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INTERACTIONS REVIEW

The Soilborne Fungus *Verticillium longisporum* and Its Interactions with the Brassicaceous Hosts

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***Verticillium longisporum*, a soilborne fungal species, is the causative agent of Verticillium stripe disease in *Brassica* species and represents a notable threat to agricultural production, particularly in regions where oilseed rape is a major crop, including Europe, North America, and Asia. The microsclerotia of this pathogen can persist in the soil for extended periods, with a potential lifespan of up to a decade, thereby posing a substantial challenge for the complete eradication of the pathogen from infested soil. The genome of *V. longisporum* is amphidiploid and resulted from the hybridization of *V. dahliae* (D genotypes) and an unidentified species (A1 genotype). At least three independent hybridization events are estimated to have occurred, resulting in three distinct lineages: A1/D1, A1/D2, and A1/D3. Genome sequence analysis revealed the presence of mating-type idiomorphs, putative cell wall-degrading enzymes, and effectors. However, due to the complexity of the genome, there is a paucity of research on the molecular interactions between *V. longisporum* and *Brassica* crops. This review summarizes the extant knowledge regarding the pathogenicity factors that *V. longisporum* deploys upon infection and the host immune responses against this attack, highlighting aspects that remain to be elucidated and the molecular tools available for studying this interaction. A better understanding of the molecular interactions in this pathosystem will contribute to developing more effective control measures against this disease in *Brassica* oilseed and cabbage crops.**

Keywords: Brassicaceae, hybrid, oilseed rape, soilborne pathogen, *Verticillium longisporum*

Background

The genus *Verticillium* comprises 10 species and is a class of soilborne plant-pathogenic fungi identified as causing disease in over 200 plant species (Inderbitzin et al. 2011a; Pegg and Brady 2002). The most notable pathogens are *Verticillium dahliae*, *Verticillium longisporum*, *Verticillium albo-atrum*, *Verticillium tricorpus*, and *Verticillium alfalfae* (Inderbitzin et al. 2011a). The genus name *Verticillium* is derived from the distinctive morphology of the conidiophores, which are branched and occur in multiple levels, forming a "verticilliate" configuration. The most notorious of these pathogens is *V. dahliae*, which causes typical wilting symptoms in a plethora of hosts. In contrast to *V. dahliae*, *V. longisporum* does not cause wilting and primarily infects plants belonging to the Brassicaceae family. Infection caused by *V. longisporum* has been recognized as a distinct disease, termed Verticillium stripe, reflecting the grayish-colored "stripes" often associated with its development (Depotter et al. 2016a). However, there has been confusion in the literature regarding the differentiation of the two *Verticillium* species (Depotter et al. 2016a). Nevertheless, genomic analyses (Fogelqvist et al. 2018) and molecular tools (Tzelepis et al. 2017) have enabled the differentiation of these two species and enhanced diagnostic accuracy.

The genus *Brassica* comprises an abundance of phenotypically diverse species that have evolved and explored during breeding into various vegetable, oilseed, and condiment crops (Wells 2024). Records of *Brassica* crops extend back to ancient times, with different species being appreciated and cultivated in many geographical regions. The historical development of these crops is reflected in the contemporary distribution of soilborne pathogens, particularly those capable of forming hardy resting structures. The intensive cultivation of "*Brassica* cash crops" has further contributed to the enrichment of soil with these pathogens. Currently, oilseed rape (*Brassica napus*) is the most prominent *Brassica* oil crop, with both spring and winter forms cultivated in temperate regions of Australia, Asia, Europe, and North America. The initial documentation of a novel soilborne pathogen responsible for wilting disease in oilseed *B. rapa* and *B. napus* occurred over five decades ago (Kroeker 1970), well before double low varieties, which exhibit low levels of erucic acid in the oil and low amounts of glucosinolates in the meal, were introduced into the market.

Yield losses caused by *V. longisporum* in oilseed rape range from 10 to 50% in Europe and Canada (Dunker et al. 2008; Wang et al. 2023). The annual yield of winter oilseed rape in Northern Europe is approximately one metric ton less than that in Central Europe (http://ec.europa.eu/eurostat), and part of this yield reduction is attributed to soil infestation by *V. longisporum.* In Asia, Chinese cabbage (*B. rapa* type) is the most important vegetable *Brassica* species, whereas cauliflower, broccoli, Brussels sprouts, and white/red cabbage (*B. oleracea* type)

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predominate in Europe and North America. The occurrence of both *V. dahliae* and *V. longisporum* in cabbage has been previously documented (Banno et al. 2015; Ikeda et al. 2012). It is also important to note that Verticillium stem stripes can be misidentified as Phoma stem cankers caused by the fungal pathogen *Leptosphaeria maculans* (anamorph *Phoma lingam*). However, Phoma stem cankers are associated with premature crop ripening (Wang et al. 2023). Phoma lesions are typically limited in length and are characterized by pink spore exudates. The co-occurrence of these diseases, often in combination with Sclerotinia stem rot and Fusarium wilt, forms a wilting disease complex. The progression of these diseases and potential yield losses are primarily driven by moisture and temperature (Wang et al. 2023).

