# DESIGN AND IMPLEMENTATION OF A ROBUST METRIC TO QUANTIFY SOILBORNE *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* RACE 4 INOCULUM DENSITY

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## Abstract

Fusarium wilt is a geographically widespread disease of cotton (*Gossypium* spp.) with symptoms presenting throughout the growing season as characteristic wilt, chlorosis, stunting, and root rot. In the United States, this disease has been identified in both Upland (*G. hirsutum*) and Pima (*G. barbadense*) cotton and throughout the cotton production region. Fusarium wilt of cotton is caused by races of the soilborne fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). Upon introduction, races of FOV can persist in soils indefinitely as chlamydospores and may act as saprophytes in absence of a suitable host. Typically, for FOV to infest cotton and for symptoms manifest, the co-infection of the root knot nematode (*Meloidogyne incognita*) is required. Race 4, however, is distinguished from the other races because it can infest cotton without the formation a disease complex with the root knot nematode. In infested fields, inoculum is heterogeneously distributed due to multiple potential introductory events. As a result of numerous disease foci within individual fields, inoculum density is highly variable at scale. Furthermore, symptom expression, which has been used as an estimate of inoculum density, is not directly correlated with severity because of the variability of symptoms between cotton cultivars that co-occur in research fields involved in variety trials. To understand the spatial dynamics of inoculum in cotton fields, a method to directly detect and quantify inoculum in environmental samples was developed using a polymerase chain reaction (PCR) based approaches. Sequence specific primers were developed to detect and quantify genomic DNA of FOV4 from soil samples using quantitative real-time PCR (qPCR) and droplet digital PCR (ddPCR). To determine the efficacy of the primer pair, mycelia were weighed and spiked into sterilized field soils collected in Fabens, Texas. The quantities of mycelia added to the sterile soils were analyzed using qPCR and were correlated with the starting quantities ($R^2 = 0.943$ $p < 0.0001$). Upon confirmation of the efficacy of the metric with spiked samples, additional field soil samples were collected to quantify inoculum with other background microbial soil inhabitants. Serial dilutions of plate grown extracted FOV4 genomic DNA that were used to construct a standard curve were correlated between the two analytical techniques ($R^2 = 0.9899$ $p = 0.005$). Genomic DNA was extracted from soil samples and analyzed using both qPCR and ddPCR, where quantities of FOV4 detected in the soil samples were correlated ($R^2 = 0.8551$ $p < 0.0001$). These results demonstrate that soilborne FOV4 inoculum can be reliably detected and quantified using this DNA-based metric.