VIRULENCE
2026, VOL. 17, NO. 1, 2682654
https://doi.org/10.1080/21505594.2026.2682654

Taylor & Francis
by informa

FUNGAL AND PARASITIC PATHOGENESIS

Pathogenicity and Virulence of *Fusarium oxysporum* f. sp. *vasinfectum* Race 4: Understanding an Emerging Threat to Cotton from Field to Genome

Yi Zhou[a], Huan Zhang[a], Timothy K. Martin[a], Jennifer Chagoya[b], Thomas Isakeit[a], and Won Bo Shim [a]

[a]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA; [b]Department of Plant Pathology and Microbiology, Texas A&M AgriLife Research and Extension Center at Lubbock, Lubbock, TX, USA

ABSTRACT

Fusarium oxysporum f. sp. *vasinfectum* race 4 (*Fov*4) is a highly virulent pathogen threatening cotton production globally, particularly in the US. This review synthesizes current knowledge of *Fov*4 biology, pathogenesis, and genomic features that drive host specificity and virulence. *Fov*4 life cycle, infection mechanisms, environmental factors influencing disease, and host resistance in Pima and Upland cotton varieties are discussed. Genomic resources reveal that *Fov*4 genomes are larger and more repeat-rich than those of several other *Fov* races, although chromosome-scale core versus accessory architecture remains unresolved. Studies have identified candidate race-specific virulence factors, including race-specific SIX effectors and secondary-metabolite genes in *Fov*4 and related *Fov* races. Challenges remain in managing cotton diseases caused by *Fov*4 due to its soil persistence and genetic adaptability. Integrated management combining resistant varieties, cultural practices, and precision monitoring ensures sustainable disease control, while advancing knowledge of *Fov*4 virulence mechanisms enhances future management strategies through innovative approaches.

ARTICLE HISTORY
Received 23 October 2025
Revised 14 March 2026
Accepted 27 May 2026

KEYWORDS
Cotton Fusarium wilt; *Fusarium oxysporum* f. sp. *vasinfectum* race 4; virulence factors; SIX effectors; host resistance; integrated management

Introduction

Cotton Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), is a globally significant disease that threatens both yield and fiber quality in *Gossypium* spp. It occurs across all major cotton-producing regions, including the United States, China, India, Pakistan, Australia, and several African countries. In the US, the disease has been detected in multiple cotton-growing states, with particularly virulent outbreaks of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 (*Fov*4) reported in California (2001), Texas (2017), and New Mexico (2019) [1–3]. These outbreaks have led to severe production losses, with some regions experiencing complete field failure. In 2022 alone, *Fusarium* wilt caused an estimated $21 million in damages in the US [4].

Fov is part of the *Fusarium oxysporum* species complex (FOSC), a large and genetically diverse group of soilborne fungi [5]. FOSC includes plant pathogens, opportunistic pathogens of humans and animals, and nonpathogenic strains. These fungi affect more than 100 plant species and exhibit remarkable host specificity. Individual isolates often show strong host adaptation, hypothesized as the result of rapid evolutionary processes occurring within the last 30 million years [6]. The term forma specialis (f. sp.) is used to describe strains adapted to specific hosts. Comparative genomics has revealed that horizontally acquired lineage-specific (LS) chromosomes play a pivotal role in host specificity and pathogenicity [7]. These LS chromosomes carry predicted virulence genes and effector proteins that enable adaptation to different hosts. Their transfer between isolates explains the rapid emergence of new pathogenic lineages and the polyphyletic nature of many formae speciales [8–12].

Fov exhibits remarkable diversity, with eight nominals races distinguished by their ability to infect various plant species, including soybean, tobacco, okra, lupine, and alfalfa, as well as specific *Gossypium* spp. cultivars [13,14]. Phylogenetic and genomic analyses group these races into four principal lineages, although traditional race classification remains based on pathogenicity patterns and differential disease severity in cotton [15–17]. While most *Fov* races induce mild to severe symptoms, particularly in conjunction with root-knot nematodes (*Meloidogyne incognita*) [18], *Fov*3, *Fov*4, and *Fov*7 stand out as exceptionally capable of causing severe wilt even in the absence of nematodes [2,19]. *Fov*4 was first recorded in India in 1908, with formal pathotype characterization established in 1960 [20]. It is now recognized as one of the most virulent races, capable of causing rapid and severe wilt in both Pima (*G. barbadense*) and Upland cotton (*G. hirsutum*).

CONTACT Won Bo Shim wbshim@tamu.edu

In the US, *Fov*4 was first detected in San Joaquin Valley of California, between 2001 and 2003, where it became a significant threat [21,22]. It was later confirmed in El Paso and Hudspeth counties of Texas in 2017 and in New Mexico in 2019, with evidence suggesting it may have been present in those areas for several years prior to the official identification (Parris et al. 2023; [1,3]). *Fusarium* wilt has long been a concern in US cotton production. According to the National Cotton Council of America, over a 59-year monitoring period (1965–2024), this disease accounted for an average annual loss of 71,133 bales, corresponding to an estimated value of $18.8 million [4. These consistent losses emphasize the need for sustained efforts in breeding, management, and disease surveillance. However, despite the availability of *Fov*4 genome assemblies and field observations, there is still limited functional validation of *Fov*4-specific virulence factors, incomplete resolution of its genome architecture, and a lack of integrative frameworks linking pathogenicity mechanisms with practical management. This review synthesizes current understanding of *Fov*4 pathogenicity and virulence mechanisms, integrating insights from its biology and genomics, with three specific objectives: (i) to summarize the biology, life cycle, and infection processes of *Fov*4 in cotton; (ii) to summarize available information on *Fov*4 virulence determinants and genomic features relative to other *Fov* races; and (iii) to outline current challenges and future directions for developing effective and sustainable cotton disease management strategies.

Biology, life cycle, and disease development of *Fov*4

Fov displays the typical morphological features of FOSC and is classified in the subdivision Deuteromycotina (Fungi Imperfecti) because its sexual reproduction has not been determined. Briefly, the colony morphology on potato dextrose agar (PDA) consists of fluffy aerial mycelia that vary in color from white to light violet with a light to dark violet or sometimes pinkish pigmentation secreted when grown on PDA. It produces three types of spores asexually: microconidia, macroconidia, and chlamydospores [23] (Figure 1). Microconidia are oval to kidney-shaped with 0 or 1 septum, and are produced from intercalary phialides, generally in false heads. Macroconidia are falcate and have three or four septa [24]. They are produced most often from terminal phialides that

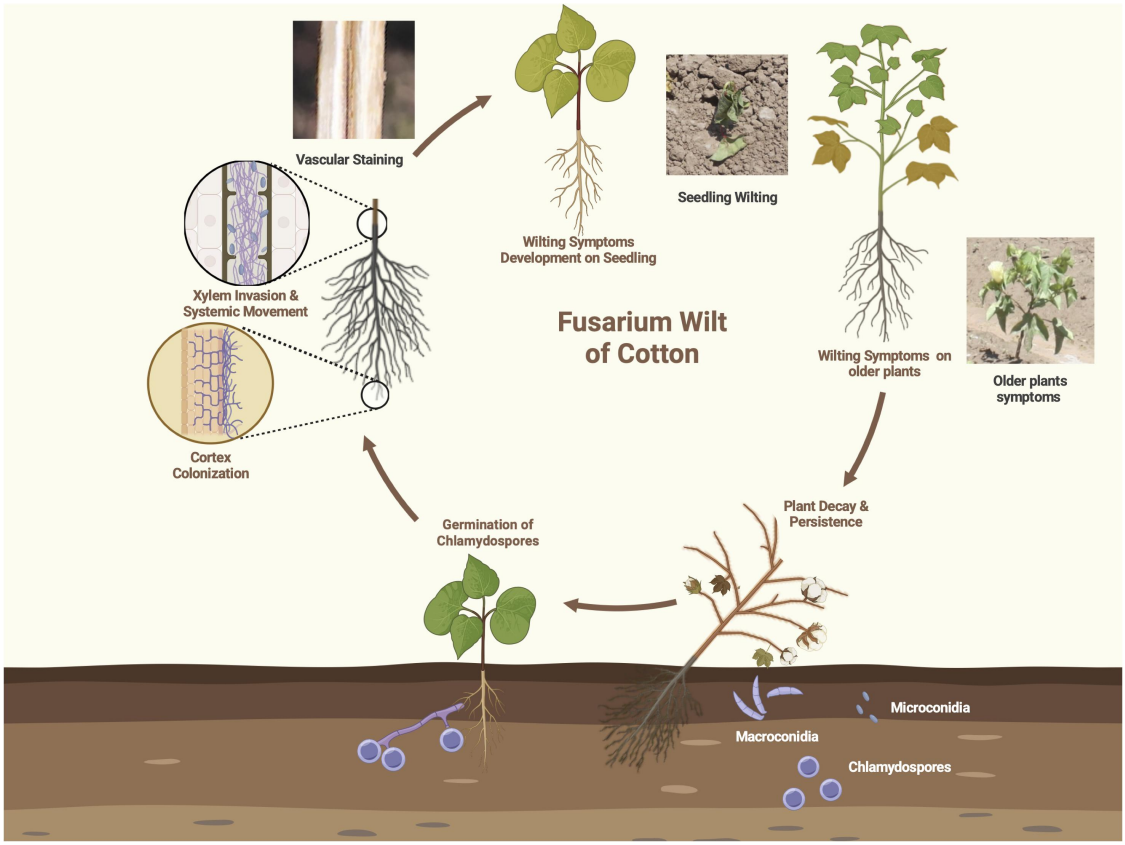

Figure 1. Infection cycle of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 in cotton.

arise from conidiophores, and at low frequencies from intercalary phialides that arise directly from hyphae. Both microconidia and macroconidia can be found on the surfaces of infected plants and serve as secondary inocula to infect neighboring host plants [25]. Chlamydospores are round-shaped with thick walls and are generally developed through the modification of hyphal and conidial cells via condensation of their contents [24]. These spores can survive in the soil for over a decade and act as primary inocula when a host is present [26].