Current Knowledge

Disease cycle

Regarding the lifecycle of *V. longisporum*, it is an anamorphic species that produces a high quantity of conidia. The conidia have been demonstrated to function as infection propagules. Their size, measuring 7 to 9 μm, exceeds the typical length of *V. dahliae* conidia (Karapapa et al. 1997). However, microsclerotia, the fungal resting structures essential for the long-term survival of *V. longisporum*, play the most prominent role in the spread of the disease (Fig. 1). Root infection and internal progression in *Brassica* species are known to be slow processes that progress without obvious external symptoms. At the senescence stage of the plants, microsclerotia protrude from the stem tissue, become visible, and begin to fall to the ground. They are typically observed on the stubble and leaf litter following harvest, and they become incorporated into the soil during the subsequent soil management operation (Fig. 1). Upon germination, fungal hyphae penetrate openings or wounds in the root, and the pathogen progresses into the xylem tissue (Fig. 1). The fungus can persist in the vascular tissue for extended periods, rendering it undetectable during field plant inspections. As the plants mature, grayish stripes become visible, and beneath the epidermis layer, the microsclerotia are formed (Fig. 1). Upon harvesting, the microsclerotia are dispersed, and contaminated debris falls to the ground, followed by soil infestation (Fig. 1), a process that initiates a new cycle of infection when conditions become conducive (Depotter et al. 2016a; Sayari et al. 2024).

Genome organization

Whole-genome duplications have occurred frequently in nature (Fox et al. 2020) and are a key feature, particularly of plants (Halabi et al. 2023); the Brassicaceae family is not an exception to this phenomenon (Das Laha et al. 2020). The self-compatible species *Arabidopsis thaliana* underwent three whole-genome duplication events. The most recent of these events is estimated to have occurred 47 million years ago. The temporal alignment of environmental instability with chromosomal rearrangements suggests a potential correlation between these events and enhanced evolutionary adaptability. In contrast, ploidy patterns observed in fungi remain largely unreported. Most of the available knowledge originates from unicellular species such as *Candida*

