Fungal Biology Reviews 55 (2026) 100471

Contents lists available at ScienceDirect

Fungal Biology Reviews

journal homepage: www.elsevier.com/locate/fbr

Dematophora necatrix: From taxonomy to molecular advances

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ARTICLE INFO

Keywords:
Dematophora necatrix, Genetic transformation
Genomes
Proteomics
Rosellinia necatrix
Secondary metabolites
Transcriptomics
White root rot

ABSTRACT

Dematophora necatrix Hartig is a destructive soil-borne fungus responsible for White Root Rot (WRR), affecting more than 350 plant species across 51 countries, including many economically important crops. The pathogen's persistence in soil and broad host range makes it especially challenging to control. Over the past decade, molecular studies have significantly advanced our understanding of the pathogen's biology and its interactions with host plants. These developments underscore the need for a comprehensive review to consolidate recent scientific progress. First, we outline the taxonomy, biology, disease symptoms, hosts and global distribution, and current management strategies of *D. necatrix*. We then focus on recent molecular advances, highlighting how genomics, transcriptomics, proteomics and metabolomics studies have improved our understanding of the pathogen's virulence and pathogenicity. A high-quality, chromosome-level genome assembly has enabled more precise annotation and gene prediction. Transcriptomic analyses have identified candidate pathogenicity-related genes and putative effectors, while secretome proteomic studies suggest the production of antimicrobial proteins which may facilitate infection by suppressing microbial competitors. Secondary metabolites, such as cytochalasin E, have been implicated in virulence, although their precise roles in pathogenicity remain unresolved. Improved transformation protocols now permit targeted gene manipulation, creating new opportunities for functional studies. Lastly, this review highlights key knowledge gaps and calls for integrated multi-omics approaches to better understand *D. necatrix* pathogenicity and virulence, long-term survival, and environmental adaptation. Such insights are critical for the development of durable, targeted strategies to manage WRR.

1. Introduction

Dematophora necatrix Hartig (Xylariaceae, Xylariales, Sordariomycetes, Ascomycota) is a highly destructive necrotrophic, soil-borne root pathogen that causes White Root Rot (WRR) in a wide range of commercially important plant species, including fruit, nut and forest trees, as well as various crops and ornamental plants (Arjona-López, 2022; CABI, 2022; USDA Fungal Database). WRR is associated with substantial economic losses, with recent estimates indicating annual reductions in fruit production equalling approximately €5.7 million in Portugal (Pimentel et al., 2021).

Infection begins in the roots and progresses to cause a suite of aboveground symptoms, including chlorosis, wilting, and dieback, ultimately resulting in the death of the host plant (Sztejnberg et al., 1987; Pérez-Jiménez, 2006; Pliego et al., 2009; Arjona-Girona and López-Herrera, 2018; Sawant et al., 2021; Fusco et al., 2022; Hartley et al., 2022; Balocchi et al., 2025; van den Berg et al., 2025). Once established in the soil, *D. necatrix* is extremely difficult to eradicate (Khan, 1959; ten Hoopen and Krauss, 2006; Ruano-Rosa et al., 2018), and management of WRR typically relies on integrated control strategies that combine chemical, physical, and cultural methods (Ruano-Rosa et al., 2018; Magagula et al., 2021).

In recent years, there has been a growing shift toward molecular approaches to better understand the mechanisms underlying the pathogenicity and virulence of *D. necatrix* (Pliego et al., 2009; Shimizu et al., 2014, 2018; Arjona-Girona et al., 2017; Kim et al., 2017; Eom et al., 2018; Zumaquero et al., 2019a; Pourmoghaddam et al., 2022; Wingfield et al., 2022; Chavarro-Carrero et al., 2024). These studies aim to uncover how the fungus interacts with its host and environment at a genetic and molecular level, paving the way for the development of more targeted and sustainable management strategies.

This review provides an updated synthesis of the current knowledge

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https://doi.org/10.1016/j.fbr.2026.100471
Received 21 July 2025; Received in revised form 28 November 2025; Accepted 13 January 2026

on *D. necatrix*, covering its taxonomy, biology, host range, global distribution, and disease management strategies. We further explore recent advances in molecular studies, including genome sequencing, transcriptomic and proteomic analyses, the role of secondary metabolites in virulence, and the development of genetic transformation systems and functional gene studies. The review concludes with a discussion on future research directions and highlights knowledge gaps that warrant further investigation.

2. Taxonomy and name change of *Rosellinia necatrix*

D. necatrix Hartig is also known as *Rosellinia necatrix* Berl. ex Prill. in literature. The coexistence of these two names stems from the historical use of dual nomenclature in fungal taxonomy, where separate names were assigned to the teleomorph (sexual) and anamorph (asexual) stages of a fungus, as permitted under Article 59 of the former International Code of Botanical Nomenclature. Under this system, *R. necatrix* Berl. ex Prill. referred to the teleomorph (Prillieux, 1904), while *D. necatrix* Hartig designated the anamorph (Hartig, 1883). Over time, *R. necatrix* became more commonly used in scientific literature. However, ongoing taxonomic revisions have contributed to the confusion regarding the species' correct nomenclature.

Hartig first described the organism in 1883 and established the name *D. necatrix* (Hartig, 1883) but in 1892, Berlese proposed that it should be classified within the genus *Rosellinia* based on his studies of *Rosellinia aquila* (Fr.) Ces. & De Not. (Xylariaceae, Xylariales, Sordariomycetes, Ascomycota) ascocarps (Berlese, 1892b, 1892a). His taxonomic findings were subsequently confirmed when Prillieux found perithecia (sexual fruiting bodies) on tree roots infected with *D. necatrix* and named the teleomorph *R. necatrix* (Prillieux, 1902, 1904).

A more recent phylogenetic study, incorporating both molecular and morphological data, showed that several species with *Dematophora*-like asexual morphs clustered in a distinct clade separate from other *Rosellinia* species (Wittstein et al., 2020). Based on these findings, the genus *Dematophora* was reinstated, and several plant pathogens, including *R. necatrix*, were reclassified under this name (Wittstein et al., 2020). As a result, *D. necatrix* is now the accepted taxonomic designation.

3. Biology

This fungus displays white, cottony mycelia with hyphal diameters of 2–5 μm when grown on potato dextrose agar (PDA) (Fig. 1A), which darken and increase in diameter to 4–8 μm as the culture ages. Optimal *in vitro* growth occurs at 22–25 °C in the dark (García-Jiménez et al., 2004; Dafny-Yelin et al., 2018). A key microscopic feature used in species identification is the presence of pear-shaped (pyriform) swellings near the septa of the hyphae (Fig. 1B). These swellings are more pronounced in the surface mycelium than in the submerged mycelium on PDA (Khan, 1959). *D. necatrix* produces three spore types, namely chlamydospores, conidiospores and ascospores, but their role in disease epidemiology remains unclear. Instead, transmission primarily occurs through direct root-to-root contact between infected and healthy plants (Pliego et al., 2009).

D. necatrix exhibits a complex life cycle that includes both asexual and sexual reproductive stages. During the asexual phase, two main spore types are produced: chlamydospores and conidiospores (Pliego et al., 2012). Chlamydospores, which are spherical and approximately 15 μm in diameter, arise from the condensation of pyriform swellings along the hyphal cell wall (Makambila, 1976). Although these structures are presumed to function in fungal survival, they are rarely observed under either natural or artificial conditions (Pérez-Jiménez et al., 2003) and their ability to germinate remains unconfirmed. Conidiospores, measuring 2–2.5 μm in width and 3–4.5 μm in length, are produced by tightly packed upright conidiophores (synoecia or coremata) which can reach up to 1.5 mm in height (Petrini, 1993). These structures typically emerge from sclerotia or compact brown mycelial masses and are found at the collar region of infected dead plants, near the soil surface.