*Fov*4 usually survives in soil and plant debris as thick-walled chlamydospores, which can stay viable for more than ten years in soil that has not been planted with cotton [27,28]. These spores germinate when they encounter root exudates, such as sugars and amino acids, from both host and non-host plants [29–31]. *Fov* spore germination is triggered by root exudates from cotton and other related hosts [30]. In the absence of a suitable host, germ tubes either die or form new chlamydospores, enabling long-term persistence in soil [29,32].

The resulting germ tubes penetrate roots either through wounds or directly through the epidermis (Figure 1). After attaching to cotton roots, germinated spores begin to colonize the root surface by forming compact mycelial mats [33, 34]. At this stage of hyphal colonization, the fungus does not cause visible damage to the root tissues. Instead, the interaction of *F. oxysporum* is classified as an endotrophic or biotrophic stage, where the pathogen coexists with the host without immediately inducing disease symptoms [35] . Once the mycelial mat is formed, the hyphae enter a parasitic stage. At this stage, the hyphae gain entry through root hairs, epidermal cracks, or wounds and then advance through the cortex and endodermis into the vascular system. Hydrolytic enzymes produced by the fungus could play an important role in penetration [36].

Once the hyphae reach the vascular tissue, many pathogenic *F. oxysporum* strains rapidly spreads through xylem vessels and colonizes the vascular bundles in roots. In the xylem vessels, microconidia produced germinate into new hyphae, which penetrate adjacent vessel elements to further proceed with colonization, resulting in increased spread of the pathogen [37,38]. Symptom expression in cotton occurs when hyphae spread into the cell apoplast [36]. Vascular systems are clogged by fungal mycelia and spores (Figure 1), which is exacerbated by host defense responses such as tyloses and gum deposition. These developments impair water and nutrient transport, resulting in wilt and eventually death of the plant [36,38] (Figure 1). Finally, as the diseased plant decays, the spores are released back into the soil and remain dormant until the next cycle [39].

*Fov*4 can infect cotton at any growth stage, and cotton growth stages may play an important role in *Fov*4-associated disease development. In seedlings, infection causes wilted cotyledons, root rot, plant wilt, and death before producing any bolls. These symptoms are similar to those caused by other pathogens such as *Rhizoctonia* species and *Pythium* species. However, these pathogens mainly cause seedling damping-off or root rot, producing sunken lesions or water-soaked roots, but without vascular discoloration. *Fov*4 can be differentiated from these pathogens by the presence of vascular discoloration in the xylem of the hypocotyl [14].

In older plants, *Fov*4 causes symptoms such as wilting, leaf chlorosis and necrosis, stunting, defoliation, reduced yield, and plant death, although death is less frequent [11,40] (Figure 1). Surviving older plants may produce cotton bolls, but with significantly reduced yield and poorer quality fiber [14]. *Fusarium* wilt is diagnosed by cutting roots, stems, or branches longitudinally. Dark brown discoloration in the vascular system of roots, lower main stems, or branches appears as visible lines or dots in cross sections [37,41]. Under wet conditions, sporulation may also appear as white, pink, or orange fungal growth on root and stem surfaces [42]. At the field scale, scattered patches of wilted seedlings first appear, then expand over successive seasons due to movement of infested soil by irrigation water and farm equipment [43]. It may take several years to spread through the field. Infected seed also provides an important route for long-distance dissemination [21].

This illustration presents the infection cycle of *Fov*4 in cotton, beginning with the release of root exudates that stimulate fungal germination. The pathogen forms long-lived survival structures (chlamydospores) in the soil, which germinate upon sensing host signals. Germ tubes penetrate the root epidermis, initiating hyphal colonization of cortical tissues. The fungus then invades the vascular system, producing microconidia and macroconidia that facilitate systemic spread. As the xylem becomes obstructed by fungal growth and host defense responses, wilt symptoms manifest, including vascular staining, leaf chlorosis, and plant death. The cycle concludes with formation of chlamydospores and sporulation on decaying tissue and the return of propagules to the soil, enabling long-term persistence.

Fusarium wilt symptom development is influenced by many factors including cotton genotype, pathogen virulence, environmental conditions, inoculum density, soil type, interaction with nematodes or other

pathogens, and crop management practices [14,44]. Disease pressure can vary from year to year based on weather conditions, even in the same field. In susceptible *G. barbadense* (Pima cotton), infection often leads to rapid seedling death, while resistant *G. hirsutum* (Upland cotton) may remain non-symptomatic aboveground but display extensive vascular browning [2,40].

A study comparing *Fov*4 infection in resistant Pima PHY 841 RF and susceptible Pima S-7 using hydroponic inoculation and microscopy showed that PHY 841 RF exhibited delayed penetration, reduced conidial attachment and fungal growth, and complete blockage of xylem invasion, confirming its resistance derived from PHY 800 and Pima S-6 [34]. Similarly, resistance in FM 2334GLT (a resistant upland cotton line) was similar to that in Pima PHY 841 RF but with fewer hyphae in the cortex, suggesting possible different resistant mechanisms [45].

*Fov*4 infection and symptom development are favored by relatively low temperatures around 23°C [46], while other races of *Fov* are favored by higher temperatures of 25–28°C [37,47,48]. Since Fusarium wilt of cotton is an inoculum density-dependent disease, inoculum density in soil is important for disease development [18,49,50]. If conidia levels are low or field conditions are not ideal for the pathogen, plants may not show any symptoms. Thresholds between 10^3 and 10^4 conidia per gram of soil are required for disease development in the susceptible Pima cultivar DP 744 [49], while nematode presence can significantly lower this threshold [18,49]. Higher inoculum densities exceeding 10^5 conidia per milliliter are needed to cause above-ground Fusarium wilt symptoms for a virulent *Fov* strain, and much higher concentrations of 10^6 conidia per milliliter are required to develop apparent symptoms on resistant cotton cultivars [50]. Zhang et al. [51] observed an approximately linear increase in disease severity rating across 10^4 –10^7 conidia ml^{-1}, with 10^6 –10^7 conidia ml^{-1} generally producing higher mean disease scores than 10^4 –10^5 conidia ml^{-1} across planting dates and cultivars. Together, these studies indicate that optimal or discriminating inoculum densities depend on multiple factors, such as cultivar susceptibility, growth stage at inoculation, temperature, and soil properties, rather than a single fixed threshold. Due to its persistence in soil and ability to colonize resistant and tolerant cultivars at sub-symptomatic levels, eradication of *Fov*4 from infested fields is not feasible [40]. Therefore, further study to unveil its molecular mechanisms and develop resistant varieties is essential.

Genomics and phylogenomics of Fov4

The genomes of FOSC isolates display a characteristic two-speed architecture, consisting of highly conserved core chromosomes and more variable, repeat-rich LS or accessory chromosomes [7,12,52]. Core chromosomes typically consist of 10–12 conserved chromosomes that encode essential housekeeping and metabolic functions and show synteny across FOSC [52]. In contrast, LS chromosomes are characterized by significantly lower gene density (150–200 genes per megabase), elevated transposable element content (often > 70% of total repeats), and highly dynamic structure [7,11]. These chromosomes are enriched in virulence factors, including secreted in xylem (SIX) effector proteins, cell wall degrading enzymes, and secondary metabolite biosynthesis clusters [53–55]. The horizontal transferability of entire LS chromosomes or chromosomal segments represents a key mechanism driving the rapid evolution of new pathogenic lineages and explains the frequent polyphyletic nature of many formae speciales within FOSC [7,9,12,56].

Genome sizes across FOSC members range from approximately 50–70 Mb, with the reference strain *F. oxysporum* f. sp. *lycopersici* (Fol4287) containing 15 chromosomes (~54 Mb total), including four LS chromosomes [7,55,57]. For *Fov*4, available draft assemblies provide estimates of genome size, gene content, and repeat abundance and indicate that race 4 has a larger, more repeat-rich genome than some other *Fov* races, consistent with an expanded accessory genome (Table 1). However, because these assemblies remain at contig level, *Fov*4 core and lineage-specific chromosomes have not yet been comprehensively identified or characterized.

Recent high-quality assemblies, such as the gap-free genome of strain Fo5176, have revealed 18–19 complete chromosomes totaling ~70 Mb, demonstrating the genomic plasticity within the complex [52,63–65].

More high-quality assemblies have become available for several *Fov* races, including *Fov*1, *Fov*4, *Fov*5, *Fov*7, *Fov*8, and Australian biotypes [17,58–62] (Table 1). These assemblies vary in completeness, sequencing technology, and annotation depth, but collectively they illuminate both conserved and race-specific genome architecture features across the FOSC. Genome assemblies of *Fov* span ~49.9 to 68.7 Mb across races, but *Fov*4 isolates consistently fall toward the upper end of this range. While *Fov*1 genomes are typically ~50–52 Mb with ≤18,000 predicted genes and <10% repetitive DNA, *Fov*4 assemblies exceed 61 Mb and often encode > 20,000 genes, with repeat content reaching 18.4% in the California isolate 89-1A [58–60]. This expansion is mirrored in the large genomes of *Fov*5 (68.7 Mb,

Table 1. Summary of genome assemblies of *Fusarium oxysporum* f. sp.*vasinfectum* Races and isolates.