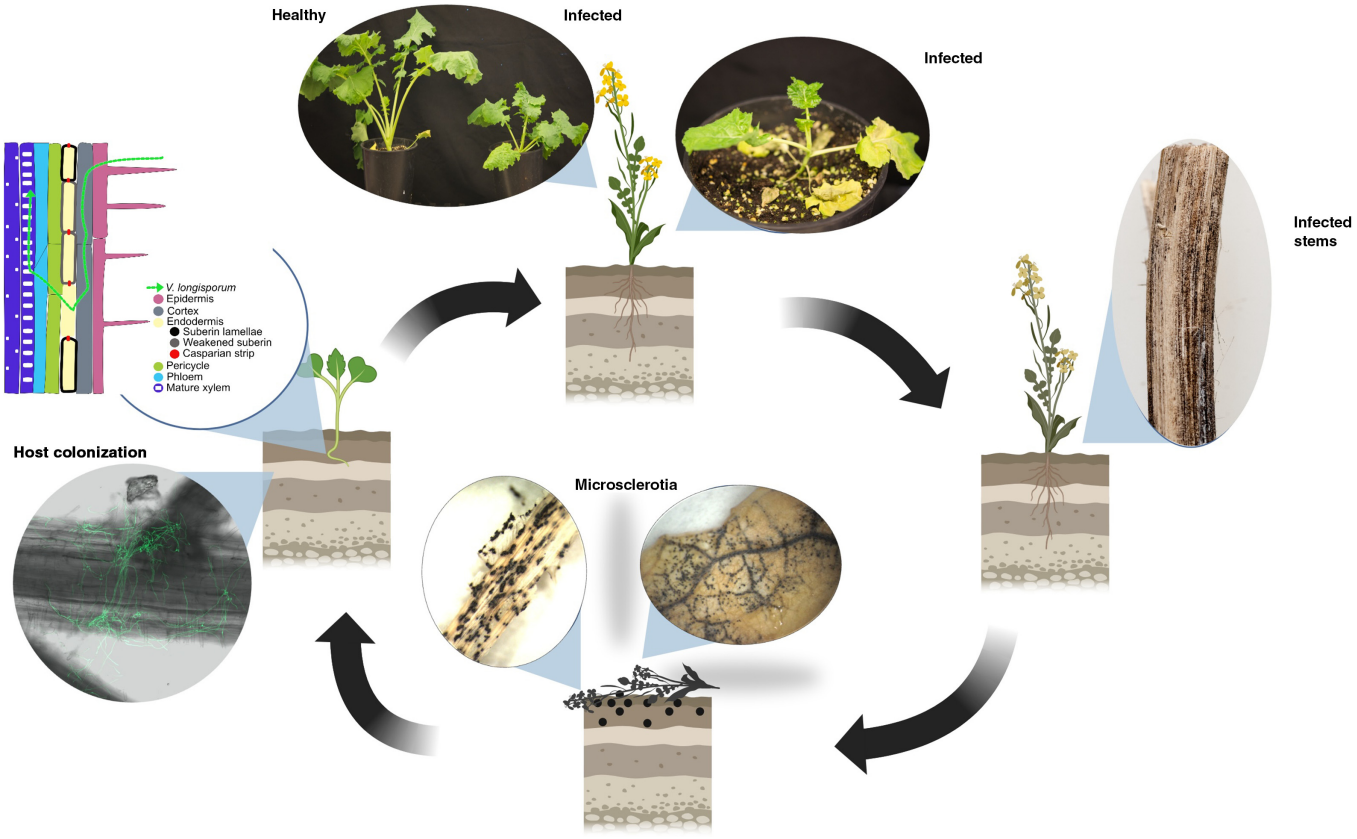

Fig. 1. Life cycle of *Verticillium longisporum*-infected oilseed rape. When *Brassica napus* plants start to senesce, microsclerotia protrude from the stem tissue, become visible, and begin to fall to the ground. Commonly, microsclerotia are observed or spotted on the stubble and leaf debris after harvest and become mixed into the soil, following the next soil management step. The microsclerotia are resistant structures and can survive in the soil for many years under adverse conditions. Upon germination, fungal hyphae enter openings or wounds in the root, and the fungus progresses into the xylem tissue. *V. longisporum* can remain in the vascular tissue for a long time, making it invisible during field plant inspections. Upon plant maturation, grayish stripes appear on the ripening stems by densely formed microsclerotia. They can start dropping to the ground and will be ready to initiate a new round of infections when the conditions become favorable. Figure created in BioRender.

orthopsilosis, most isolates of which are hybrids, a characteristic shared by related *Candida* species (del Olmo et al. 2023; Gerstein and Sharp 2021; Wertheimer et al. 2016). These *C. orthopsilosis* isolates are believed to have descended from at least four distinct hybridization events, each involving two distinct parental lineages. Among plant pathogens, lineages of *Ophiostoma* that cause Dutch elm disease have hybridized, leading to increased virulence (Steensels et al. 2021). Powdery mildew poses a significant threat to food security, and aggressive *Blumeria graminis* f. sp. *triticale* strains have evolved as a result of hybridization events between rye-specific and wheat-specific mildew genotypes. These strains have adapted to formerly resistant triticale crops (Menardo et al. 2016). It appears that during the hybridization process, genomes undergo substantial reorganization, including the gain and loss of genes, activation of transposons, and alterations in the transcriptome (Wu et al. 2022). It seems plausible that hybridization may have contributed to enhanced fitness, thereby enabling the hybrid species to demonstrate superior adaptation compared with its haploid progenitors (Depotter et al. 2016b). This indicates that extensive genetic recombination may be a pivotal factor in the narrow host specificity of *V. longisporum* against Brassicaceae hosts.