The sexual spores, known as ascospores, are typically between 30 and 50 μm in length and 5–8 μm in width (Fig. 1C) (Petrini, 1993; Pérez-Jiménez et al., 2003; Pliego et al., 2012). These spores are produced in groups of eight within elongated, cylindrical asci measuring 250–380 μm long and 8–12 μm wide. The asci are enclosed within perithecia (Fig. 1D, red arrow) found inside the stromata (Fig. 1D, white arrow), which are 1–2 mm in diameter and embedded in host plant tissue (Fig. 1E) (Pliego et al., 2012). Perithecium formation requires prolonged incubation of infected roots under high humidity, either in natural environments or under controlled *in vitro* conditions (Nakamura et al., 2000; Pérez-Jiménez et al., 2003). In nature, these sexual reproductive structures have been observed on apple, pear, tea shrubs, loquat and avocado (Lin and Duan, 1988; Teixeira de Sousa and Whalley, 1991; Nakamura et al., 2000; Pérez-Jiménez et al., 2003; Sun et al., 2008). However, inducing sexual reproduction *in vitro* has proven difficult (Watanabe, 1992; Pliego et al., 2012), which has hindered detailed study of the sexual cycle. Consequently, fundamental aspects of sexual reproduction in *D. necatrix* remain unresolved, including whether the species is heterothallic or homothallic.

Although evidence has suggested a heterothallic life cycle, definitive confirmation is lacking. Kanda et al. (2003) observed genetic variation between a presumed parental strain and F1 perithecia progeny collected from infected roots. Using RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) analyses, they showed that the progeny were genetically distinct from the presumed parent, suggesting the involvement of a second, genetically different strain in mating. Similarly, Ikeda et al. (2011) used universal primer PCR to detect DNA polymorphisms in ascospores from field-collected perithecia, concluding that each ascospore inherited genetic material from two distinct parents. While both studies support the hypothesis of heterothallism, their reliance on field-collected material and assumed parental strains limits the strength of their conclusions.

The hypothesis of a heterothallic life cycle in *D. necatrix* was challenged by findings from Robinson and Natvig (2019), who demonstrated that several Xylariales species, including *D. necatrix*, lack key

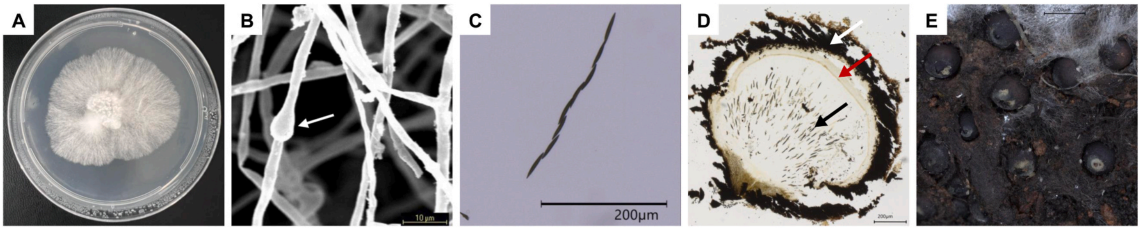

Fig. 1. Morphological traits of *Dematophora necatrix*. **(A)** Colony morphology of *D. necatrix* strain CMW50482 grown on Potato Dextrose Agar (PDA) for 7 days. **(B)** Scanning electron micrograph (SEM) of the hyphal network formed by *D. necatrix* on avocado roots. Pyriform swellings (white arrow) are diagnostic microscopic features used for species identification. **(C)** Digital microscopy image of eight, dark ascospores lined up in a row. **(D)** Digital microscopy image of a cross-section through *D. necatrix* stromata (white arrow) showing perithecia (red arrow) and several ascospores (black arrow). The ascospores are not grouped in eight as the asci were disrupted during sectioning. **(E)** Stromata embedded on the underside of a decaying tree branch.

mating-type genes essential for heterothallic reproduction. Using BLAST searches with the conserved genes (*SLA2*, *APN2*, *ACP5*, *COX13*) flanking the mating-type (*MAT1*) locus as well as known mating-type genes from Sordariomycetes, they found no association between the flanking genes and any mating-type loci, and no homologs of *MAT1-1-1* or *MAT1-1-2* were identified. Although homologs of *MAT1-2-1* and *MAT1-1-3* were detected, these genes were likely involved in non-mating-related functions. Based on these results, the authors proposed that the mating-type loci in Xylariales may have been lost or have diverged substantially, implying that sexual development in this order could be governed by alternative regulatory mechanisms. They further proposed unisexual reproduction, a form of homothallism, as a potential sexual strategy, emphasizing the persistent uncertainty surrounding the sexual biology of *D. necatrix*.

4. Hosts and geographical distribution of White Root Rot

D. necatrix has been confirmed in 465 ost-pathogen interactions (Thomas et al., 1953; van der Merwe and Matthee, 1974; Marais, 1980; Denardi and Bretón, 1995; Teixeira de Sousa et al., 1995; Sasaki et al., 2005; ten Hoopen and Krauss, 2006; Takemoto et al., 2014; Arjona-López, 2022; CABI, 2022; Polat et al., 2022; USDA Fungal Database at https://fungi.ars.usda.gov/). It should be noted that this figure includes multiple records of identical host-pathogen interactions reported in different countries, particularly within the USDA Fungal Database, which currently lists 443 such interactions (accessed November 5, 2025). Based on our analysis, *D. necatrix* affects 353 unique plant species across 167 genera, and this number continues to rise. Its host range spans a diverse array of commercially important fruit and nut trees, crops and ornamental plants (Fig. 2; Arjona-López, 2022; CABI, 2022; USDA Fungal Database). Additional hosts not shown in Fig. 2 include kiwi, pomegranate, raspberry, strawberry (fruit); macadamia, pistachio (nut trees); cedar, oak, pine (forest trees); potato (food crops); hemp (industrial crops); daffodil, carnation, jasmine, lavender (ornamental plants) as well as various weed species (Shiragane et al., 2019; Arjona-López, 2022; CABI, 2022; USDA Fungal Database). *D. necatrix* has been reported in 51 countries across five continents ranging from temperate to tropical climatic zones (Fig. 3; Arjona-López, 2022; CABI, 2022; USDA Fungal Database). Owing to its broad geographic distribution and extensive host range, *D. necatrix* ranks as one of the most devastating plant pathogens globally.

Despite limited quantitative data on economic losses caused by WRR, the broad host range of *D. necatrix* and the increasing incidence of the disease underscore its potential to cause significant economic impact. In Portugal, recent estimates show that on average *D. necatrix* affects 5 % of fruit orchards, resulting in an annual reduction of about 16 500 tonnes of fruit. These losses correspond to an estimated €5.7 million in direct losses for producers, while the broader value chain incurs an approximate €22.28 million in downstream losses. Growers also spend about €192 500 annually on control costs, including chemical treatments and other management practices (Pimentel et al., 2021). In Australia's Granite Belt, 54 % of apple orchards were affected in 2003, with an average annual loss of 200 trees per orchard (Stephens, 2003). Earlier reports from the 1990s showed that 42 % of apple orchards in the Alcobaça region of Portugal were infected (Teixeira de Sousa et al., 1995). Given the wide range of susceptible host species and the rapid global spread of WRR as illustrated in Fig. 3, the overall economic burden, albeit not fully quantified, is undoubtedly significant, encompassing not only direct crop yield reductions but also increased costs associated with disease management and orchard replacement.