Genotype	Isolate / Strain	Geographic Origin	Genome Size (Mb)	Gene Count	Sequencing Technology	Assembly Level	Chromosomes / Contigs	GenBank Accession	Repetitive Sequence	Reference
*Fov*1	TE1	USA	50	17,975	PacBio	Scaffolds	17	GCA_009602505.1	3.1 Mb	[58]
*Fov*1	ME23	California, USA	49.9	16,305	PacBio	Scaffolds	94	GCA_030719095.1	11.7 Mb	[59]
*Fov*1	NRRL 25,420	USA	52	16,384	PacBio	Scaffolds	26	GCA_012610785.1	4.6 Mb	[60]
*Fov*4	89-1A	California, USA	63.3	20,222	PacBio + Illumina	Contigs / Scaffolds	54 / 30	GCA_038050535.1	11.8 Mb	[58]
*Fov*4	NRRL 25,434	India	67.8	20,259	Nanopore	Contigs	13	GCA_012610825.1	NA	[60]
*Fov*4 (N-type)	Tm2	California, USA	61	17,418	Nanopore	Contigs	861	GCA_032991405.1	NA	[61]
*Fov*4 (T-type)	15-2J	California, USA	64.5	18,074	Nanopore	Contigs	634	GCA_032878545.1	NA	[61]
*Fov*5	NRRL 25,432	Sudan	68.7	20,609	Nanopore	Contigs	16	GCA_012610825.1	NA	[60]
*Fov*7	F17	China	64.3	19,633	Nanopore	Contigs	18	GCA_038050555.1	NA	[17]
*Fov*8	NRRL 31,665	China	54.9	16,839	Nanopore	Contigs	581	GCA_012610815.1	NA	[60]
MDS-12	LA1E	California, USA	53.5	19,058	Nanopore	Contigs	433	GCA_012610815.1	NA	[58]
VCG-01111	VCG-01111	Australia	56.3	14,495	PacBio	Chromosome	15	GCA_049307005.1	NA	[62]
VCG-01112	VCG-01112	Australia	56.3	15,342	PacBio	Chromosome	15	GCA_049306905.1	NA	[62]

20,609 genes) and *Fov*7 (64.3 Mb, 19,633 genes) but contrasts sharply with the more compact *Fov*8 genome (54.9 Mb, 16,839 genes). Notably, phylogenomic analyses place *Fov*4 and *Fov*7 as closest relatives, and both share elevated repeat proportions and accessory sequence content, consistent with a "two-speed" genome architecture enriched in transposable elements [17]. In contrast, Australian VCG01111/01112 isolates (~56.3 Mb) possess fewer genes and a distinct set of accessory chromosomes, highlighting divergent evolutionary trajectories [62]. These patterns suggest that the large, repeat-rich genomes of *Fov*4 and its close relatives may underpin their adaptive potential and pathogenic versatility in cotton-growing regions. Overall, the availability of multiple *Fov* genomes creates an opportunity for systematic pan-genome and comparative analyses to distinguish core from accessory content and to pinpoint race-specific virulence loci. However, we recognize that the contribution of specific repetitive elements and accessory regions to fitness and virulence remains incompletely resolved. Also, the available whole-genome resources still represent only a small fraction of known *Fov* diversity, both in terms of lineages and races. Moreover, differences in assembly contiguity, completeness, and annotation consistency across isolates can complicate fine-scale comparisons of effector clusters and secondary-metabolite loci. Integrating short- and long-read sequencing with chromosome-conformation capture and optical mapping would enable chromosome-scale assemblies for *Fov*4, which will help researchers define core versus lineage-specific regions and lead to precise mapping of candidate effector clusters and secondary-metabolite genes.

The availability of high-quality whole-genome assemblies of *Fov* has also provided resources for phylogenetic analysis with better resolution. Previously, efforts at race and population classification in *Fov* relied on single-gene and multilocus sequence markers, including translation elongation factor (EF-1α), nuclear rDNA intergenic spacer region (IGS), nitrate reductase (NIR), phosphate permease (PHO), mitochondrial small subunit (mtSSU), β-tubulin, and ITS, often in combination with morphological or vegetative compatibility group (VCG) assays [2,66–68].

These studies demonstrated that the classic race system, which is based on pathogenicity to host differentials, does not always reflect evolutionary history. Most *Fov* races are polyphyletic, meaning they do not form a single clade but rather arise independently across the broader FOSC. Skovgaard et al. [67] used combined EF-1α, NIR, PHO, and mtSSU rDNA sequence data from 28 isolates of *Fov* and revealed four major phylogenetic lineages that correlated with differences in virulence and geographic origin. Lineage I included race 3 (mostly from Egypt) and race 5 (from Sudan); lineage II contained races 1, 2, and 6 from North and South America and Africa; lineage III encompassed race 8 from China; and lineage IV comprised isolates of races 4 and 7 from India and China, respectively.

Kim et al. [2] confirmed substantial diversity among Californian *Fov* isolates, with race 4-like types clustering separately from other races by sequence. Population-level work by Bennett et al. [66] further

documented regional emergence of "race 4-like" lineages in the southeastern US, underscoring the recurrent and independent origins of virulent *Fov* lineages. More recent whole-genome sequencing and phylogenomic studies reinforce these findings [17,61,62]. These genome-scale analyses indicate that *Fov* races occupy several distinct genomic lineages, with races 4, 7, and 8 forming a closely related group and races such as 1 and 5 falling into separate clades [17]. These results highlight that race definitions in *Fov* alone are insufficient for tracing evolutionary relationships, and that horizontal transfer, particularly of lineage-specific chromosomes, further scrambles simple genealogical patterns. Together, these analyses show that *Fov* pathogenicity on cotton has arisen multiple times, shaped by both vertical descent and horizontal gene/chromosome transfer within a globally interconnected species complex [62].

*Fov*4 displays considerable genotypic diversity, with four primary genotypes commonly recognized: N (no transposon insertion), T (Tfo1 transposon insertion), MT (MULE insertion with Tfo1), and MiT (MITE insertion in Tfo1) [21,69]. These genotypes are defined by the presence or absence of specific transposable element insertions in the PHO gene, which can be discriminated using a multiplex PCR assay with defined primer sets and corresponding amplicon sizes. The T genotype is predominant in California, while the N, MT, and MiT types occur less frequently and have broader distributions across the US cotton-growing regions [21]. Virulence differences are marked across *Fov*4 genotypes. N-type isolates typically display high aggressiveness on cotton, especially in California, while MT and MiT genotypes show moderate to low virulence [21,70]. Race 4-like isolates such as MDS-12 in the southeastern US are less pathogenic or even nonpathogenic, highlighting epidemiological relevance for disease management and resistance breeding [70]. Molecular surveillance indicates that *Fov*4 genotypes have distinct evolutionary backgrounds and that their introduction into the US likely resulted from multiple independent events, possibly via contaminated seed or agricultural equipment (Ortiz et al. 2017; [21]). Genotype diversity seen today reflects dynamic adaptation and migration of the pathogen between major cotton-producing regions [21].

Determinants of virulence

Advances in FOSC functional genomics have revealed that the diversity and abundance of virulence factors, especially those located on LS chromosomes, are key to race emergence and pathogenic adaptation. Among the most notable are the SIX proteins, enzymes involved in degrading plant cell walls (CWDEs), and clusters responsible for the biosynthesis of secondary metabolites. The SIX proteins, a group of fungal effectors initially isolated from *F. oxysporum*-infected xylem sap, are important for infection but can also trigger plant defense responses [71–74]. Jobe et al. [75] reported that some of the most virulent *Fov* races, *Fov*4 and *Fov*7, share a common SIX effector SIX9. This effector is largely absent in other *Fov* races in North America, making SIX9 a potential target for rapid detection of these highly virulent strains and enabling race-specific quantification in infected host plants. However, through inoculation experiments, Yang et al. [17] discovered that knockout of this gene did not affect the pathogenicity of *Fov*7 on cotton plants. Instead, they identified a significant role for *Fov*SIX16 in *Fov*7 virulence and demonstrated its necessity for full virulence, noting that it shares the same sequence as the one found in *Fov*4. These results suggest that SIX16-like effectors are strong candidates for contributing to *Fov*4 pathogenicity, but their roles have not yet been directly validated in *Fov*4 isolates. Gardiner et al. [76] used an effector clustering pipeline on Australian cotton-associated *F. oxysporum* and showed that the pathogenic *Fov* isolate SG1 carries multiple SIX genes on a repeat-rich contig absent from the nonpathogenic isolate *F. oxysporum* BRF1, which encodes no SIX homologues. Other cotton isolates such as LA3B [58] also lack SIX genes, and SIX presence – absence profiles are incongruent with genome-wide phylogeny, indicating that host specificity and virulence can arise independently of canonical SIX repertoires in some lineages. Thus, while SIX genes are important virulence factors in some *Fov* lineages, they are neither universally required for pathogenicity nor sufficient to explain *Fov*4 virulence, underscoring the need to characterize additional *Fov*4-specific effectors and metabolic genes.

