1383 F*usarium oxysporum* f. sp. *vasinfectum* Race 4 in California

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A brief review of research on *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyder & H.N. Hans. race 4 in California is presented. Fusarium wilt has recently emerged as the dominant disease concern for cotton (*Gossypium hirsutum* L., *G. barbadense* L.) growers in California. An especially virulent form of *F. oxysporum* f. sp. *vasinfectum* was discovered in 2001 and has since spread to major cotton-producing counties in the San Joaquin Valley. Pathogenicity tests and genetic analyses revealed the causal agent to be *F. oxysporum* f. sp. *vasinfectum* race 4, a race originally described from India in 1960. Screening trials conducted since 2003 have shown that nearly all of the commercially available *G. barbadense* varieties are highly susceptible, and that many varieties of *G. hirsutum* also exhibit significant disease symptoms under certain conditions. Race 4 infection in acid-delinted cottonseed has been observed, however, seed infection in California cotton appears to occur at very low frequencies, possibly because of the arid production environment. Diagnostic tools such as race 4-specific PCR primers and macroarrays for the rapid identification of all known lineages of *F. oxysporum* f. sp. *vasinfectum* have been developed. These tools should be helpful in efforts to detect and quantify *F. oxysporum* f. sp. *vasinfectum* from plant tissue and soil.

KEYWORDS

Fusarium wilt, Fusarium *oxysporum* f. sp. *vasinfectum* race 4, San Joaquin Valley

Although *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyder & H.N. Hans. was first described in 1892 , and was well-studied by plant pathologists in the southeastern U.S. in the first half of the 20th century, it was not discovered in California until 1960 . Over the next three decades, the fungus became established over a broad area of the San Joaquin Valley . However, associated yield losses in cotton (*Gossypium hirsutum* L., *G. barbadense* L.) were primarily limited to fields with sandy soils infested with the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, . The use of nematicides,

crop rotation, and nematode-resistant cultivars successfully managed the disease, and Fusarium wilt became a minor concern in California cotton production in comparison other soil-borne diseases.

In 2001, symptoms of Fusarium wilt were observed in a Fresno County field of Pima cotton (*G. barbadense*) despite conditions atypical of the disease in California – i.e., heavy clay loam soil with few root-knot nematodes. Despite initial concerns that the causal agent was one of the virulent Australian biotypes , the new strain was identified as *F. oxysporum* f. sp. *vasinfectum* race 4 based on phylogenetic analyses of three genes (β-tubulin, translation elongation factor, and phosphate permase), pathogenicity assays, and restriction digests of the intergenic spacer of nuclear rDNA . This was the first report of race 4 outside of Asia . Kim et al. (2005) also discovered that races 3 and 8 had been present in California for many years upon analyses of historical isolates from J. E. DeVay's culture collection from the University of California, Davis.

Since 2001, *F. oxysporum* f. sp. *vasinfectum* race 4 has been confirmed at a growing number of locations in the San Joaquin Valley each subsequent year. By the end of 2006, a total of 38 fields, distributed across Fresno, Tulare, Kern, and Kings Counties, had tested positive for race 4. However, these records probably represent a significant underestimation of the true distribution of race 4 in the Valley. Several growers have failed to submit plant samples for confirmation even if their fields had symptomatic plants and areas of dead plants. In addition, an increasing number of growers have limited plantings to the commercially available and highly resistant Pima variety, Phytogen 800 (Dow AgroSciences, Indianapolis, IN), or to moderately resistant Acala varieties. These practices have essentially masked race 4 infections that would otherwise be apparent in plantings of susceptible Pima varieties. Variety trials in both field and greenhouse settings are being used to identify resistant germplasm for breeding . These trials have shown that many commercial Pima varieties are highly susceptible, and that Upland varieties, while not as susceptible as most Pima varieties, are only moderately resistant .

