC, such as the use of *Saccharothrix yanglingensis* Hhs.015, *Bacillus velezensis* D4, or *Bacillus subtilis* E1R-J, have been explored (Gao et al. 2009; Li et al. 2016; Wang et al. 2016a; Yan et al. 2017; Liu et al. 2018, 2021b). Third, it is also important to reduce disease by delaying the extension of *Vm* in tree tissues. Measures that help

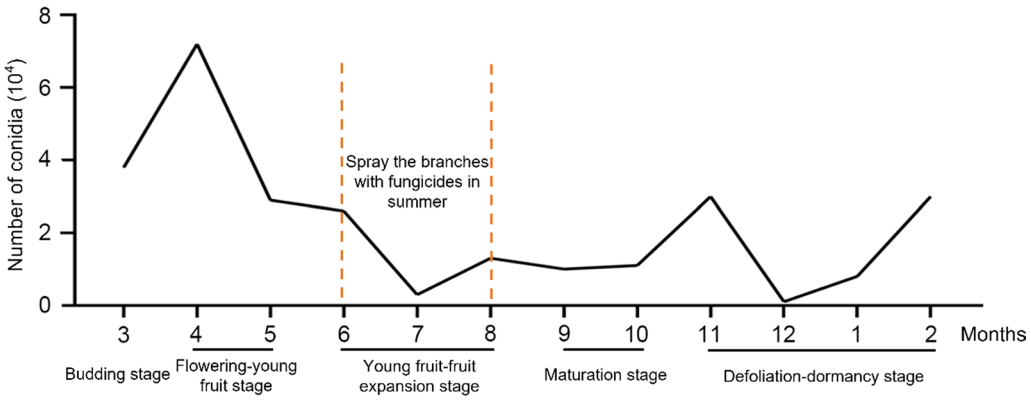

Fig. 4 Annual production and dissemination of conidia and the critical periods for preventing infection. Conidia and ascospores of *Vm* released from the bark surface during rainfall or high humidity are dispersed by raindrops, wind, and insects. Conidia can be produced in enormous numbers and dispersed throughout the year, especially during the flowering season (i.e., April in Shaanxi Province). Therefore, it is critical to protect the bark surface with fungicides such as tebuconazole, difenoconazole, and pyraclostrobin after the peak period of pathogen dissemination and infection during young fruit development (generally June–August)

to improve tree vigor can effectively suppress the extension of *Vm*. It has been found that high concentrations of potassium ions contribute to tree vigor and resistance-enhancing effects of potassium slowing pathogen extension in the infected tissue (Peng et al. 2016; Du et al. 2023). Finally, disease resistance levels can be improved by the application of biological fertilizers during the tree budding stage and resistance-inducing compounds such as chitosan oligosaccharide at young fruiting and fruit expansion stages (Darvill et al. 1992; Creelman et al. 1997; Hu et al. 2015; Yang et al. 2022).

Conclusion and future perspectives

AVC is a destructive fungal disease that severely threatens apple production in East Asian countries. This review summarizes our understanding of the pathogenic mechanisms of AVC and discusses various approaches for the disease control. Compared to many other major crop diseases, little attention has been given to AVC. More basic research is required to provide a better theoretical basis for sustainable AVC control, which will demand a clearer understanding of the pathogenesis to identify the key pathogenic factors. Based on this, disease control can be achieved by silencing critical pathogenic factors through host- or spray-induced gene silencing to prevent infection and spread of *Vm* within infection sites. At the same time, the specific pathogenic factors could also provide new targets for developing new agents. In terms of host plants, more research is needed to identify resistance genes, not just based on genetic markers of disease-resistant germplasms, but more importantly, based on the analysis of the host immune system regulated by pathogenic factors of *Vm*. The use of disease resistance genes should be increased via molecular breeding technologies. Host susceptibility genes are now being discovered in various crop plants, and there may also be opportunities for their manipulation using genome editing technology in apple plants to improve resistance levels against AVC. In terms of disease control, it is of great significance to develop accurate monitoring and early warning technology for guiding disease prevention. Meanwhile, newer technologies such as immunity induction agents and broad-spectrum bio-control agents with low ecological and environmental impact should be investigated and applied where possible.

Abbreviations

AGOs	Argonautes
AVC	Apple Valsa canker
Co	Conidia
CRISPR-Cas	Clustered regularly interspaced palindromic repeats/CRISPR-associated proteins system
CTX	Cortex
CWDEs	Cell wall degrading enzymes
DCLs	Dicers
Gt	Germ tubes
Hc	Host cell
HIGS	Host-induced gene silencing
Hy	Hyphae
MilRNAs	MicroRNA-like RNAs
NRPs	Non-ribosomal peptides
PAMP	Pathogen-associated molecular pattern
PKs	Polyketides
RdRP	RNA-dependent RNA polymerase
RNAi	RNA interference
SIGS	Spray-induced gene silencing
Vm	*Valsa mali*

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Author contributions

HF and LH were responsible for conceptualizing, integrating, writing, and revising this manuscript. CW, YH, LT, PH, and JL were responsible for finding and summarizing the literature as well as initially writing the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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