Many CWDEs are subject to carbon catabolite repression and nitrogen regulation, which are important for the infection process [77,78]. However, the precise role of individual CWDEs in FOSC pathogenicity remains unclear, as gene knockouts for various enzymes – including pectate lyases, xylanases, and polygalacturonases – often show no obvious effect on virulence. This is likely due to functional redundancy or compensation by other enzymes [79,80]. FOSC members produce a variety of secondary metabolites, including beauvericin, fusaric acid, and fumonisins. The gene clusters responsible for these compounds are typically located on LS chromosomes and contribute to pathogenicity in hosts [81–83]. Zhang et al [84][] identified a

*Fov*4-specific nonribosomal peptide synthetase (NRPS) gene, *FNP*1, that plays a critical role in the pathogenicity of *Fov*4. Mutation of this gene resulted in markedly attenuated hyphal growth on media containing cotton roots as the sole carbon source, increased sensitivity to cellular stress agents, and delayed spore germination. Furthermore, the knockout strain showed reduced virulence in cotton root rot, a significant reduction in fusaric acid production, and impaired infection progression within cotton roots. These findings, while awaiting further characterization, suggest that *FNP*1 is an important pathogenic factor contributing to both the fitness and virulence of *Fov*4 [84][]. Together, these findings underscore that only a few virulence genes have been functionally confirmed in *Fov*4, and that additional reverse genetic work will be required to distinguish determinants that are shared across *Fov* races from those that are uniquely associated with the most aggressive *Fov*4 genotypes.

Host-pathogen interactions

The interaction between *Fov* and its cotton host is a complex dynamic governed by sophisticated plant defense signaling and structural fortification mechanisms. Understanding these host responses, particularly against the highly virulent *Fov*4, is paramount to augmenting crop resilience. Cotton plants utilize intricate molecular pathways to transduce external pathogenic signals into internal defense responses, often involving phytohormones, transcription factors, and specialized defense proteins. Phytohormones are central to shaping plant – pathogen interactions. Salicylic acid (SA) generally functions to reduce plant susceptibility, while hormones such as jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), and auxins exhibit complex and sometimes antagonistic effects [85]. The upregulation of genes involved in flavonoid biosynthesis, promoted by group IIc WRKY transcription factors, leads to flavonoid accumulation, thereby enhancing cotton resistance to *Fov* [86]. The germin-like protein GhABP19 also plays a role in modulating resistance against *Fov* pathogens, partly through its regulation of JA pathways [87]. The Mitogen-Activated Protein Kinase (MAPK) cascade is an essential conduit for transmitting external stress signals into internal biotic and abiotic responses. The gene GhMPK20 is a key player in regulating cotton resistance to *F. oxysporum* [88]. Studies using virus-induced gene silencing (VIGS) showed that silencing GhMPK20 enhanced cotton tolerance to *F. oxysporum*, while its overexpression disrupted the SA-mediated defense pathway. The scaffold protein GhMORG1 has been identified as enhancing resistance by significantly amplifying the activity of the GhMKK6–GhMPK4 cascade [88,89]. Conversely, silencing GhMPK4 reduced tolerance to Fusarium wilt and led to decreased expression of resistance genes. The regulation of MAPK cascades also involves negative regulators, such as GhAP2C1; silencing this gene enhanced resistance to Fusarium wilt, while its overexpression led to increased sensitivity, underscoring its intricate interactions with GhMPK4.

Cotton employs sophisticated recognition systems, such as the chitin sensing and signaling system, which involves the dimerization and phosphorylation of GhLYK5–GhCERK1 alongside the wall-associated kinase GhWAK7A, playing a significant role in responding to *Fov* infections [90]. Additionally, molecular evidence supports the involvement of polygalacturonase-inhibiting proteins (PGIPs) in enhancing resistance to *Fov*. Specifically, GhPGIP1 inhibits pathogenic endopolygalacturonases secreted by the fungal pathogen, and its overexpression triggers the expression of pathogenesis-related proteins (PRs) and defense-related genes [91]. Structural defenses in cotton are crucial for restricting pathogen movement after invasion, often manifested visibly through vascular responses. A hallmark symptom of Fusarium wilt is the reddish-brown discoloration observed within the vascular tissues of sectioned stems and roots. A comparative analysis of the infection process by virulent *Fov*4 in Pima cotton varieties revealed key structural defense differences. In the resistant variety PHY 841 RF, germination of conidia on the root surface was significantly lower, and the penetration of *Fov*4 into the root epidermis was notably delayed compared with the susceptible Pima S-7 [34]. Subsequent infection stages showed that the resistant PHY 841 RF restricted the fungal ability to infiltrate the xylem by exhibiting reduced hyphal growth and reproduction, with delayed infection, inhibited fungal growth, and prevention of xylem invasion contributing to resistance [34,40]. Similar structural defense patterns are also evident in Upland cotton. Zhu et al. [45] showed that the resistant cultivar FM 2334GLT(R) had the fewest conidia on the root surface, delayed hyphal penetration, and greatly reduced cortical colonization compared with susceptible PHY 725 RF, FM 2334GLT(S), and Acala 1517–08. In susceptible cultivars, hyphae reached and colonized xylem vessels by 7–15 dpi, whereas in FM 2334GLT(R) hyphae were not detected in the xylem, highlighting delayed infection and blockage of xylem invasion as key components of *Fov*4 resistance in Upland cotton.

Consistent with these structural defenses, QTL and GWAS studies in tetraploid cotton have identified *Fov*4

resistance loci on several D-subgenome chromosomes, including major QTLs on D02, D03, D05, D06, D08, D11, and D13, and a major *Fov*4 resistance gene in Pima cotton mapped near a region on chromosome D02 (Abdelraheem et al. 2020; [34,92]). Recent work has further extended the genetic basis of *Fov*4 resistance into diploid cotton. A genome-wide association study in 246 accessions of Gossypium arboreum identified 24 QTLs for *Fov*4 resistance, including three major QTLs on chromosomes A04, A06, and A11, and showed in a BC_2F_7 population that resistance from diploid species such as *G. arboreum* and *G. aridum* can be transferred into Upland cotton [93]. These findings, together with the structural and signaling defenses described earlier, highlight that *Fov*4 resistance in cotton is controlled by a complex network of defense pathways and multiple loci across both tetraploid and diploid genomes, providing diverse targets and germplasm sources for breeding.

Environmental conditions contributing to the virulence of Fov4

To date, *Fov*4 in the US has been found only in California, New Mexico, and Texas ([1,94], Zhu et al. 2020). Each of these states shares similar climatic characteristics in their cotton-growing regions, being largely dry and warm environments. Historically, similar climates have been used for growing cotton in the US, although owing to irrigation practices, heat accumulation has been of greater importance in developmental studies of cotton than water uptake. For *G. hirsutum* L. and *G. barbadense* management in the US, a base 60 °F degree-day model has been commonly deployed as a method of predicting cotton development [95, 96]. In practice, time spent above 15.5 °C is assumed to be beneficial to cotton growth and development, such that days spent above this threshold are recorded as degree-days, and the accumulation of degree-days is correlated to developmental stages. Degree-day modeling of developmental thresholds is particularly important in cotton cultivation because many of its pests and pathogens are most destructive at particular life stages. *Fov*4, for example, is described as a seedling disease because it is most destructive to cotton during the seedling stage. Although *Fusarium* infection may occur throughout the life cycle of the plant, older plants are often tolerant of infection. Little work exists specifically investigating degree-day models for *Fov*4; however, other races have been shown to positively correlate with the number of cotton growing degree-days experienced in a research field [15]. Furthermore, Zhang et al. [97] identified 23°C as the laboratory temperature at which disease develops most readily and as one of the temperatures at which the fungus grows most quickly on plates. These temperatures closely align with those realized in the field at the start of the growing season in the regions of the US where *Fov*4 is found. To the extent that *Fov*4 has been studied, synchronicity of phenology is an apparent contributor to disease development.

Synchronous phenology is only useful to the pathogen if it exists in the same space as its hosts, in sufficient quantity to cause disease. Fusarium wilt of cotton is recognized as an inoculum density-dependent disease, and much of the existing evidence supports this interpretation. In earlier phases of *Fov*4's invasion into the US, experimental evidence emerged that the probability of infection increased logistically as a function of inoculum density [49]. Despite identifying that the disease is inoculum density-dependent, another problem persists. Within agricultural fields, there is significant spatial heterogeneity of inoculum, such that characterizing where in a field inoculum exists has been a topic of interest to assist in management decisions. Davis et al. [98] observed spatial autocorrelation indicative of clustered patches of *Fov* inoculum density throughout the study field. Given that disease is dependent on inoculum density and that inoculum density exists in an aggregated distribution in the field, it may be possible to improve management strategies by predicting the location of the patches within the field. Using the geostatistical interpolation technique kriging has demonstrated some success in identifying patches of increased inoculum density (Parris et al., 2023). At the small scale of an individual field, appropriately resolved sampling may enable description of *Fov*4 inoculum density by interpolation.

Disease management

Management of *Fov* can be grouped into four categories: host resistance, nematode management, cultural control, and chemical and biological treatments [41,44]. Co-infection with root-knot nematodes (RKN, *Meloidogyne incognita*) can increase the severity of *Fov*4; however, unlike races 1 and 2, nematode interaction is not required for disease development [21,70]. By contrast, in Australian cotton systems nematodes are generally a minor concern for Fusarium wilt, and severe disease has been managed primarily through resistant cultivars, on-farm hygiene, summer flooding, and crop rotation that limit inoculum build-up and spread [99]. Consequently, it is important to recognize that cultivar resistance to *Fov*4 remains the foundation of Fusarium wilt disease management.