Phylogenetic analysis of several *Verticillium* isolates and five single loci indicated that *V. longisporum* comprised two progenitor genomes (A1 and D1) and two *V. dahliae* lineages (D2 and D3) (Inderbitzin et al. 2011b). Notably, A1 and D1 do not correspond to any known *Verticillium* species, which could indicate the involvement of extinct or hitherto uncharacterized species. Combinations of A1/D1, A1/D2, and A1/D3 differ in their virulence and pathogenicity (Novakazi et al. 2015; Vega-Marin and von Tiedemann 2023). A1/D1 lineages have been identified as the most pathogenic, whereas A1/D3 lineages are less capable of causing diseases in oilseed rape (Novakazi et al. 2015). A 20-kilobase sequence has been identified to be associated with virulence in an A1/D1 lineage as compared with A1/D3 (Harting et al. 2021). As for the A1/D2 lineages, they have only been identified in horseradish in Illinois (U.S.A.), and they are considered to be the least virulent (Novakazi et al. 2015). Genetic studies have indicated that this pathogen forms distinct genetic groups based on its geographic origin (Fahleson et al. 2003; Gladders et al. 2011; Steventon et al. 2002). Further analysis of 80 A1/D1 isolates revealed the presence of two distinct subgroups, designated as the "East" and "West" groups. The East A1/D1 group contains isolates primarily from the United Kingdom, Belgium, the United States of America, and Japan, whereas the West group contains isolates primarily from Sweden, Germany, France, and Latvia (Depotter et al. 2017). Additionally, a distinct A1/D1 subgroup was identified, predominantly comprising isolates from Canada, Germany, Sweden, Poland, and Latvia (Vega-Marin et al. 2025). Recent genomic information on *V. dahliae* from cotton revealed a partial overlap with two other species, *Plectosphaerella cucumerina* and *V. alfalfae*, and a whole-genome duplication event in the *V. dahliae* genome, the genomic consequences of which are unknown (Yang et al. 2023).

The genome size of *V. longisporum* is approximately 70 Mb (Fogelqvist et al. 2018), and the A1 genome is more ancient than the D1 genome, with the latter being more closely related to *V. dahliae*. *Verticillium* mitochondria carry 15 protein-coding genes (Shi-Kunne et al. 2018), and the mitochondrial genome of *V. longisporum* is similar to that of *V. dahliae* (Depotter et al. 2021; Fogelqvist et al. 2018). Long-read sequencing of three distinct *V. longisporum* isolates, representing the A1, D1, and D3 subgenome combinations, corroborated the genome size, which is distributed across 15 to 17 chromosomes (Depotter et al. 2021). Chromosome conformation capture (Hi-C) analysis has substantiated the dissection of chromosomal rearrangements, thereby providing profound insights into the mosaic structure of *V. longisporum* genomes (Depotter et al. 2021). The presence of two complete sets of chromosomes enables allele-specific gene expression, enhancing the capacity of an organism to adapt to altered environments (hybrid fitness advantage). In addition, a comprehensive Hi-C analysis identified distinct three-dimensional genomic structures or adaptive genomic regions from topologically associating domains in nine *Verticillium* species, including *V. longisporum* (Torres et al. 2024). The conservation of adaptive genomic regions across different *Verticillium* species suggests their fundamental role in adaptation and evolution. The three-dimensional chromosome organization, sequence specificity of the boundary regions, and overall chromosomal locations are believed to influence fungal evolution and transcript regulation. However, the precise mechanisms underlying these phenomena remain unclear.

Verticillium mating types

Sexual reproduction in ascomycetes is controlled by a small mating-type locus (*MAT*) with dissimilar sequences (idiomorphs). *MAT1-1* carries an α1-box in one idiomorph. This gene, called *MAT1-1-1*, defines the *MAT1-1* idiomorph, whereas the other idiomorph, *MAT1-2*, is characterized by the presence of a *MAT1-2-1* gene that encodes a transcription factor with a MATA_HMG domain (Turgeon and Yoder 2000). Both idiomorphs have been previously identified in *V. dahliae* (Short et al. 2014). Chemotactic growth assays further suggested that *V. dahliae* recognized two pheromones from *Fusarium oxysporum* f. sp. *vasinfectum*, a species that could be an ancient gene donor candidate (Zhang et al. 2024). Sequence searches of the *V. longisporum* genome revealed the presence of two copies of *MAT1-1-1* and one of the *MAT1-2-1* gene (Fogelqvist et al. 2018). Phylogenetic analysis indicated that *V. longisporum MAT1-1-1* was more closely related to the *V. dahliae* homolog, suggesting a D subgenome origin (Fogelqvist et al. 2018; Harting et al. 2021), whereas *MAT1-2-1* most likely originated from the A subgenome (Fogelqvist et al. 2018). When *V. longisporum* genomes from different isolates were compared, the two alleles of the *MAT1-1* idiomorph appeared to be conserved. The presence of *MAT1-2-1*, in contrast, varies between isolates (Harting et al. 2021). An *MAT1-1-3* sequence encoding another HMG domain was also identified. In conclusion, *V. longisporum* carried two *MAT1-1* alleles derived from each parent, whereas the opposite *MAT1-2* idiomorph was absent. However, as previously stated, the genus *Verticillium* is anamorphic, and no instances of sexual reproduction have yet been observed among its species.