5. Disease symptoms

WRR disease typically begins with below-ground symptoms, such as dead or damaged roots (Figure 4A - 4C), which consequently lead to above-ground symptoms including wilting, leaf chlorosis and necrosis,

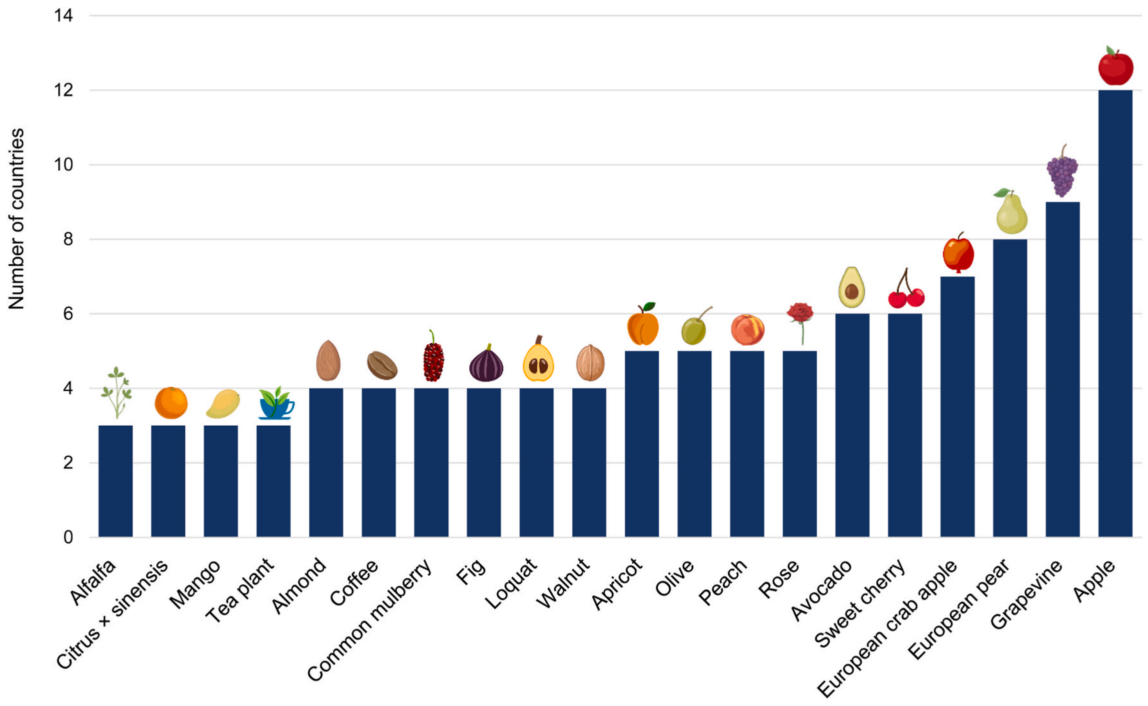

Fig. 2. Economically important plant species affected by *Dematophora necatrix*, ranked by the number of countries with reported infections.
The x-axis lists plant species with confirmed *D. necatrix* infections in at least three countries, while the y-axis indicates the number of countries where each species has been reported as a host. Plant species affected are (from left to right): alfalfa, citrus x sinensis, mango, tea plant, almond, coffee, common mulberry, fig, loquat, walnut, apricot, olive, peach, rose, avocado, sweet cherry, European crab apple, European pear, grapevine and apple. Figure created with Biorender.com. Data obtained from USDA Fungal Database (https://fungi.ars.usda.gov/); Arjona-López (2022); CABI, 2022; Denardi and Bretón (1995); Marais (1980); Sasaki et al. (2005); Teixeira de Sousa et al. (1995); Thomas et al. (1953); van der Merwe and Matthee (1974).

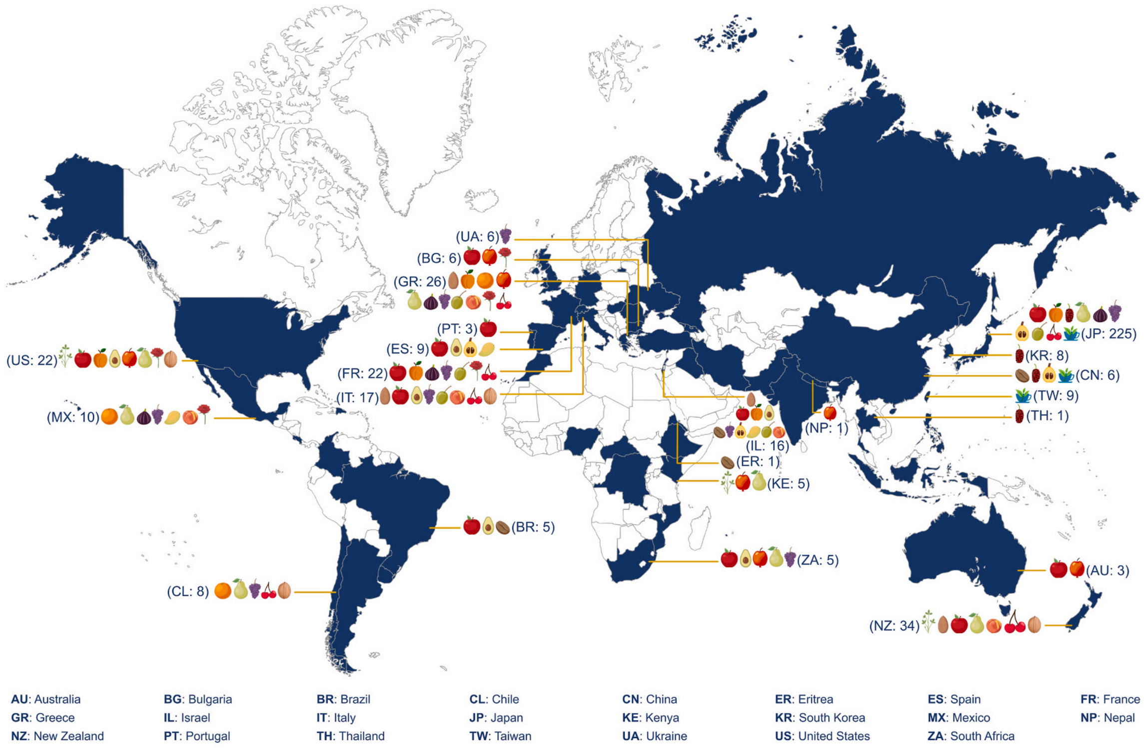

Fig. 3. Global distribution of *Dematophora necatrix* and the worldwide infection of economically important plant species.
Countries shaded in blue indicate the confirmed presence of *D. necatrix*. Countries where economically important plant species (as shown in Fig. 2) are affected are additionally marked with yellow lines, with fruit icons representing the specific plant species impacted in each of these countries. Country abbreviations and total number of affected host species per country are shown in brackets. A legend explaining country abbreviations is provided at the bottom of the figure. Figure created with Biorender.com. Data obtained from USDA Fungal Database (https://fungi.ars.usda.gov/); Arjona-López (2022); CABI, 2022; Denardi and Bretón (1995); Marais (1980); Sasaki et al. (2005); Teixeira de Sousa et al. (1995); Thomas et al. (1953); van der Merwe and Matthee (1974).