In Pima cotton (*G. barbadense*), resistance shows a dominant inheritance pattern resulting in highly resistant cultivars, but susceptible Pima cultivars are extremely vulnerable [92]. Pima S-6 was the first cultivar identified with strong *Fov*4 resistance [100], though it was released decades before the pathogen appeared in the US [101]. PHY 800 was the first commercial cultivar marketed for *Fov*4 tolerance [102], and subsequent releases PHY 881 RF, PHY 807 RF, and PHY 861 RF (PhytoGen Cottonseed, Corteva Agriscience) combine *Fov*4 and glyphosate tolerance and are widely adopted in Pima-producing regions. Publicly available Pima germplasm lines have been released to diversify the genetic base of resistance [103–105].

In Upland cotton (*G. hirsutum*), the predominant cotton planted in the US, resistance is recessive or incompletely dominant, with few resistance sources available and no marketed *Fov*4-tolerant commercial cultivars [40]. Severity in susceptible Upland cultivars is typically less than that of susceptible Pima, but severe disease can still occur under conducive conditions, and all commercial Upland cultivars have shown some degree of susceptibility [106,107]. Obsolete Upland germplasm was screened for *Fov*4 resistance using an SNP marker, but only 0.86% of accessions possessed the resistance allele [97]. Progress has been achieved through public breeding programs, including the release of NuMex COT 17 GLS, with *Fov*4 resistance introgressed from Pima, and Acala 1517–20, with a *Fov*7-resistant source that confers moderate resistance to *Fov*4 [11,108]. Seventeen germplasm lines were publicly released to enhance *Fov*4 resistance and improve fiber quality in Upland cotton [109].

Because disease severity is linked to soil inoculum density [49], remaining management strategies rely on limiting the buildup and persistence of viable propagules in infested fields. Cultural controls such as soil solarization, deep tillage, and crop rotation with non-host species may reduce inoculum levels. Avoiding movement of infested soil via irrigation water and farm equipment is critical to prevent field-to-field spread. Seed sanitation and certification programs can help limit long-distance dissemination, as infected seed can serve as a source of new inoculum for *Fov*4 [21]. Chemical treatments, including fungicides and soil fumigants, have shown limited efficacy against *Fov*4 due to its deep soil persistence and systemic infection pathway. Biological control agents, such as *Trichoderma* spp. and *Bacillus* spp., have demonstrated antagonistic activity to *Fov* in vitro and in greenhouse trials [110 ,111]. However, experimental work on *Fov*4-specific biocontrol agents remains limited, and robust field-level efficacy data are still lacking. Recent work has also begun to examine the *Fov*4 hyphosphere as a potential management target [112]. An integrated metabarcoding and culture-based study showed that the *Fov*4 hyphosphere is dominated by a single *Pseudomonas* amplicon sequence variant and a functionally enriched bacterial community, and that several hyphosphere isolates, including *Pseudomonas laurylsulfatiphila*, can significantly promote *Fov*4 hyphal extension *in vitro*. These findings support the emerging concept of a hyphosphere pathobiome and highlight microbial associations as promising, though still experimental, targets for future *Fov*4 disease management strategies. Integrated disease management strategies that combine resistant cultivars, cultural practices, and targeted monitoring of inoculum density offer the most promising approach for long-term control of *Fov*4 in cotton production systems.

Current gaps and research needs: Unresolved questions in Fov4 biology and host interaction

Although substantial progress has been made in characterizing the biology and genomics of *Fov*4, several key questions remain. The molecular mechanisms underlying host specificity and resistance breakdown are still poorly understood, and only a few virulence genes have been functionally characterized. Genome comparisons and functional studies indicate that some SIX effectors, such as FovSIX16, contribute to virulence in highly aggressive *Fov* races, whereas others like SIX9 appear to be useful molecular markers for *Fov*4 and *Fov*7 but are dispensable for pathogenicity [17,75]. For *Fov*4 specifically, the chromosomal context of these effectors and the broader set of race-specific virulence factors need to be better defined. The biological drivers of inoculum density variation in field soils, such as saprotrophic growth, seed-borne transmission, and spatial heterogeneity, remain largely uncharacterized [113,114]; Parris et al. 2023). These gaps hinder predictive modeling and limit the precision of field-level management strategies. Resistance breeding also faces challenges. In Pima cotton, resistance is dominantly inherited and well-characterized [92,100], but in Upland cotton, resistance is recessive or incomplete, with limited sources available and no commercial cultivars currently marketed for *Fov*4 tolerance [40,97]. The genetic basis of resistance in Upland cultivars and the mechanisms by which *Fov*4 overcomes partial resistance remain urgent areas for investigation.

Recent research efforts have significantly advanced our understanding of cotton's resistance to *Fov*4, revealing key genetic and structural defense mechanisms[115]. demonstrated that resistant *G. barbadense*

cultivars activate genes involved in reactive oxygen species metabolism, chitinase activity, and Casparian strip biosynthesis, reinforcing vascular barriers against fungal invasion. Abdelraheem et al. [93] identified 24 resistance-associated QTLs in *G. arboreum* through genome-wide association studies, with successful introgression into *G. hirsutum*, offering a genetic roadmap for breeding *Fov*4-resistant Upland cotton. Complementing these findings Popa-Baez et al. [62] used comparative genomics to uncover unique gene clusters in resistant cultivars enriched in metabolic and pathogenicity-related pathways, highlighting host-pathogen co-evolution and the role of accessory chromosomes in. shaping resistance traits. Together, these studies provide critical insights into the molecular and genomic architecture of cotton defense, guiding future strategies for durable resistance.

Emerging technologies: Genomics, gene editing, and diagnostics

Recent advances in sequencing technologies have enabled high-resolution genome assemblies for multiple *Fov* races, revealing genome plasticity, repeat-rich LS regions, and race-specific virulence factors [58,60,62]. *Fov*4 genomes consistently exhibit larger sizes and higher gene counts than other races, with repeat content exceeding 18% in some isolates [59]. However, functional genomics, such as transcriptomics, proteomics, and gene knockout studies, remains underutilized in dissecting virulence pathways. CRISPR/Cas9 and other gene editing tools offer promising avenues for functional validation and resistance engineering, but their application to *Fov*4 and cotton remains limited. Rapid diagnostics based on race-specific markers like SIX9 [75] could enable early detection and quantification of *Fov*4 in soil and plant tissues.

High-throughput functional genomics as well as other omics approaches now provide researchers more robust tools to study *Fov*4 pathogenicity. CRISPR-based mutagenesis, host-induced gene silencing, and transcriptome analyses allow systematic validation of large sets of *Fov*4-specific candidate effectors and metabolic genes predicted from comparative genomics. Integrating these data into co-expression and protein-protein interaction networks can help pinpoint key regulatory hubs and pathways underlying virulence, rather than studying single genes in isolation. Machine-learning and AI-assisted tools for effector prediction, structure modeling, network inference, and genotype–phenotype association could then prioritize the most promising virulence factors for mechanistic studies and for development of diagnostic markers and resistance-breeding targets in cotton.

Global collaboration and policy: Coordinated response to Fov4 threat

The emergence of *Fov*4 in multiple cotton-producing regions, including India, California, Texas, and New Mexico, underscores the need for coordinated international efforts. Harmonized protocols for resistance screening, genome annotation, and field surveillance would accelerate progress and reduce redundancy. Public breeding programs have released resistant germplasm for both Pima and Upland cotton [109], but broader collaboration among academic institutions, industry stakeholders, and regulatory agencies is essential to scale these efforts.

Policy frameworks that support seed certification, pathogen monitoring, and open data sharing are critical to managing long-distance dissemination and ensuring biosecurity. As climate change and global trade reshape agricultural landscapes, proactive and unified strategies will be key to mitigating the impact of *Fov*4 and safeguarding cotton production worldwide.

Acknowledgements

Y. Z. and W.B.S. conceptualized and prepared the outline of this review. Y. Z., H. Z., T. M., J. C., T. I. and W.B. wrote the original manuscript. T.I. and W.B.S. administered the project and acquired funding. All authors have read and approved the final manuscript.

Funding

This work was funded by the United States Department of Agriculture National Institute of Food and Agriculture (2022–67013-36836 and 2023–67013-40174) and Cotton Incorporated (project 24-807TX).

Disclosure statement

Grammarly (version 1.151.1.0) was used to assist in reviewing the spelling and grammar when completing this manuscript. Figure 1(Infection Cycle) was created with the authors' original photographs and comprehension of the Fusarium wilt disease using bioRENDER application (www.biorender.com), and subsequently reviewed and edited by the authors to ensure scientific accuracy.

Data availability statement

No new data were generated as part of this review article.

ORCID

Won Bo Shim http://orcid.org/0000-0002-1423-7813

References

[1] Halpern HC, Bell AA, Wagner TA, et al. First report of Fusarium wilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum* race 4 in Texas, U.S.A. Plant Dis. 2018;102(2):446–446. doi: 10.1094/PDIS-07-17-1084-PDN

[2] Kim Y, Hutmacher RB, Davis RM. Characterization of California isolates of *Fusarium oxysporum* f. sp *vasinfectum*. Plant Dis. 2005;89(4):366–372. doi: 10.1094/PD-89-0366

[3] Zhu Y, Abdelraheem A, Lujan P, et al. Detection and characterization of fusarium wilt (*Fusarium oxysporum f. sp. vasinfectum*) race 4 causing fusarium wilt of cotton seedlings in New Mexico. Plant Dis. 2021;105(11):3353–3367. doi: 10.1094/PDIS-10-20-2174-RE

[4] Faske, T. and Sisson, A. 2023. Cotton disease loss estimates from the United States - 2022. Crop Protection Network. CPN-7001–22. doi: 10.31274/cpn-20230405-0.