The production of hybrid plant varieties is contingent on genetic systems that can restore fertility, which are often found in the nucleus of the mitochondrial donor genotype. Fertility restorer lines are typically developed by rigorous backcrossing or alternative methods (Bashir et al. 2018). However, information on nuclear-mitochondrial interactions in the fungal kingdom remains limited. Restoration of fertility in yeast hybrids has underscored the significance of nuclear-mitochondrial-dependent traits, and sterility has been surmounted by enabling continuous multigenerational breeding (Naseeb et al. 2021).

Secreted fungal proteins

Pathogenic fungi secrete various proteins to perforate the plant cuticle and epidermal cell layers, facilitating the invasion of apoplastic and cytoplastic spaces. Among secreted proteins, carbohydrate-active enzymes (CAZymes), which are responsible for plant cell wall degradation, are significant components of the fungal secretome (Drula et al. 2022; Rafiei et al. 2021).

The genome of *V. longisporum* encodes fewer polysaccharide lyases and carbohydrate-binding modules than those predicted for *L. maculans* (Fogelqvist et al. 2018). Several genes harboring LysM motifs and necrosis-inducing *Phytophthora*

proteins or NPP1-domains are also present (Fogelqvist et al. 2018). KOG analysis of the secreted proteins revealed approximately twice as many proteins involved in ion transport and metabolism, lipid transport, and metabolism as in *V. dahliae*, including abundant groups of small cysteine-rich effector candidates, of which few have been studied in more detail (Fogelqvist et al. 2018). Furthermore, proteomic analysis of *V. longisporum*, grown on media containing xylem sap from *B. napus* and pectin-rich media, revealed enrichment of CAZymes, such as glycoside hydrolases, polysaccharide lyases, and carbohydrate esterases (Leonard et al. 2020).

There is a paucity of functional studies of *V. longisporum* effectors, which may be attributed to the intricacies of the diploid genome and the absence of a transformation protocol. Among the few available studies, Rafiei et al. (2022a) showed that *V. longisporum Vl3720*, which encodes a putative endolysin protein, was significantly induced upon infection with *B. napus*. Furthermore, the overexpression of this gene leads to enhanced virulence in *A. thaliana* Col-0 plants (Rafiei et al. 2022a). Although the precise function remains to be determined, it can be speculated that fungal endolysins facilitate the degradation of host cell walls, thereby enabling the translocation of other pathogenic factors into the cytoplasm. A phospholipase A2 plays a significant role in the virulence of this fungal species (Rafiei et al. 2023). This phospholipase has been identified as a component that targets the host nucleus, leading to alterations in the expression of genes, including MYB transcription factors, receptor-like protein kinases, and subtilisin-like proteases, which are involved in the basal induction of plant immunity (Rafiei et al. 2023). This process is thought to be accomplished by the hydrolysis of phospholipids in the nuclear envelope. However, the underlying mechanism of this phospholipase remains unclear.

Tran et al. (2014) showed that the transcriptional activator *Vta2* is a crucial factor in the pathogenicity of *V. longisporum*. This regulator appears to play a pivotal role in facilitating fungal adhesion to plant roots during the early stages of infection by inducing the expression of adhesin genes and suppressing the formation of microsclerotia. By balancing adhesion and microsclerotia formation, *Vta2* is a critical regulator of both systemic infection and asexual reproduction and, thus, an important virulence factor for *V. longisporum*. Proteomic analysis of *B. napus* sap demonstrated enrichment of known *Verticillium* effector proteins, including cerato-platanins, necrosis and ethylene-inducing protein-like proteins, and metalloproteases. Functional analysis of homologous genes in *V. dahliae* revealed involvement in virulence (Leonard et al. 2020). A comparison of the genomes of *V. longisporum* and other *V. dahliae* races showed the presence of a homolog to the Ave2 effector from race 2 of *V. dahliae*, which is recognized by the *V2* tomato plants (Chavarro-Carrero et al. 2021). In contrast, no homologs have been identified for the Ave1 from race 1, which displays an antimicrobial activity (de Jonge et al. 2012; Snelders et al. 2020), or for the race 3-specific VdR3e effector (Tan et al. 2023) in the published *V. longisporum* genomes.

Host-*V. longisporum* interactions

In addition to having a physical anchoring role, roots facilitate nutrient and water acquisition in the above parts of the plant. Radially, the root cells are organized in concentric cylinders surrounding the stele. Root cell anatomy varies among species, root types, and plant developmental stages (Kawa and Brady 2022). Accordingly, responses to different microorganisms depend on the root cell type they attempt to invade. This response can vary from a physical blockage by the endodermal barrier and Casparian strip to a lack of recognition of the effectors and chitin.