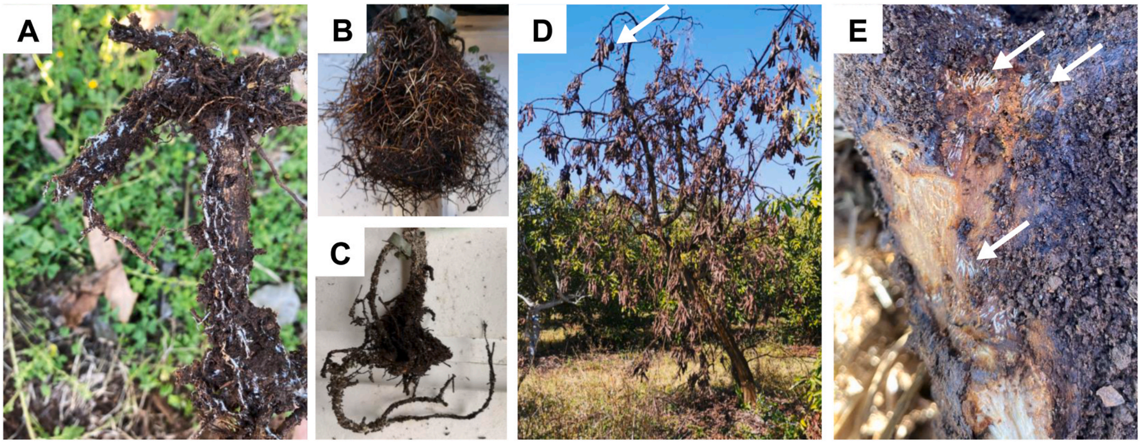

Fig. 4. Typical disease symptoms on avocado plants infected with *Dematophora necatrix*. (A) Below-ground symptoms of White Root Rot (WRR) showing mycelia covering the root surface of an adult plant. **(B)** Uninoculated controls plants exhibiting healthy roots during a glasshouse inoculation trial on five-year-old plants. **(C)** Inoculated plants displaying severe root decay during the same glasshouse trial. **(D)** Aerial symptoms of WRR include plant death often occurring with avocado fruit still attached (indicated by arrow). **(E)** *D. necatrix* produces characteristic white, fan-shaped mycelia between the wood and bark of woody plant roots (indicated by arrows).

leaf drop, branch dieback and ultimately plant death (Fig. 4D) (Sztejnberg et al., 1987; Pérez-Jiménez, 2006; Pliego et al., 2009; Arjona-Girona and López-Herrera, 2018; Sawant et al., 2021; Fusco et al., 2022; Hartley et al., 2022; Balocchi et al., 2025; van den Berg et al., 2025); these manifestations are consistent among all affected plant hosts. The infection process starts with the appearance of mycelial aggregates on the root surface or in the surrounding soil (Pérez-Jiménez, 2006; Pliego et al., 2009; van den Berg et al., 2025). *D. necatrix* infects the roots either directly or through wounds and natural openings, entering at multiple sites. In young seedling roots, it penetrates via penetration cones, while in thicker roots, infection occurs through penetration sclerotia (Makambila, 1976). The fungus typically colonizes the area between the wood and the bark, forming characteristic white fan-shaped mycelia (Fig. 4E). Once the infection reaches this stage, root rot sets in, leading to the full spectrum of symptoms (Pliego et al., 2009, 2012).

The pathogen spreads via contaminated roots and soil, with healthy plants becoming infected either through mycelial spread underground or root-to-root contact, making it difficult to trace the original source of infection (Pérez-Jiménez, 2006; Pliego et al., 2009). Aerial symptoms resulting from infected roots may appear suddenly, with rapid plant decline, or the disease may progress slowly, weakening the plant over several years until it eventually dies (Guillaumin et al., 1982). Although tree death within weeks of symptom onset has been reported (van den Berg et al., 2018), not all infected trees show visible signs of disease (Pliego et al., 2012), complicating disease diagnosis and making symptomatic assessments unreliable.

While the life cycle of *D. necatrix* and its infection process on avocado roots have been described in detail, including the formation of infection structures and host colonisation dynamics (Pliego et al., 2009, 2012), the molecular processes governing its pathogenicity and virulence remain poorly understood. To date, there is limited insight into the specific genes, effectors or signalling pathways involved in host manipulation, defence suppression or tissue degradation. Nevertheless, transcriptomic analyses have begun to identify candidate effector and cell wall-degrading enzyme (CWDE) genes that may be associated with these pathogenic processes (Kim et al., 2017; Shimizu et al., 2018; Zumaquero et al., 2019a), but functional evidence is still lacking. Comprehensive molecular and genomic studies are therefore required to elucidate the infection strategies employed by *D. necatrix* and to enhance our understanding of the mechanisms underlying its broad host range and necrotrophic lifestyle.

6. Management strategies of White Root Rot

Managing WRR remains challenging due to the pathogen's ability to penetrate deep into the soil, survive in acidic and dry conditions, and resist fungicides (Khan, 1959; ten Hoopen and Krauss, 2006; Ruano-Rosa et al., 2018). Although integrated control strategies combining chemical, physical and cultural methods can reduce symptoms (Ruano-Rosa et al., 2018; Magagula et al., 2021), none have proven fully effective.

Chemical control includes soil treatments with fungicides like Fluazinam (FRAC code 29: 2,6-dinitro-anilines) (Sugimoto, 2002; Arjona-López et al., 2020; van den Berg et al., 2025) and the soil fumigant Chloropicrin (trichloronitromethane) (Magagula et al., 2021), which offer moderate to good efficacy. However, their long-term use is unsustainable due to environmental risks, regulatory restrictions, and the potential for resistance development. Physical methods exploit the pathogen's sensitivity to heat. Soil solarization is costly and prone to reinfection (Pliego et al., 2012), while hot water treatments are constrained by host's heat tolerance (Eguchi et al., 2008). Cultural practices such as weed removal and burning of infected material, reduce disease spread (Pérez-Jiménez, 2006) as some weeds can act as asymptomatic reservoirs (Shiragane et al., 2019). Despite these interventions, the pathogen can persist for years on root debris and in the soil as saprophytic mycelia (Pérez-Jiménez, 2006) or pseudosclerotia (proposed specialised survival structures based on morphological and field evidence) (Shimizu et al., 2014). Consequently, crop rotation or fallowing is recommended before replanting (Pasini et al., 2016).

Biological control of *D. necatrix* is being actively investigated. The pathogen is sensitive to antagonistic bacteria, with *Pseudomonas chlororaphis* demonstrating strong efficacy in avocado trials (Tienda et al., 2020). Fungal antagonists, particularly *Trichoderma* spp., have also shown effectiveness in controlling the disease in apple (Sztejnberg et al., 1987) and avocado (Cazorla et al., 2006; Cazorla et al., 2007; Pliego et al., 2007; Ruano and López Herrera, 2009). In addition, hypovirulence-inducing mycoviruses such as *Rosellinia necatrix* hypovirus 2 have shown potential by reducing *in vitro* colony growth and lowering virulence during avocado infection (Arjona-López et al., 2021). Despite the promise of these biological approaches, most studies remain confined to *in vitro* or greenhouse trials.

The use of resistant or tolerant rootstocks is arguably the most promising long-term solution. Resistance has been explored across multiple crops, including apple (Lee et al., 2000), avocado (Barceló-Muñoz et al., 2007; Zumaquero et al., 2019b), citrus (Arjona-López et al., 2022), grapevine (Mansoori and Dorostkar, 2008), pear (Sharma et al., 2017), plum and prune (Dastjerdi and Pirkhezri, 2023).

Climate change is expected to exacerbate WRR incidence and severity. Rising temperatures and drought conditions promote tissue necrosis, facilitate pathogen colonisation and weaken plant immune responses (Desaint et al., 2021; Suraj and van Kan, 2024). These conditions favour necrotrophs such as *D. necatrix*, which can survive in dry soils for extended periods (Khan, 1959). This makes sustainable control strategies increasingly urgent.

Ultimately, prevention remains a cornerstone of management. Avoidance of infected plant material and contaminated soil is essential. Early detection tools, including PCR-based assays (Schena et al., 2002; Shishido et al., 2012) and semi-selective media (Hartley et al., 2022), offer support. Nevertheless, deeper insight into the molecular processes underlying WRR disease is essential for the development of targeted approaches, including resistant genotypes and integrated pest management.