[5] DeIulio Gregory A, Guo L, Zhang Y, et al. Kinome expansion in the *Fusarium oxysporum* species complex driven by accessory chromosomes. mSphere. 2018;3(3):231–00218. doi: 10.1128/mSphere.00231-18

[6] Ma L-J, Geiser DM, Proctor RH, Donnell K, Trail F, Gardiner DM, Manners JM, & Kazan K. Fusarium pathogenomics. Annu Rev Microbiol. 2013;67(1):399–416. doi: 10.1146/annurev-micro-092412-155650

[7] Ma L-J, van der Does HC, Borkovich KA, et al. Comparative genomics reveals mobile pathogenicity chromosomes in fusarium. Nature. 2010;464(7287):367–373.

[8] Henry PM, Pincot DDA, Jenner BN, et al. Horizontal chromosome transfer and independent evolution drive diversification in *Fusarium oxysporum* f. sp *fragariae*. New Phytol. 2021;230(1):327–340.

[9] Vlaardingerbroek I, Beerens B, Schmidt SM, et al. Dispensable chromosomes in *Fusarium oxysporum* f. sp *lycopersici*. Mol Plant Pathol. 2016;17(9):1455–1466.

[10] Zhang Y, Liu S, Mostert D, et al. Virulence of banana wilt-causing fungal pathogen *Fusarium oxysporum* tropical race 4 is mediated by nitric oxide biosynthesis and accessory genes. Nat Microbiol. 2024;9(9):2232–2243. doi: 10.1038/s41564-024-01779-7

[11] Zhang J, Abdelraheem A, Zhu Y, et al. Pedigree selection under field conditions within Acala 1517–08 and its glandless derivatives for development of cotton resistant to Fusarium wilt caused by Fusarium oxysporum f. sp. vasinfectum race 4. Euphytica. 2020;216(10):155. doi: 10.1007/s10681-020-02691-x

[12] van Dam P, Fokkens L, Ayukawa Y, et al. A mobile pathogenicity chromosome in *Fusarium oxysporum* for infection of multiple cucurbit species. Sci Rep. 2017;7(1):9042. doi: 10.1038/s41598-017-07995-y

[13] Atkinson GF. Some diseases of cotton. III frenching. Alabama Exp Sta Bul. 1892;41:19–29.

[14] Davis RM, Colyer PD, Rothrock CS, et al. Fusarium wilt of cotton: population diversity and implications for management. Plant Dis. 2006;90(6):692–703. doi: 10.1094/PD-90-0692

[15] Dyer DR, Newman M, Lawrence KS. Diversity and temporal distribution of *Fusarium oxysporum* f. sp. *vasinfectum* races and genotypes as influenced by Gossypium cultivar. Front Fungal Biol. 2022;3:1022761. doi: 10.3389/ffunb.2022.1022761

[16] Liu J, Wagner T A. (2022). Detection and genotyping of *Fov*4 (Race 4, VCG0114), the Fusarium Wilt pathogen of cotton.Methods Mol Biol, 2391 191–205. doi: 10.1007/978-1-0716-1795-3_16

[17] Yang D, Zhang X, Ming Y, et al. Characterization of the high-quality genome sequence and virulence factors of *Fusarium oxysporum* f. sp. vasinfectum race 7. J Fungi (Basel). 2024;10(4):242. doi: 10.3390/jof10040242

[18] Garber R, Jorgenson E, Smith S, et al. Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. J Nematol. 1979;11(2):133.

[19] Bell AA, Kemerait RC, Ortiz CS, et al. Genetic diversity, virulence, and Meloidogyne incognita interactions of *Fusarium oxysporum* isolates causing cotton wilt in Georgia. Plant Dis. 2017;101(6):948–956. doi: 10.1094/PDIS-09-16-1382-RE

[20] Armstrong GM, Armstrong JK. American, Egyptian, and Indian cotton-wilt fusaria: their pathogenicity and relationship to other wilt fusaria. US Department Agriculture Technical Bull. 1960; 1219: 1–19.

[21] Bell AA, Gu A, Olvey J, et al. Detection and characterization of *Fusarium oxysporum* f. sp. *vasinfectum* VCG0114 (race 4) isolates of diverse geographic origins. Plant Dis. 2019;103(8):1998–2009. doi: 10.1094/PDIS-09-18-1624-RE

[22] Diaz J, Garcia J, Lara C, et al. Characterization of current *Fusarium oxysporum* f. sp. *vasinfectum* isolates from cotton in the San Joaquin Valley of California and lower valley El Paso, Texas. Plant Dis. 2021;105(7):1898–1911. doi: 10.1094/PDIS-05-20-1038-RE

[23] Nelson PE. Chapter 3 – Life cycle and epidemiology of *Fusarium oxysporum*. In: Mace ME, AA Bell CH Beckman, editors. Fungal wilt diseases of plants. (NY): Academic Press; 1981. p 51–80.

[24] Isakeit T. *Fusarium oxysporum* f.sp. *vasinfectum* (Fusarium wilt). Wallingford, UK: CABI Compendium; 2022.

[25] Rekah Y, Shtienberg D, Katan J. Disease development following infection of tomato and basil foliage by airborne conidia of the soilborne pathogens *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *basilici*. Phytopathology. 2000;90(12):1322–1329. doi: 10.1094/PHYTO.2000.90.12.1322

[26] Leslie JF, Summerell BA. The fusarium laboratory manual. Oxford, UK: John Wiley & Sons; 2008.

[27] Beckman CH. The nature of wilt diseases of plants. St. Paul (MN): APS Press; 1987. p 175.

[28] Smith SN, DeVay JE, Hsieh W-H, et al. Soil-borne populations of *Fusarium oxysporum* f. sp. *vasinfectum*, a cotton wilt fungus in California fields.

Mycologia. 2001;93(4):737–743. doi: 10.1080/00275514.2001.12063205
[29] Gordon TR. *Fusarium oxysporum* and the fusarium wilt syndrome. Annu Rev Phytopathol. 2017;55(1):23–39. doi: 10.1146/annurev-phyto-080615-095919
[30] Li X, Zhang YN, Ding C, et al. Declined soil suppressiveness to Fusarium oxysporum by rhizosphere microflora of cotton in soil sickness. Biol Fertili Soils. 2015;51(8):935–946. doi: 10.1007/s00374-015-1038-8
[31] Steinkellner S, Mammerler R, Vierheilig H. Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. Eur J Plant Pathol. 2008;122(3):395–401. doi: 10.1007/s10658-008-9306-1
[32] Chawla S, Woodward JE, Wheeler TA, et al. Effect of *Fusarium oxysporum* f. sp. *vasinfectum* inoculum density, *Meloidogyne incognita* and cotton cultivar on Fusarium wilt development. Tex J Agriculture Nat Resour. 2012;25:46–56.
[33] Elzein A, Heller A, Ndambi B, De Mol M, Kroschel J and Cadisch G. (2010). Cytological investigations on colonization of sorghum roots by the mycoherbicide *Fusarium oxysporum* f. sp. *strigae* and its implications for Striga control using a seed treatment delivery system. Biological Control, 53(3), 249–257. doi: 10.1016/j.biocontrol.2010.02.002
[34] Zhu Y, Abdelraheem A, Cooke P, et al. Comparative analysis of infection process in Pima cotton differing in resistance to fusarium wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* race 4. Phytopathology. 2022;112(4):852–861.
[35] Lyons R, Stiller J, Powell J, Rusu A, Manners J M, Kazan K and Blazquez M A. (2015). *Fusarium oxysporum* triggers tissue-specific transcriptional reprogramming in *Arabidopsis thaliana*. PLoS ONE, 10(4), e0121902 doi: 10.1371/journal.pone.0121902
[36] Walter S, Nicholson P, Doohan FM. Action and reaction of host and pathogen during Fusarium head blight disease. New Phytol. 2010;185(1):54–66. doi: 10.1111/j.1469-8137.2009.03041.x
[37] Hillocks R. Fusarium wilt. In: Hillocks RJ, editor. Cotton diseases. Wallingford, UK: CAB International; 1992. p 432.
[38] Okungbowa F, Shittu H. Fusarium wilts: an overview. Environ Res J. 2012;6:83–102.
[39] Fassihiani A. Symptomless carriers of the causal agent of tomato wilt pathogen. J Agriculture Sci Technol. 2000;2:27–32.
[40] Ulloa M, Hutmacher RB, Schramm T, et al. Sources, selection and breeding of Fusarium wilt (Fusarium oxysporum f. sp. vasinfectum) race 4 (FOV4) resistance in Upland (Gossypium hirsutum L.) cotton. Euphytica. 2020;216(7):109. doi: 10.1007/s10681-020-02643-5
[41] Cianchetta AN, Davis RM. Fusarium wilt of cotton: management strategies. Crop Prot. 2015;73:40–44. doi: 10.1016/j.cropro.2015.01.014
[42] Mariyappa RB, Manjunatha L, Naik MK, et al. Fusarium, compendium of phytopathogenic microbes in agro-ecology: vol. 1 fungi. London, UK: Springer; 2025. p 329–362.
[43] Liu S, Zhang X, Xiao S, et al. A single-nucleotide mutation in a glutamate receptor-like gene confers resistance to Fusarium wilt in *Gossypium hirsutum*. Adv Sci. 2021;8:2002723. doi: 10.1002/advs.202002723
[44] Sanogo S, Zhang J. Resistance sources, resistance screening techniques and disease management for Fusarium wilt in cotton. Euphytica. 2016;207(2):255–271. doi: 10.1007/s10681-015-1532-y
[45] Zhu Y, Abdelraheem A, Cooke P, et al. Comparative analysis of infection process in upland cottons differing in resistance to fusarium wilt caused by Fusarium oxysporum f. sp. vasinfectum race 4. Crop Sci. 2023;63(3):1330–1343. doi: 10.1002/csc2.20965
[46] Zhang J, Abdelraheem A, Zhu Y, et al. Importance of temperature in evaluating cotton for resistance to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* race 4. Crop Sci. 2021;61(3):1783–1796. doi: 10.1002/csc2.20446
[47] Cao Jun CJ, Gao ZhiMou GZ, Wang Tao WT, Pan YueMin PY, Ji WenFei JW, Zhang Le ZL. Biological characteristics and esterase isozyme analysis of the pathogenic fungus causing Fusarium wilt of cotton in Anhui Province. J Anhui Agric Univ. 2005;32(2):224–229. (Chinese).
[48] Liu F. A brief analysis on impact factors of cotton fusarium wilt occurring. J Anhui Agric Sci. 2010;38:3234–3235.
[49] Hao JJ, Yang ME, Davis RM. Effect of soil inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 on disease development in cotton. Plant Dis. 2009;93(12):1324–1328. doi: 10.1094/PDIS-93-12-1324
[50] Wang B, Dale M, Kochman J. Studies on a pathogenicity assay for screening cotton germplasms for resistance to *Fusarium oxysporum* f. sp. vasinfectum in the glasshouse vasinfectum the glasshouse. Australian J Exp Agriculture. 1999;39(8):967–974. doi: 10.1071/EA99070
[51] Zhang J, Abdelraheem A, Zhu Y, et al. Studies of evaluation methods for resistance to Fusarium wilt race 4 (*Fusarium oxysporum* f. sp. *vasinfectum*) in cotton: effects of cultivar, planting date, and inoculum density on disease progression. Front Plant Sci. 2022;13:13. doi: 10.3389/fpls.2022.900131
[52] Armitage AD, Taylor A, Sobczyk MK, et al. Characterisation of pathogen-specific regions and novel effector candidates in *Fusarium oxysporum* f. sp. cepae. Sci Rep. 2018;8(1):13530. doi: 10.1038/s41598-018-30335-7
[53] Jangir P, Mehra N, Sharma K, et al. Secreted in xylem genes: drivers of host adaptation in *Fusarium oxysporum*. Front Plant Sci. 2021;12:628611. doi: 10.3389/fpls.2021.628611
[54] Witte TE, Villeneuve N, Boddy CN, et al. Accessory chromosome-acquired secondary metabolism in plant pathogenic fungi: the evolution of biotrophs into host-specific pathogens. Front Microbiol. 2021;12:2021. doi: 10.3389/fmicb.2021.664276
[55] Yang H, Yu H, Ma LJ. Accessory chromosomes in *Fusarium oxysporum*. Phytopathology. 2020;110(9):1488–1496. doi: 10.1094/PHYTO-03-20-0069-IA
[56] Czislowski E, Fraser-Smith S, Zander M, et al. Investigation of the diversity of effector genes in the banana pathogen, *Fusarium oxysporum* f. sp. *cubense*,