Plant hormones. In studies on root development and growth, particularly in *A. thaliana*, much emphasis has been placed on hormone homeostasis and crosstalk. Among the most important root hormones are auxins and the common indole-3-acetic acid (Rayle and Cleland 1992). The establishment of a root-to-shoot auxin gradient and local auxin maxima by auxin influx and efflux carriers controls root cell division and differentiation, including the formation of lateral roots from pericycle cells (Dubrovsky et al. 2008; Grieneisen et al. 2007). Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are the three major hormones that regulate defense signaling (Glazebrook 2005). These three hormones also defend against *V. longisporum* (Johansson et al. 2006; Ralhan et al. 2012; Ratzinger et al. 2009). Phytohormones, including abscisic acid, brassinosteroids, gibberellic acid, and cytokinin, modulate the SA and ET/JA signaling pathways (Li et al. 2019). Enhanced drought tolerance in *A. thaliana*, triggered by the vascular-related NAC domain transcription factor, was an early finding in this pathway (Reusche et al. 2012). The following finding was that abscisic acid plays a vital role in the stomatal immune response to *V. longisporum* (Roos et al. 2014) but is repressed during early infection in *B. napus* (Behrens et al. 2019). Several defense factors against *V. longisporum* have been identified, among which JA is involved in transcriptional regulation (Dölfors et al. 2024; Roos et al. 2015).

The role of phytohormones, their crosstalk, and their impact on a range of transcription factors in *V. longisporum* interactions is a complex and multifaceted process, one that is susceptible to disruption and may result in adverse consequences (Schenke and Cai 2020). The use of diverse materials, developmental stages, and cultivation regimens further complicates the comparison of valuable targets demonstrated by research teams. Another challenge that must be addressed before large-scale breeding initiatives can be undertaken is the translation of observations from controlled to field conditions (Lopisso et al. 2017; Zheng et al. 2019). *Arabidopsis thaliana* has a generation time of approximately 6 weeks, whereas winter oilseed rape in the field requires 10 to 12 months from seed to seed. During this long growth period, the oilseed crop encounters many challenges compared with the controlled environments applied for the *A. thaliana* plants.

Glucosinolates (GLSs). Volatiles are released in response to various forms of damage, including mechanical stress and herbivore attacks (Danner et al. 2018). Many of these volatiles are derived from the shikimic acid, isoprenoid, and lipoxygenase pathways (Dudareva et al. 2013). The availability of biosynthetic precursors, the presence of an endogenous circadian clock, and the activity of transporters influence the synthesis and release of these molecules. GLSs represent a substantial group of nitrogen- and sulfur-rich plant secondary metabolites predominantly found within the family Brassicaceae (B. Zhu et al. 2023). The bioactivities of GLSs are predominantly ascribed to their catabolism, which is catalyzed by a specialized group of β-glucosidases known as myrosinases (Andersen et al. 2013; Wittstock et al. 2016). The composition and content of GLSs exhibit significant variation between different plant tissues and between species, such as *A. thaliana* and *B. napus* (Kittipol et al. 2019; Kliebenstein et al. 2001; Sønderby et al. 2010; Y. Zhu et al. 2023). GLS biosynthesis is regulated by a complex network of transcription factors (Mitreiter and Gigolashvili 2021). The MYB-DOMAIN PROTEIN (MYB) transcriptional regulatory family plays a central role in this process. The accumulation of glucosinolates is also influenced by hormones such as abscisic acid, JA, ET, and SA. The products resulting from GLS hydrolysis exhibit a high degree of diversity, and many of these compounds are highly reactive (Wittstock et al. 2016). Two plasma membrane-localized glucosinolate importers, GTR1 and GTR2, have been identified as key players in the transport of aliphatic GLSs (Xu et al. 2017). This finding is significant for advancing our understanding of rhizosecretion of root-synthesized phyto-

chemicals, facilitating a more comprehensive analysis of the intricate processes occurring within plant roots.