7. Molecular biology studies of *Dematophora necatrix* to date

Molecular biology has greatly advanced our understanding of plant-pathogen interactions and pathogen pathogenicity and virulence by revealing the underlying genetic and biochemical mechanisms (Chavarro-Carrero et al., 2024). High-throughput approaches such as genomics, transcriptomics, proteomics and studies of secondary metabolites and gene function have become essential for dissecting these complex interactions at a molecular level. In the case of *D. necatrix*, such molecular studies have become increasingly important for elucidating its pathogenesis and impact on host plants. While early studies focussed on biology, disease symptoms and control strategies, the past 25 years have seen a growing body of molecular work (Fig. 5). This section reviews key advances from these studies that have advanced our knowledge of this root-rot pathogen.

7.1. Genomes of Dematophora necatrix

The advent of high-throughput sequencing technologies has greatly accelerated the characterisation of plant pathogenic fungi by enabling whole-genome sequencing and facilitating the identification of genes associated with virulence and pathogenicity (Dean et al., 2012). Early draft genome assemblies of *D. necatrix* strain W97 (Shimizu et al., 2018) and CMW50482 (Wingfield et al., 2022) were highly fragmented, with each consisting of more than 1200 contigs (Table 1). The assembled genome size of CMW50482 was roughly 48.79 Mb, which is larger than that of strain W97 (44.3 Mb), but still within range observed for other strains isolated from rose, indicative of genome composition variability within the species (Wingfield et al., 2022). In addition, the CMW50482 mitochondrial genome (mitogenome) comprises 14 protein-coding genes, 26 tRNA-encoding genes and single copies of *rnl*, *rns*, *rps3* and *nat1* (van der Nest et al., 2024). It also harbours numerous group I introns encoding homing endonucleases or reverse-transcriptases, which contribute to genome expansion, rearrangement, and genetic diversity (van der Nest et al., 2024). The availability of the *D. necatrix* mitochondrial genome offers insights into its evolutionary history and adaptive potential, and may serve as a source of molecular markers for diagnostics or future disease management strategies.

Recognising the limitations of previous fragmented assemblies, a recent study produced high-quality genome assemblies from several strains isolated in Spain and Mexico (Table 1; Chavarro-Carrero et al., 2024). Among these, strain R18 isolated from rose (GenBank accession GCA_021209875.1) was assembled to chromosome-level resolution

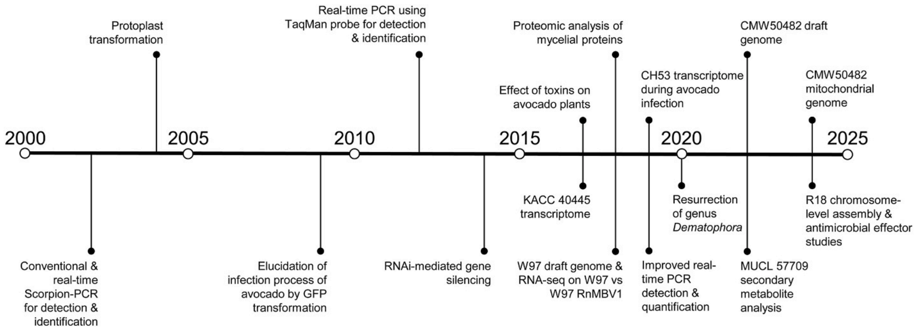

Fig. 5. Timeline of molecular studies on *Dematophora necatrix* (2000–2025).
This timeline illustrates major molecular advances in *D. necatrix* research over the past 25 years. Key developments include: developing of identification and detection methods using conventional and real-time Scorpion-PCR (Schena et al., 2002); establishing a protoplast transformation method (Kanematsu et al., 2004); elucidating the infection process of avocado roots with GFP transformed *D. necatrix* (Pliego et al., 2009); developing a real-time PCR assay with a TaqMan probe for detection and quantification (Shishido et al., 2012); performing functional gene analysis using RNAi-mediated gene silencing (Shimizu et al., 2014); investigating the effects of toxins on avocado plants (Arjona-Girona et al., 2017); conducting transcriptome studies on strain KACC 40445 (Kim et al., 2017); analysing the proteome of mycelial proteins (Eom et al., 2018); generating a draft genome sequence and conducting transcriptional analysis of strain W97 (Shimizu et al., 2018); analysing the transcriptome of strain CH53 during infection of a susceptible avocado rootstock (Zumaquero et al., 2019a); developing an improved real-time PCR protocol for accurate detection and quantification (Arjona-López et al., 2019); resurrecting the genus *Dematophora* and reclassifying *Rosellinia necatrix* (Wittstein et al., 2020); genome sequencing and assembly of strain CMW50482 (Wingfield et al., 2022); evaluation of secondary metabolites from strain MUCL 57709 (Pourmoghaddam et al., 2022); mitochondrial genome sequencing and assembly of strain CMW50482 (van der Nest et al., 2024); and assembling a chromosome-level genome of strain R18 and functionally analysing its antimicrobial proteins (Chavarro-Carrero et al., 2024).

spanning and proposed as the reference genome for *D. necatrix*. This assembly represents the most comprehensive genomic resource to date and provides an invaluable framework for dissecting the molecular mechanisms underpinning pathogenicity. Although the assemblies of other strains in the study were similar in size (48.2 Mb–49.9 Mb; Table 1), they did not reach chromosome-level resolution (Chavarro-Carrero et al., 2024) but nonetheless offer useful genomic information.

Furthermore, the R18 genome also provides a foundation for large-scale studies, such as phylogenomics (Ma et al., 2025) and population genomic studies (McDonald, 2015), which are essential for understanding genomic diversity, population structure, and evolutionary history across different regions and host species. These insights are critical for disease management, as they can reveal genetic exchange events across the genome that lead to novel allele combinations that facilitate virulence gain or evade host recognition (Amezrou et al., 2024), and may also contribute to the emergence of fungicide resistance (Einspanier et al., 2022).

7.2. *Transcriptomics and differential gene expression in Dematophora necatrix*

Transcriptomic analyses offer critical insights into the molecular dynamics of plant-pathogen interactions by identifying differentially expressed genes (DEGs) during infection. These studies reveal how pathogens potentially manipulate host gene expression to suppress immune responses and facilitate colonisation, while also uncovering pathogen genes involved in pathogenicity, virulence, metabolism and stress adaptation. In *D. necatrix*, transcriptomics has been instrumental in identifying candidate genes potentially involved in pathogenicity and host colonisation, although functional validation remains limited.

Early transcriptomic efforts aimed at elucidating the genetic basis of pathogenicity in *D. necatrix*. In one such study, the transcriptome of strain KACC 40445 was analysed using PacBio single-molecule sequencing, resulting in the identification of 10 616 novel full-length transcripts (Kim et al., 2017). This dataset enabled the prediction of numerous candidate pathogenicity-related genes, including putative effector-encoding genes and 74 novel genes encoding CWDEs (Kim et al., 2017). Despite their potential relevance to infection processes, these genes were not functionally characterised, underscoring a critical gap between transcriptomic prediction and experimental validation.

A subsequent study explored the impact of mycoviral infection on gene expression in *D. necatrix*. By comparing a hypovirulent W97 strain infected with *Rosellinia necatrix* megabirnavirus 1 (RnMBV1) to its virus-free counterpart, the authors identified 545 upregulated and 615 downregulated genes (Shimizu et al., 2018). The hypovirulent RnMBV1-infected strain was shown to have reduced virulence on apple rootstocks and reduced mycelial growth *in vitro* (Chiba et al., 2009). Downregulated transcripts were enriched for genes involved in primary and secondary metabolism, transcriptional regulation and CWDEs. In addition, the RnMBV1-infected W97 strain displayed downregulated expression in nine genes homologous to the previously characterised cytochalasin biosynthetic gene cluster of *Aspergillus clavatus* NRRL 1 (Aspergillaceae, Eurotiales, Eurotiomycetes, Ascomycota) (Qiao et al., 2011) and produced significantly less cytochalasin E toxin compared to the virus-free W97 strain (Shimizu et al., 2018). Although reduced cytochalasin E may affect virulence, prior research showed that this toxin is not essential for *D. necatrix* pathogenicity (Kanematsu et al., 1997). Downregulation of these functions in the hypovirulent strain provided early evidence that the abovementioned genes play a central role in *D. necatrix*'s virulence, thereby affecting disease severity. However, this study did not capture the transcriptional dynamics of *D. necatrix* during actual host infection.