reveals evidence of horizontal gene transfer. Mol Plant Pathol. 2018;19(5):1155–1171.

[57] Ayhan DH, López-Díaz C, Pietro AD, et al. Improved assembly of reference genome *Fusarium oxysporum* f. sp. lycopersici strain Fol4287. Microbiol Resour Announc. 2018;7(10):00918. doi: 10.1128/MRA.00910-18

[58] Seo S, Pokhrel A, Coleman JJ. The genome sequence of five genotypes of *Fusarium oxysporum* f. sp. *vasinfectum*: a resource for studies on fusarium wilt of cotton. MPMI. 2020;33(2):138–140. doi: 10.1094/MPMI-07-19-0197-A

[59] Jobe TO, Ulloa M, Ellis Margaret L. A high-quality whole-genome sequence, assembly, and gene annotation of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) race 1 from California. Microbiol Resour Announc. 2023;13(1):e00702–00723. doi: 10.1128/mra.00702-23

[60] Srivastava SK, Zeller KA, Sobieraj JH, et al. Genome resources of four distinct pathogenic races within *Fusarium oxysporum* f. sp vasinfectum that cause vasc wilt disease cotton. Phytopathol. 2021;111(3):593–596.

[61] Jobe TO, Abdurakhmonov IY, Ulloa M, et al. Molecular characterization of fusarium isolates from upland cotton roots in Uzbekistan and whole-genome comparison with isolates from the United States. Phytopathology. 2025;115(1):54–65.

[62] Popa-Baez AD, Smith LJ, Stiller WN, et al. Comparative genomics reveals ancient and unique pathogenicity features in Australian *Fusarium oxysporum* f. sp *vasinfectum*. J Fungi. 2025;11(7):481.

[63] Fokkens L, Guo L, Dora S, et al. A chromosome-scale genome assembly for the *Fusarium oxysporum* strain Fo5176 to establish a model Arabidopsis-fungal pathosystem. G3: Genes | Genomes | Genet. 2020;10(10):3549–3555. doi: 10.1534/g3.120.401375

[64] Khayi S, Armitage AD, Gaboun F, et al. Chromosome-scale assembly uncovers genomic compartmentation of *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of Bayoud disease in date palm. Front Microbiol. 2023;14:2023.

[65] Krasnov GS, Pushkova EN, Novakovskiy RO, et al. High-quality genome assembly of *Fusarium oxysporum* f. sp *lini*. Front Genet. 2020;11:- 2020.

[66] Bennett RS, Scott TZ, Lawrence KS, et al. Sequence characterization of race 4-like isolates of *Fusarium oxysporum* from Alabama and Mississippi. J Cotton Sci. 2013;17:125–130.

[67] Skovgaard K, Nirenberg HI, O'Donnell K, et al. Evolution of *fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology. 2001;91(12):1231–1237. doi: 10.1094/PHYTO.2001.91.12.1231

[68] Wang B, Brubaker CL, Summerell BA, et al. Local origin of two vegetative compatibility groups of *Fusarium oxysporum* f. sp *vasinfectum* in Australia. Evol Appl. 2010;3(5–6):505–524.

[69] Ortiz C S, Bell A A, Magill C W and Liu J. (2017). Specific PCR Detection of *Fusarium oxysporum* f. sp. *vasinfectum* California Race 4 Based on a Unique Tfo1 Insertion Event in the PHO Gene. Plant Disease, 101 (1), 34–44. doi: 10.1094/PDIS-03-16-0332-RE

[70] Wagner TA, Duke SE, Davie SM, et al. Interaction of Fusarium wilt race 4 with root-knot nematode increases disease severity in cotton. Plant Dis. 2022;106(10):2558–2562. doi: 10.1094/PDIS-12-21-2725-SC

[71] Houterman PM, Speijer D, Dekker HL, et asl. The mixed xylem sap proteome of *Fusarium oxysporum*-infected tomato plants. Mol Plant Pathol. 2007;8(2):215–221. doi: 10.1111/j.1364-3703.2007.00384.x

[72] Rep M, Van Der Does HC, Meijer M, et al. A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for I-3-mediated resistance in tomato. Mol Microbiol. 2004;53(5):1373–1383. doi: 10.1111/j.1365-2958.2004.04177.x

[73] Schmidt SM, Houterman PM, Schreiver I, et al. Mites in the promoters of effector genes allow prediction of novel virulence genes in *Fusarium oxysporum*. BMC Genomics. 2013;14(1):119. doi: 10.1186/1471-2164-14-119

[74] van der Does HC, Lievens B, Claes L, et al. The presence of a virulence locus discriminates *Fusarium oxysporum* isolates causing tomato wilt from other isolates. Environ Microbiol. 2008;10(6):1475–1485. doi: 10.1111/j.1462-2920.2007.01561.x

[75] Jobe TO, Urner M, Ulloa M, et al. Secreted in xylem (SIX) gene SIX9 is highly conserved in *Fusarium oxysporum* f. sp. *vasinfectum* race 4 isolates from cotton in the United States. PhytoFrontiers. 2024;4(4):476–481. doi: 10.1094/PHYTOFR-11-23-0143-SC

[76] Gardiner DM, Aitken EAB, van Dam P, et al. De novo long-read assembly and annotation for genomes of two cotton-associated *Fusarium oxysporum* isolates. Australas Plant Pathol. 2025;54(3):377–386.

[77] Ospina-Giraldo MD, Mullins E, Kang S. Loss of function of the *Fusarium oxysporum* SNF1 gene reduces virulence on cabbage and Arabidopsis. Curr Genet. 2003;44(1):49–57. doi: 10.1007/s00294-003-0419-y

[78] Rocha ALM, Di Pietro A, Ruiz-Roldán C, et al. Ctf1, a transcriptional activator of cutinase and lipase genes in *Fusarium oxysporum* is dispensable for virulence. Mol Plant Pathol. 2008;9(3):293–304.

[79] Calero-Nieto F, Di Pietro A, Roncero MIG, et al. Role of the transcriptional activator XlnR of *Fusarium oxysporum* in regulation of xylanase genes and virulence. Mol Plant-Microbe Interact. 2007;20(8):977–985.