Interactions between GLS and its breakdown products with respect to nearly all *Brassica* pathogens have been the subject of many studies over the years. In the case of *V. longisporum*, the GLS breakdown products could impede the fungal infection process (Witzel et al. 2015). The influence of members of the large ET response factor family on indole GLS production in plants, in turn, influences the susceptibility of plants to *V. longisporum* (Fröschel et al. 2019). However, numerous aspects of GLS biology, including regulation, signal mediation, and cellular transport, remain unclear. The MYB transcription factor family, which comprises approximately 200 members in *A. thaliana*, regulates diverse processes beyond GLSs (Biswas et al. 2023). MYB108 induces reactive oxygen species generation by interacting with the promoter of the respiratory burst oxidase homolog in *B. rapa* (Su et al. 2023). The resulting reactive oxygen species impedes the growth of *V. longisporum* during the nascent stage. However, the question remains of whether *V. longisporum* undergoes a biotrophic phase inducing a hypersensitive response in the host. The progression of fungal growth in vascular tissues has been the subject of divergent studies (Depotter et al. 2016a; Reusche et al. 2014). The process of plant senescence is intricate and influenced by numerous factors (Guo et al. 2021; Miryeganeh 2021; Zhang et al. 2023). The initiating factors of microsclerotia development and growth in the outer stem cell layer, where they are clearly visible, remain unclear.

Terpenes. Terpenes, a diverse class of volatile organic compounds, play a major role in the defense mechanisms against *V. longisporum* (Rafiei et al. 2022b; Roos et al. 2015) These compounds, particularly monoterpenes, are produced in response to pathogen infection and function as antimicrobial agents that can disrupt fungal cell walls or inhibit fungal growth. Terpenes play a major role in plant-pathogen interactions, particularly in the rhizosphere, where plants secrete them to influence the microbial community (Boncan et al. 2020). The role of monoterpene production in *A. thaliana* susceptibility to *V. longisporum* has been the subject of investigation. A previous study identified the monoterpene synthase genes *TPS23/27* as critical determinants in the initial stages of fungal infection and revealed significant alterations in monoterpene production, particularly 1,8-cineole, which stimulates the germination of *V. longisporum* conidia and hyphal growth (Roos et al. 2015). The regulation of this pathway is linked to JA signaling, which is mediated by the transcription factor MYC2 (Roos et al. 2015). A recent study has demonstrated the involvement of monoterpenes, α-pinene, β-pinene, and *3*-carene, in *V. longisporum* infection in oilseed rape. Of these, β-pinene has been identified as a particularly effective agent in the plant defense mechanisms against this pathogen (Rafiei et al. 2022b).

Lectins. Calreticulin (CRT) and calnexin are homologous lectins that function as glycoprotein molecular chaperones in the endoplasmic reticulum of eukaryotic cells. Genes encoding CRT in *A. thaliana* and *B. napus* reduce susceptibility to mutagenesis (Pröbsting et al. 2020). Inactivation of *CRT* genes triggers the ET signaling pathway, potentially influencing additional defense responses. Conversely, a combination of target mutations in candidate genes can potentially result in *Brassica* crops exhibiting improved resistance to *V. longisporum*.

Other host defense responses. Using tagged ribosomes expressed in a cell-type-specific manner has facilitated the isolation of ribosome-bound mRNA, thereby yielding cell layer translatomes (TRAP-seq, translating ribosome affinity purification, and RNA sequencing). This approach has further elucidated the intricacies of cellular events associated with pathogenic infections (Fröschel et al. 2021). In this study, *V. longisporum* was shown to suppress the endodermal barrier, which restricts the invading hyphae from reaching the central cylinder of the roots. This tissue consists of the pericycle, xylem, and phloem. The nitrate transporter NPF5.12 and the major latex-like protein

Table 1. Genetic resources available for *Verticillium longisporum*-Brassicaceae interactions