Mechanisms of pathogen dispersal throughout the San Joaquin Valley likely include the movement of infested soil and infected seed. Several of the confirmed, race 4-positive locations are within close proximity. It is also common to rotate cotton with high-value vegetable crops such as tomato (*Solanum lycopersicum* (L.) Karsten ex Farwell), whose production practices favor movement of infested soil. Like our Australian colleagues , we have observed expansions of disease foci in some fields to be oriented in the direction of irrigation water flow and/or the path of soil cultivation equipment. Standard containment procedures such as equipment sanitation have been recommended for several years. However, a rigorous containment policy similar to the Australian industry's "come clean, go clean" campaign has yet to be widely adopted.

Dispersal through infected seed is a likely explanation for confirmed, race 4-positive fields characterized by continuously planted cotton and few but widely-dispersed disease foci. While we have confirmed the presence of *F. oxysporum* f. sp. *vasinfectum* race 4 in acid-delinted and surface-sterilized seed, seed infection appears to occur infrequently in California. Smith et al. screened >3,000 acid-delinted seeds collected from infected plants; no *F. oxysporum* f. sp. *vasinfectum*-infected seeds were found even though peduncles and a few funiculi were infected. Subsequently, we used Petri plate assays to examine approximately 10,000 seeds collected from infected susceptible and resistant plants during the 2005 field season. Very few *Fusarium*-infected seeds, and none infected by isolates of race 4, were observed. During the 2006 field season, seed was collected from the lower canopy of a susceptible Pima variety that had been briefly overhead-irrigated during the

flowering and boll formation periods. Only one of >5000 seeds assayed was found to contain *F. oxysporum* f. sp. *vasinfectum* race 4. Seed infection by race 4 was also confirmed though mass isolations by culturing 400 seeds in 500ml of liquid media made selective for *F. oxysporum* by the addition of PCNB. Eight mass isolations were examined before DNA isolated from the resulting mass of fungal mycelia tested positive with race 4-specific primers. Although seed infection appears to be rare in the San Joaquin Valley, high commercial plant populations permit nearly non-detectable levels of seed infection to remain epidemiologically significant. For example, a seedlot with a 0.0033% infection level (i.e., one infected seed out of 30,000) from which 120,000 seeds were planted to a single hectare would be expected to produce four disease loci. Development of effective treatments for eliminating *F. oxysporum* f. sp. *vasinfectum* from seed while maintaining seed vigor is a high priority of researchers in the U.S., as it has been in Australia .

Considerable progress has been made in developing diagnostic methods, with the ultimate goal of rapid screening of seeds and soil. Reliable *F. oxysporum* f. sp. *vasinfectum* race 4-specific primers have been developed which work well on sonicated mycelia and young infected plant tissue, as well as with pure DNA. More recently, a rapid, high-throughput method of simultaneously detecting all known races of *F. oxysporum* f. sp. *vasinfectum* was developed using a macroarray technique . In this method, DNA oligomers composed of race/lineage-specific sequences are blotted onto a nylon membrane. DNA from the sample of interest is amplified with standard primers, labeled using a chemoluminescent labeling kit, and then hybridized to the membrane. If *F. oxysporum* f. sp. *vasinfectum* is present in the sample, the matching race-specific oligomer blots become luminescent. In the future, the macroarray approach can be modified to detect more pathogens, such as *Pythium* and *Rhizoctonia*, or to provide quantitative estimates of the pathogens in the sample .

The arrival of *F. oxysporum* f. sp. *vasinfectum* race 4 poses significant challenges for the San Joaquin Valley cotton industry, and many basic questions about its biology and management (e.g., longevity of chlamydospores, optimal combinations of soil treatments, host resistance, and rotation crops) remain. Host resistance offers promise as a long-term solution, but until highly resistant and commercially acceptable varieties are widely available, pathologists must continue work to develop economically feasible approaches for managing the disease. Much work has been done on *F. oxysporum* f. sp. *vasinfectum* race 1 and the Australian biotypes – we must now catch up with race 4.