[80] Pietro AD, Madrid MP, Caracuel Z, et al. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. Mol Plant Pathol. 2003;4(5):315–325. doi: 10.1046/j.1364-3703.2003.00180.x

[81] Li C, Zuo C, Deng G, et al. Contamination of bananas with beauvericin and fusaric acid produced by *Fusarium oxysporum* f. sp *cubense*. PLOS ONE. 2013;8(7):e70226. doi: 10.1371/journal.pone.0070226

[82] López-Díaz C, Rahjoo V, Sulyok M, et al. Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. Mol Plant Pathol. 2018;19(2):440–453. doi: 10.1111/mpp.12536

[83] Rheeder JP, Marasas WF, Vismer HF. Production of fumonisin analogs by Fusarium species. Appl Environ Microbiol. 2002;68(5):2101–2105. doi: 10.1128/AEM.68.5.2101-2105.2002

[84] Zhang H, Zhou Y, Bedsole C Oliver and Shim W Bo. (2026). *Fusarium oxysporum* f. sp. *vasinfectum* Race 4 (*Fov*4) FNP1 , a Nonribosomal Peptide Synthetase Gene, Plays an Important Role in Cotton Fusarium Wilt. MPMI, doi: 10.1094/MPMI-01-26-0004-R

[85] Di X, Takken FL, Tintor N. How phytohormones shape interactions between plants and the soil-borne fungus *Fusarium oxysporum*. Front Plant Sci. 2016;7:170. doi: 10.3389/fpls.2016.00170

[86] Wang L, Guo D, Zhao G, et al. Group IIc WRKY transcription factors regulate cotton resistance to *Fusarium oxysporum* by promoting GhMKK2-mediated flavonoid biosynthesis. New Phytol. 2022;236(1):249–265. doi: 10.1111/nph.18329

[87] Pei Y, Li X, Zhu Y, et al. Ghabp19, a novel germin-like protein from *Gossypium hirsutum*, plays an important role in the regulation of resistance to Verticillium and Fusarium wilt pathogens. Front Plant Sci. 2019;10:-2019 doi: 10.3389/fpls.2019.00583

[88] Wang C, He X, Li Y, et al. The cotton MAPK kinase GhMPK20 negatively regulates resistance to *Fusarium oxysporum* by mediating the MKK4–MPK20–WRKY40 cascade. Mol Plant Pathol. 2018;19(7):1624–1638. doi: 10.1111/mpp.12635

[89] Guo D, Hao C, Hou J, et al. The protein phosphatase GhAP2C1 interacts together with GhMPK4 to synergistically regulate the immune response to *Fusarium oxysporum* in cotton. Int J Mol Sci. 2022;23(4):2014. doi: 10.3390/ijms23042014

[90] Wang P, Zhou L, Jamieson P, et al. The cotton wall-associated kinase ghwak7a mediates responses to fungal wilt pathogens by complexing with the chitin sensory receptors. Plant Cell. 2020;32(12):3978–4001. doi: 10.1105/tpc.19.00950

[91] Liu N, Zhang X, Sun Y, et al. Molecular evidence for the involvement of a polygalacturonase-inhibiting protein, GhPGIP1, in enhanced resistance to Verticillium and Fusarium wilts in cotton. Sci Rep. 2017;7(1):39840. doi: 10.1038/srep39840

[92] Ulloa M, Hutmacher RB, Roberts PA, et al. Inheritance and QTL mapping of Fusarium wilt race 4 resistance in cotton. Theor Appl Genet. 2013;126(5):1405–1418. doi: 10.1007/s00122-013-2061-5

[93] Abdelraheem A, Zhu Y, Zeng L, et al. A genome-wide association study for resistance to Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*) race 4 in diploid cotton (*Gossypium arboreum*) and resistance transfer to tetraploid *Gossypium hirsutum*. Mol Genet Genomics. 2024;299(1):30. doi: 10.1007/s00438-024-02130-9

[94] Bennett RS, Colyer PD. Dry heat and hot water treatments for disinfesting cottonseed of *Fusarium oxysporum* f. sp. vasinfectum. Plant Disease. 2010;94 (12):1469–1475. doi: 10.1094/PDIS-01-10-0052

[95] Raper T B *et al.* (2023). Reevaluation of the degree day base 60°F concept in US cotton (*Gossypium hirsutum* L.) production. Agronomy Journal, 115(6), 3045–3061. doi: 10.1002/agj2.21480

[96] Viator R P, Nuti R C, Edmisten K L and Wells R. (2005). Predicting Cotton Boll Maturation Period Using Degree Days and Other Climatic Factors. Agronomy Journal, 97(2), 494–499. doi: 10.2134/agronj2005.0494

[97] Zhang J, Zhu Y, Wheeler T, et al. Targeted development of diagnostic SNP markers for resistance to Fusarium wilt race 4 in upland cotton (*Gossypium hirsutum*). Mol Genet Genomics. 2023;298(4):895–903. doi: 10.1007/s00438-023-02024-2

[98] Davis R L, Isakeit T and Chappell T M. (2022). DNA-Based Quantification of Fusarium oxysporum f. sp. vasinfectum in Environmental Soils to Describe Spatial Variation in Inoculum Density. Plant Disease, 106(6), 1653–1659. doi: 10.1094/PDIS-08-21-1664-RE

[99] Allen S, Lonergan P. Control strategies for Fusarium wilt of cotton in Australia. In: 2000 Beltwide Cotton Conferences; Memphis (TN); 2000.

[100] Ulloa M, Hutmacher RB, Davis RM, et al. Breeding for Fusarium wilt race 4 resistance in cotton under field and greenhouse conditions. J Cotton Sci. 2006;10:114–127.

[101] Feaster CV, Turcotte EL. Registration of Pima S-6 cotton. Crop Sci. 1984;24(2):382. doi: 10.2135/cropsci1984.0011183X002400020045x

[102] Mahill DM, Hutmacher RB, Marsh BH, et al. Phy 800 Pima: a new pima variety from PhytoGen. In: Proceeding of the Beltwide Cotton Conferences; Memphis, TN. National Cotton Council of America; 2005. p. 61–65.

[103] Ulloa M, Percy R, Zhang J, et al. Registration of four Pima cotton germplasm lines having good levels of Fusarium wilt race 4 resistance with moderate yields and good fibers. J Plant Regist. 2009;3(2):198–202. doi: 10.3198/jpr2008.09.0530crg

[104] Ulloa M, Hutmacher RB, Percy R, et al. Registration of five Pima cotton germplasm lines (Pima SJ-FR05–Pima SJ-FR09) with improved resistance to Fusarium wilt race 4 and good lint yield and fiber quality. J Plant Regist. 2016;10(2):154–158. doi: 10.3198/jpr2015.07.0043crg

[105] Ulloa M, Abdurakhmonov IY, Hutmacher R, et al. Registration of three *Gossypium barbadense* L. American pima-like germplasm lines (PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03) with improved resistance to Fusarium wilt race 4 and good fiber quality. J Plant Regist. 2022;16(3):626–634. doi: 10.1002/plr2.20230

[106] Isakeit T, Flores C. Reaction of commercial upland cotton varieties to Fusarium oxysporum f. sp. vasinfectum race 4. In: Proceeding of the Beltwide Cotton Conferences; Memphis, TN: National Cotton Council of America; 2020. p. 243–246.

[107] Isakeit T, Arce G. Evaluation of upland cotton varieties for resistance to Fusarium oxysporum f. sp. vasinfectum race 4. In: Proceeding of the Beltwide Cotton Conferences; Memphis, TN: National Cotton Council of America; 2021. p. 488–491.

[108] Zhang J. Registration of upland cotton cultivar 'Acala 1517-20' resistant to Fusarium wilt race 4. J Plant Regist. 2020;14(1):10–18. doi: 10.1002/plr2.20022

[109] Ulloa M, Hutmacher R, Zhang J, et al. Registration of 17 upland cotton germplasm lines with improved resistance to Fusarium wilt race 4 and good fiber quality. J Plant Regist. 2023;17(1):152–163. doi: 10.1002/plr2.20258

[110] Sivan A. (1989). The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. Phytopathology, 79(2), 198 doi: 10.1094/Phyto-79-198

[111] Zhang J, Howell CR, Starr JL. Suppression of Fusarium colonization of cotton roots and Fusarium wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis*. Biocontrol Sci Technol. 1996;6(2):175–188. doi: 10.1080/09583159650039377

[112] Antony-Babu S, Abeysinghe G, Thomas VE, et al. Integrated metabarcoding and culture-dependent assessments reveal Pseudomonas as dominant hyphosphere-pathobiont in race 4 Fusarium wilt pathogen of cotton. Front Microbiol. 2025;16:- 2025. doi: 10.3389/fmicb.2025.1661556

[113] Davis R L, Hayter J T, Marlino M L, Isakeit T and Chappell T M. (2023). Pathogenic and saprophytic growth rates of *Fusarium oxysporum* f. sp. vasinfectum interact to affect variation in inoculum density and interannual infection risk. Phytopathology, 113 (8), 1447–1456. doi: 10.1094/PHYTO-11-22-0407-R

[114] Bennett RS, Hutmacher RB, Davis RM. Seed transmission of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 in California. J Cotton Sci. 2008;12:160–164.

[115] Parris S, Kumar S, Li Z, Olvey J, Olvey M, Jones D C and Saski C A. (2025). Casparian strip fortification as a defense mechanism to *Fusarium oxysporum* f. sp. *vasinfectum* race 4 infection in a highly resistant *Gossypium barbadense* cultivar. Genes, 16(10), 1158 doi: 10.3390/genes16101158