Database	BioProject	Citation	Link	Resources
NCBI	GSE62537	Roos et al. 2015	https://doi.org/10.1111/tpj.12752	Microarray raw data on *Arabidopsis thaliana* lines upon *V. longisporum* infection
NCBI	KY828946–KY828954	Depotter et al. 2017	https://doi.org/10.1111/1462-2920.13801	Simple sequence repeat markers for *V. longisporum* A1/D1 and A1/D3 lineages
NCBI	SRR5430591– SRR5430596, SRR5435055– SRR5435060	Depotter et al. 2017	https://doi.org/10.1111/1462-2920.13801	Illumina genome sequence raw data of VLB2 and VL20 isolates
ENA	GCA_001268145	Fogelqvist et al. 2018	https://doi.org/10.1186/s12864-017-4407-x	Illumina genome sequence raw data of VLI and VL2 isolates
NCBI	PRJNA419857, PRJNA563868	Behrens et al. 2019	https://doi.org/10.1111/mpp.12867	RNA sequencing raw data of *A. thaliana* and *Brassica napus* upon *V. longisporum* infection
NCBI	PXD018533	Leonard et al. 2020	https://doi.org/10.3389/fmicb.2020.01876	Proteomics raw data of *V. longisporum* upon exposure to plant xylem media
NCBI	PRJNA473305	Depotter et al. 2021	https://doi.org/10.1128/mbio.01496-21	PacBio genome sequence raw data of the VLB2, VL20, and PD589 isolates from lineages A1/D1 and A1/D3
NCBI	PRJNA643983–PRJNA643985	Harting et al. 2021	https://doi.org/10.1111/mpp.13071	Illumina genome sequence raw data of the Vl42 and Vl43 isolates from lineages A1/D1 and A1/D3
NCBI	GSE15 8956	Rafiei et al. 2022b	https://doi.org/10.1111/mpp.13162	RNA sequencing raw data of *V. longisporum* upon exposure to *B. napus* β-pinene
NCBI	PRJNA995408	Su et al. 2023	https://doi.org/10.1016/j.celrep.2023.112938	RNA sequencing raw data upon infection of *B. rapa* with *V. longisporum*
NCBI	PRJNA1101731	Vega-Marin et al. 2025	https://doi.org/10.1111/ppa.14009	Raw sequence reads from 142 *V. longisporum* isolates sequenced with Illumina
–	Upon request	Wang et al. 2024	https://doi.org/10.3389/fpls.2024.1436982	Genome-wide association study analysis of *V. longisporum* resistance in *Brassica* genotypes

MLP6 are crucial in enabling plants to defend themselves against *V. longisporum*. Gene editing to mutate both genes in *B. napus* resulted in increased fungal colonization and growth, emphasizing their involvement in plant defense against the fungus (Dölfors et al. 2024). These proteins function in a coordinated manner in the plasma membrane and endoplasmic reticulum, thereby fortifying the cellular defense.

Available Resources and Tools

The genome assemblies of several *V. longisporum* isolates, RNA sequencing data, and proteomics data can be accessed via BioProjects at the National Center for Biotechnology and Information (NCBI, https://www.ncbi.nlm.nih.gov/) and the European Molecular Biology Laboratory (EMBL, https://www.embl.org). These resources are summarized in Table 1. Transcriptomic data from the host plants of *V. longisporum*, such as the RNA sequencing analyses of *Brassica* species and *A. thaliana*, are also available (Table 1). A genome-wide association analysis of *Brassica* accessions was conducted to identify resistant loci against this pathogen (Table 1). A variety of molecular techniques have been employed, including heterologous expression of pathogenicity factors in other organisms, construction of overexpressed fungal strains, deletion of homologous genes in the parental *V. dahliae* species, and construction of mutants in *Brassica* plants (Dölfors et al. 2024; Leonard et al. 2020; Rafiei et al. 2022b, 2023). All these available recourses and tools could contribute toward a better understanding the molecular interactions within this pathosystem.

Knowledge Gaps and Future Research

The diploid nature of the *V. longisporum* genome has resulted in a paucity of available functional studies, which is a significant limitation. To date, only a limited number of pathogenic factors have been characterized for this pathogen, primarily through heterologous expression in other systems. This is due to the inherent complexity of constructing deletion strains using the classical homologous recombination strategy. Consequently, developing an efficient CRISPR/Cas9 or other gene-editing system, as previously developed for the diploid oomycete *Phytophthora* species (Cao et al. 2022; Mendoza et al. 2025), would facilitate a more comprehensive understanding of the numerous aspects of *V. longisporum* infection biology.

Moreover, it is imperative to elucidate the mechanisms by which *V. longisporum* loses its capacity to infect a diverse range of hosts akin to the progenitor *V. dahliae.* Therefore, it is necessary to conduct extensive evolutionary studies to ascertain whether hybridization influences host specificity. The reduction in the host range observed in this species represents an intriguing phenomenon worthy of further investigation. Identifying the pathways and molecular factors responsible for host specificity and virulence can provide valuable insights into the mechanisms underlying these processes.

A comprehensive understanding of the lifecycle of *V. longisporum* is essential to develop an effective management strategy for this disease. For example, microsclerotia play a significant role in the infection process of *V. longisporum*, as they can survive in soil and plant debris for extended periods under extreme climatic conditions. Microsclerotia germinate and infect the roots under conditions conducive to their proliferation. The precise mechanisms underlying their germination remain poorly understood. Further research is required in the *B. napus* rhizosphere to ascertain the exact roles of secondary metabolites in microsclerotia germination. Finally, it would be interesting to determine whether the root microbiome influences this process as well.

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