To address this limitation, Zumaquero et al. (2019a) performed the first *in planta* transcriptomic analysis of *D. necatrix* during infection of detached roots. This study compared the gene expression profiles of the virulent strain CH53 during infection of susceptible avocado roots to that of the same strain grown on PDA. Of the 12 104 total transcripts, 11 807 were expressed under both conditions, while 137 and 160 transcripts were uniquely expressed during root infection and culture growth, respectively. A total of 1937 DEGs were identified, and genes upregulated during root infection included those involved in production of CWDEs and fungal toxins, detoxification and transport proteins, proteases, gibberellin biosynthesis enzymes, and gene silencing. Among the 137 transcripts uniquely expressed during root infection, 24 candidate effector proteins were predicted, three of which exhibited homology to known fungal effectors (Zumaquero et al., 2019a). This analysis provided key insights into infection-associated gene expression, although the use of a detached root system may limit the extrapolation of results to natural disease conditions. Studies using whole-plant infection models are warranted to validate and extend these findings.

More recently, the expression of putative antimicrobial effector

Table 1
Summary of publicly available genome assemblies of *Dematophora necatrix* strains.

Strain name	W97	CMW50482	R18[b]	R18	Rn19	Ch12	Rn400	R10	R25	R27	R30	Rn19
Isolated from	Japanese pear	Avocado	Rose	Rose	Avocado	Avocado	Avocado	Rose	Rose	Rose	Rose	Rose
Country of origin	Japan	South Africa	Mexico	Mexico	Spain	Spain	Spain	Mexico	Mexico	Mexico	Mexico	Mexico
Year sequenced	2018	2022	2024	2024	2024	2024	2024	2024	2024	2024	2024	2024
Sequencing technology	Illumina HiSeq 2000	Illumina HiSeq 2500	Oxford Nanopore & Hi-C	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore	Oxford Nanopore
Coverage	160x	65x	120x	42x	45x	46x	35x	35x	45x	46x	41x	52x
No. of contigs	1209	1362	11	28	35	399	37	50	58	31	47	130
No. protein coding genes	12 444	10 714	11 760	NP[a]	NP[a]	NP[a]	NP[a]	NP[a]	NP[a]	NP[a]	NP[a]	NP[a]
Total length (Mb)	44.3	48.8	49.1	49.0	48.2	49.9	48.2	48.9	49.2	49.2	48.7	48.3
N50 (Mb)	0.55	0.12	5.10	3.10	3.40	4.60	3.80	1.90	2.50	3.2	3.4	1.6
GC (%)	50.00	46.25	45.46	45.35	46.28	46.37	45.35	45.35	45.49	45.43	45.50	45.99
BUSCO (%)	NP[a]	96.2	97.0	82.5	76.7	69.9	70.2	68.4	78.9	72.3	68.2	80.7
Reference	Shimizu et al. (2018)	Wingfield et al. (2022)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)	Chavarro-Carrero et al. (2024)

[a] NP represents Not Published.

[b] Proposed reference genome of *Dematophora necatrix* (GenBank accession GCA_021209875.1).

genes in *D. necatrix* was investigated using genome mining and RNA-seq datasets (Chavarro-Carrero et al., 2024). From the predicted secretome (all proteins secreted extracellularly) of strain R18, 26 putative genes encoding antimicrobial proteins were identified based on structural homology to known antimicrobial proteins (Chavarro-Carrero et al., 2024). Expression profiling from previous RNA-seq data revealed that five of these genes (*FUN_010039*, *FUN_004580*, *FUN_004961*, *FUN_006760*, and *FUN_011519*) were upregulated both during avocado root colonisation and in culture conditions, while four additional genes (*FUN_009266*, *FUN_005751*, *FUN_003304*, and *FUN_007808*) exhibited upregulation exclusively during avocado colonisation (Zumaquero et al., 2019a). Of particular interest, *FUN_009266* and *FUN_005751* also showed high expression during cotton colonisation at 14 and 21 days post-inoculation, suggesting a conserved role in infection across host species (Chavarro-Carrero et al., 2024). While antimicrobial proteins typically act in microbial competition in the rhizosphere, Chavarro-Carrero et al. (2024) proposed that *D. necatrix* uses them during host colonisation to modulate the host-associated microbiome and facilitate disease progression by selectively inhibiting microbes that antagonise *D. necatrix*.

Despite these advances, the broader effector repertoire and signalling networks involved in *D. necatrix* pathogenicity and virulence are still largely uncharacterised, and the study itself emphasises the need for further functional and molecular research.

With the increasing availability of transcriptomic datasets from *D. necatrix* during various stages of host colonisation, future meta-analyses hold promise for identifying conserved infection strategies and core pathogenicity genes (Cai et al., 2022). However, the field is still hindered by a lack of functional gene studies. Without targeted genetic manipulation, such as gene knockouts or knockdowns, the biological relevance of predicted effectors and DEGs cannot be confirmed. To fully unravel the molecular biology of *D. necatrix* pathogenicity and virulence, integration of transcriptomic, proteomic, and functional assays will be essential.

7.3. Proteomic studies in Dematophora necatrix

Proteomics, the large-scale study of all proteins expressed by a genome, has become an indispensable approach in understanding host-pathogen interactions. In fungal phytopathogens, proteomics allows for the identification and quantification of proteins that underpin infection strategies, contribute to virulence, and mediate interactions with host plants and microbial communities (Singh et al., 2025). It also facilitates the study of post-translational modifications which can reveal critical insights into how pathogens modulate host cellular processes, for example through the secretion of effector proteins that directly modify host proteins to subvert defences or hijack cellular machinery during infection, or how pathogens regulate their own proteins to adapt to host environments (Tahir et al., 2019; Liu et al., 2021). In addition to identifying potential effectors, proteomic methods can reveal protein-protein interactions between pathogen effectors and host targets, providing a mechanistic understanding of how effectors disrupt signalling pathways, suppress immune responses or manipulate host physiology (Doyle, 2011; Sa et al., 2025; Singh et al., 2025). Such insights are critical for informing resistance breeding and biotechnological strategies for crop protection.

Despite its significance, proteomic research in *D. necatrix* remains limited due to genomic, technical, and historical constraints. High-quality nuclear (Chavarro-Carrero et al., 2024) and mitochondrial (van der Nest et al., 2024) genome assemblies were unavailable until recently, restricting accurate protein identification and annotation. Protein extractions from filamentous fungi like *D. necatrix* are technically challenging due to their tough, melanised, polysaccharide-rich cell walls (Ning et al., 2021), especially for low-abundance secreted proteins often crucial to pathogenicity (Fernandes et al., 2014). Historically, research centred on disease symptoms, epidemiology, host range, and management rather than molecular pathogenicity mechanisms. Consequently, proteomics has contributed little to understanding this pathogen's pathogenicity and functional information on associated proteins remains lacking. Nevertheless, existing qualitative proteomic studies provide foundational insights.

Among the most studied aspects of the *D. necatrix* proteome is its secretome (Chavarro-Carrero et al., 2024). By screening the secretome for structural homologs of known antimicrobial proteins, 26 putative antimicrobial effector proteins were identified, nine of which were significantly expressed during plant infection. Notably, FUN_004580, an antifungal protein AFP1 homolog, and FUN_011519, an antimicrobial protein homolog, demonstrated selective antifungal and antibacterial activity *in vitro,* including against several bacteria that antagonise *D. necatrix in vitro*. Correspondingly, cotton plants pre-treated with these antagonistic bacteria showed reduced disease severity compared to plants pre-treated with non-antagonistic bacterial species (Chavarro-Carrero et al., 2024).These findings support a dual role for secreted antimicrobial proteins: directly contributing to host tissue colonisation and indirectly modulating the host-associated microbiota to create a more favourable niche for fungal establishment. This highlights an emerging theme in plant pathology, where pathogens may suppress or outcompete beneficial microbiota as part of their infection strategy (Snelders et al., 2020).

Beyond the secretome, mycelial proteomic analyses have yielded valuable insights into core cellular processes in *D. necatrix*. Nanoliquid chromatography combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry was employed to investigate the proteome of strain KACC 40445 (Eom et al., 2018). A total of 696 proteins were identified and 573 were categorised into at least one of 26 functional groups. The most abundant functional categories included catalytic activity (62 %), binding functions (38 %), and cellular processes (28 %) while a subset of proteins (9 %) localised to the ribosome (Eom et al., 2018), reflecting active protein synthesis. Gene ontology annotations revealed that eight proteins were associated with antioxidant activity, including superoxide dismutase and catalase. These enzymes are known to detoxify reactive oxygen species, a key component of plant immune responses. The presence of such antioxidant proteins suggests that *D. necatrix* may employ oxidative stress mitigation as a virulence strategy during the early stages of infection, a tactic characteristic of many broad host range necrotrophic fungi (Newman and Derbyshire, 2020). However, their role *in planta* remains to be experimentally validated.

Proteomic research in *D. necatrix* is increasingly supported by computational tools such as AlphaFold (Jumper et al., 2021) and EffectorP 2.0 (Sperschneider et al., 2018), which bridge the gap between genome prediction and protein function. AlphaFold, a powerful tool for predicting protein structure, has been employed to model the structures of secreted proteins from fungal phytopathogens (Seong and Krasileva, 2023) and has been used to predict the structural impact of mutations on effector proteins, providing insights into effector evolution and functional diversification (Pak et al., 2023). In the case of *D. necatrix*, Chavarro-Carrero et al. (2024) used AlphaFold2 (Jumper et al., 2021) to identify two candidate antimicrobial effectors (FUN_004580 and FUN_011519) based on structural homology with experimentally validated antimicrobial proteins. Complementary to structural prediction, EffectorP 2.0, a machine learning-based tool, has become critical for *in silico* effector discovery as it predicts fungal effectors based on protein properties and amino acid composition (Sperschneider et al., 2018). During annotation of the R18 genome, EffectorP 2.0 predicted a total of 192 candidate effector proteins (Chavarro-Carrero et al., 2024), further highlighting the vast effector potential encoded by this pathogen. Integrating these predictions with transcriptomic and functional data can prioritise candidates for experimental validation.

More comprehensive and targeted proteomic studies, especially those examining the secretome during host colonisation and protein-protein interactions with host targets, are needed to decode the molecular basis of *D. necatrix* pathogenicity and virulence. Integration with computational tools like AlphaFold and EffectorP 2.0 will further accelerate the discovery of novel effectors. Functional validation, through gene knockout or heterologous expression studies, remains the critical next step in translating proteomic predictions into a clearer understanding of disease. As the field advances, proteomics will be key to bridging the gap between genome annotation and functional biology.

7.4. Role of secondary metabolites in Dematophora necatrix virulence

Fungal secondary metabolites (SMs) are low-molecular-weight bioactive compounds that serve diverse functions. These include phytotoxins, which are secreted by plant-pathogenic fungi to damage host plants; mycotoxins, produced by fungi that contaminate crops and pose health risks to humans and animals; pigments, which are coloured compounds often possessing antioxidant activity; and antibiotics, which are natural substances that suppress or eliminate competing microorganisms (Elhamouly et al., 2022; Wadhwa et al., 2024). Accumulating evidence suggests that these compounds play a significant role in *D. necatrix* disease symptom development. Several SMs have been isolated from *D. necatrix* mycelia or culture filtrates, showing phytotoxic activity against a variety of host seedlings, including apple (Edwards et al., 2001; Kshirsagar et al., 2001), geranium (Edwards et al., 2001), wild pear (Kanematsu et al., 1997) and avocado (Arjona-Girona et al., 2017), underscoring its metabolic versatility.

Among the best-characterised SMs of *D. necatrix* is cytochalasin E (Edwards et al., 2001; Kshirsagar et al., 2001; Pourmoghaddam et al., 2022), a member of the cytochalasin family known for disrupting the eukaryotic actin cytoskeleton. Cytochalasins function by binding to F-actin filaments, leading to disorganisation of cytoskeletal structure, inhibition of cytokinesis, and disruption of cell integrity (Scherlach et al., 2010). In *D. necatrix*, cytochalasin E has been linked to virulence when it was shown that a hypovirulent strain infected with a mycovirus produced significantly lower levels of cytochalasin E compared to its uninfected, virulent counterpart (Shimizu et al., 2018). Furthermore, exogenous application of purified cytochalasin E to avocado seedlings resulted in reduced photosynthetic efficiency, suggesting that some disease symptoms like wilting, may result from toxin exposure rather than direct fungal invasion (Arjona-Girona et al., 2017). Furthermore, fluorescence microscopy confirmed that cytochalasin E from *D. necatrix* binds strongly and irreversibly to F-actin, leading to extensive cytoskeletal disruption (Pourmoghaddam et al., 2022). Interestingly, this study also found that structural analogues such as $\Delta^{6,12}$-cytochalasin E exhibit potent, irreversible effects on F-actin, whereas cytochalasin K shows weaker, reversible activity (Pourmoghaddam et al., 2022).

Despite its demonstrated phytotoxicity, cytochalasin E is not necessary for pathogenicity as no significant difference between wild-type and cytochalasin E-deficient mutant strains were observed during pathogenicity tests (Kanematsu et al., 1997), indicating that other factors likely compensate for its absence during host colonisation. Cytochalasin E also inhibited the growth of *Schizosaccharomyces pombe* (Pourmoghaddam et al., 2022), suggesting that its primary role may be ecological. It may function by suppressing microbial competitors in the soil, thereby influencing soil microbiome composition and activity. Co-culture experiments with soil microbes grown with either the wild-type or the cytochalasin E-deficient mutant strain could help clarify this potential ecological role.

Other SMs identified from *D. necatrix* include rosellichalasin (Kimura et al., 1989), rosellinic acid (Chen, 1964), diketopiperazines (Chen, 1960) and rosnecatrone (Edwards et al., 2001). Rosnecatrone, in particular, has demonstrated strong phytotoxicity and may contribute more to host damage than cytochalasin E as shown by a virulent *D. necatrix* strain that produced high levels of rosnecatrone and caused severe damage to apple and geranium seedlings, while cytochalasin E was only a minor metabolite in this strain (Edwards et al., 2001). The presence of multiple phytotoxic compounds in *D. necatrix* highlights the possibility of functional redundancy or synergy among SMs in driving disease, as seen in *Botrytis cinerea* (Sclerotiniaceae, Helotiales, Leotiomycetes, Ascomycota), another necrotrophic phytopathogen (Leisen et al., 2022). Such diversity may facilitate host range expansion or adaptability to different environmental conditions, enhancing the pathogen's fitness.

A significant breakthrough in understanding *D. necatrix* SM biosynthesis came from the genome annotation of strain R18 by Chavarro et al. (2024). Bioinformatics analysis predicted 48 SM biosynthetic gene clusters (BGCs), 13 of which showed homology to known phytotoxic BGCs, including those responsible for producing enniatin, pyriculol, naphthalene (Xu et al., 2021) and swainsonine (Cook et al., 2017). This discovery provides a foundation for dissecting the molecular pathways involved in SM production. Functional characterisation of these BGCs could uncover novel phytotoxins or regulatory networks controlling their production during host infection.

To our knowledge, no metabolomic studies have been conducted on *D. necatrix* to date. Metabolomics, a systems-level approach for characterising metabolic profiles, has emerged as a powerful tool in phytopathology. It enables the detection of dynamic metabolic changes during plant-pathogen interactions and can uncover novel bioactive compounds involved in virulence, stress tolerance and environmental adaptation. Recent advances in high-resolution mass spectrometry, nuclear magnetic resonance spectroscopy and bioinformatic pipelines have made it increasingly feasible to apply metabolomics to fungal plant pathogens (Chen et al., 2019; Castro-Moretti et al., 2020). However, several challenges complicate metabolomic investigations of soil-borne pathogens such as *D. necatrix*. In host-microbe interaction studies, samples contain metabolites from the pathogen, host plant and surrounding soil microbiota, hindering the identification of fungal-specific signatures (Castro-Moretti et al., 2020). Variability arising from differences in fungal growth conditions and host susceptibility must be minimised to ensure reproducible results (Tian et al., 2025), while the production of secondary metabolites by *D. necatrix* across infection stages necessitates precisely timed sampling (Kanematsu et al., 1997). Finally, limited metabolite reference databases for plant-pathogenic fungi, combined with the structural complexity of fungal secondary metabolites, further restrict accurate compound identification (Chen et al., 2019; Castro-Moretti et al., 2020).

Despite these challenges, applying metabolomic approaches to *D. necatrix* would significantly enhance our understanding of its SM profile. Moreover, integrating metabolomics with transcriptomics, proteomics and genomics offers the potential to generate a comprehensive view of *D. necatrix* pathogenicity and virulence.

7.5. Genetic transformation and functional gene studies in Dematophora necatrix

Initial attempts to introduce foreign genes into *D. necatrix* utilised either protoplast-mediated transformation (Kanematsu et al., 2004) or *Agrobacterium tumefaciens*-mediated transformation of mycelia (Aimi et al., 2005). However, these pioneering efforts achieved only low transformation efficiencies, limiting their practicality for functional studies.

Subsequent refinements have significantly improved transformation outcomes. Notably, Pliego et al. (2009) demonstrated successful protoplast transformation using the enhanced green fluorescent protein as a visual reporter. This allowed for the microscopic visualisation of *D. necatrix* infection dynamics in avocado roots, providing direct insights into the infection process. The *Agrobacterium*-mediated method was also optimised to achieve stable transformation of *D. necatrix* mycelia with a hygromycin B resistance gene, facilitating the selection of transformants (Kano et al., 2011). Both studies emphasised the influence of fungal strains on transformation efficiency as neither study successfully transformed all strains tested (Pliego et al., 2009; Kano et al., 2011), underscoring the need for optimised transformation protocols tailored to each *D. necatrix* strain. By using restriction enzyme-mediated integration (REMI), Attri et al. (2018) demonstrated increased transformation efficiency. REMI involves transforming cells with linearised plasmid DNA in the presence of a restriction enzyme, which introduces double-stranded breaks in the genome and generates compatible ends for integration (Kahmann and Basse, 1999). Attri et al. (2018) analysed 47 single-spore transformants, successfully amplifying the hygromycin resistance gene in 33 of them, with 19 showing single-copy integration of the gene.

The establishment of these transformation systems laid the groundwork for researchers to carry out successful transformations, thereby enabling functional gene analysis aimed at elucidating the molecular basis of pathogenicity and environmental adaptation in *D. necatrix*. However, despite the availability of these tools, only one functional gene study has been conducted to date. In this study, Shimizu et al. (2014) employed RNA interference to silence the *RnPKS1* gene, which encodes a polyketide synthase involved in melanin biosynthesis. Silencing *RnPKS1* led to a pronounced reduction in melanin accumulation and revealed that melanin is essential for the formation of pseudosclerotia and the structural integrity of conidiomata (fruiting bodies that produce asexual conidia) (Shimizu et al., 2014). These structures are vital for the survival of the fungus in soil environments, suggesting a key role for melanin in environmental resilience. Interestingly, the absence of melanin did not affect pathogenicity, indicating that while melanin contributes to fungal fitness, it is not directly involved in its ability to cause disease. This finding highlights the melanin biosynthetic pathway as a potential target for strategies aimed at reducing the persistence of *D. necatrix* in agricultural soils (Shimizu et al., 2014). Separately, although *RnPKS1* does not appear to influence pathogenicity, polyketide synthase genes in other fungal pathogens have been implicated in virulence; for example, *Verticillium dahliae* (Trichosphaeriaceae, Incertae sedis, Sordariomycetes, Ascomycota) *VdPKS9* functions as a key virulence factor (Li et al., 2022). Thus, while melanin itself is not involved in pathogenicity, other polyketide-derived metabolites may play a role.

The high-quality genome assembly of strain R18 has revealed the presence of multiple putative effector genes and SM BGCs (Chavarro-Carrero et al., 2024). However, their specific functions in pathogenicity remain to be experimentally validated. The improved transformation protocols provide the necessary tools for such functional studies, enabling targeted gene knockouts or overexpression analyses. Systematic functional characterisation of these genes will be essential for understanding the molecular mechanisms underlying *D. necatrix* pathogenicity and survival, and may ultimately contribute to the development of more effective disease control strategies.

8. Future prospects and conclusions

This review provides a comprehensive overview of the molecular biology advancements that have enhanced our understanding of *D. necatrix* virulence and pathogenicity. A major recent milestone was the generation of a high-quality, chromosome-level genome assembly, which offers an essential foundation for functional genomics and comparative studies. Transcriptomic analyses have further advanced our knowledge by identifying candidate genes potentially involved in pathogenicity; however, functional validation of their proteins remains notably limited.

Several critical knowledge gaps persist. Proteomic investigations, particularly those focusing on the secretome during host colonisation, are lacking and are essential for identifying effectors and other pathogenicity-associated proteins. Moreover, studies on protein-protein interactions between fungal proteins and host targets, as well as metabolomic profiling during infection, are needed to build a more complete picture of host-pathogen dynamics. The integration of multi-omics approaches (transcriptomics, proteomics, and metabolomics) has the potential to provide a systems-level understanding of the molecular processes underlying *D. necatrix* biology and pathogenesis.

Encouraging progress has been made in enhancing transformation efficiency. This opens new avenues for functional studies of endogenous genes through gene knockdown strategies or targeted genome editing using CRISPR-based systems.

Looking ahead, climate change poses an emerging threat by altering environmental conditions in ways that may favour the spread and persistence of *D. necatrix*. This underscores the urgent need for research not only into the pathogen's biology but also into the development of effective, sustainable control strategies.

Future studies should prioritise the identification of molecular targets for disease intervention and the breeding or engineering of resistant crop varieties. Investigating the impact of environmental factors like temperature and soil moisture on *D. necatrix* virulence gene expression and SM production will also clarify how climate-driven changes impact pathogen behaviour.

Authorship contributions

Maryke Carstens: conceptualisation, investigation, writing of original draft, reviewing and editing. Clara Pliego: Fig. 1, funding acquisition, writing, reviewing and editing. Annabel Norval: writing, reviewing and editing. Noëlani van den Berg: funding acquisition, writing, reviewing and editing.

Funding declaration

Maryke Carstens, Annabel Norval and Noëlani van den Berg was funded by the Hans Merensky Legacy Foundation. Clara Pliego's INV2023.014 project was co-financed by the European Regional Development Fund within the Andalusia ERDF 2021–2027 Programme.

Declaration of competing interest

None.

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