THE PAST AND CURRENT IMPACT OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* RACE 4 ON COTTON PRODUCTION IN CALIFORNIA

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Abstract

Fusarium oxysporum f. sp. *vasinfectum* (Fov) race 4 is an extremely virulent wilt pathogen to susceptible cotton varieties. Within the United States, Fov race 4 currently is geographically limited, first identified in California in 2001 and confirmed in Texas in 2017. Since the introduction of Fov race 4 in California, it has been a recurring and expanding threat to California's cotton production. Work done in California with Fov race 4 by various researchers during the past decade has produced evidence that cultural and chemical control methods (seed or in-furrow chemical or biological treatments, altered planting dates, crop rotations) typically have not adequately controlled infestations of Fov race 4 or adequately limited crop losses when growing Fov race 4 susceptible or moderately susceptible cultivars. Recommended strategies for improving crop survival and limiting economic damages have focused on identification of and growing more Fov race 4 tolerant cultivars, and growers to a large extent have taken this approach. More recently, in California, we have identified that our Fov populations in production fields are more diverse than previously reported. Our current research is focused on a survey of the cotton production fields in CA, and to molecularly characterize the Fov isolates collected during the survey. This current work will be important for the continued breeding efforts to develop superior cotton cultivars with high levels of resistance to Fov race 4.

Summary

Fusarium wilt of cotton, caused by *Fusarium oxysporum f.* sp. *vasinfectum* (Fov), is one of the most important diseases in cotton producing regions. Previous surveys across cotton growing regions in the United States have identified five nominal races of Fov, races 1, 2, 3, 4, and 8, all of which have been identified in California (Kim et al., 2005; Cianchetta et al., 2015). Fov race 4 is a particularly virulent wilt pathogen to susceptible cotton cultivars. Since 2001, Fov race 4 was geographically limited to the state of California, until its confirmation in Texas in 2017 (Halpern et al., 2017). The severity and type of plant symptoms induced by Fov race 4 are dependent on several factors including susceptibility of cotton cultivar, the pathogen genotype present in the field, environmental factors, and inoculum density (Araujo et al., 2016; Hillocks, 1992; Hutmacher et al., 2005; Ulloa et al., 2006). Pima cotton (*Gossypium barbadense* L.) varieties when susceptible, develop more severe symptoms than several Upland cotton (*G. hirsutum* L.) varieties (Hutmacher et al., 2005; Kim et al., 2005; Ulloa et al., 2006). In susceptible Pima, foliar symptoms develop as marginal chlorosis and necrosis, whereas the same marginal foliar symptoms do not develop in Upland varieties. In some cases, no foliar symptoms develop in Upland varieties even when vascular symptoms are severe (Hutmacher et al., 2005; Kim et al., 2005). Under favorable environmental conditions and when high levels of inoculum are present Fov race 4 can also cause of damping off and seedling mortality early in the growing season. In a study by Hao et al., (2009), a threshold for disease development with Fov race 4 was found to be between $1x10^3$ and $1x10^4$ conidia per gram of potting mix for susceptible cotton varieties. This threshold is similar to that for disease development with Fov race 1 in the presence of nematodes, however, nearly ten times more inoculum of Fov race 1 would be needed for similar disease development in the absence of nematodes.

Host-plant resistance has been the only cost-effective long-term management solution for Fov race 4. There has been great success in the development of resistant Pima cotton (Ulloa et al., 2006; Ulloa et al., 2009; Ulloa et al., 2010), but development of resistant Upland cotton is seriously impeded by the narrow genetic base of Upland cotton (Ulloa et al., 2010). Greenhouse screening of germplasm is an important step in finding new sources of resistance to Fov race

4, both in the initial and later stages. Greenhouse screening is especially important for the confirmation of field results, since many environmental and biological factors, such as multiple pathogens, can impact results. Currently, a root dip inoculation such as the one described by Kim et al. (2005) is used for large scale greenhouse screenings of cotton germplasm for resistance to Fov. However, there are some major disadvantages to this method such as not representing how plants would be infected in the field and predisposing the plants to transplant stress after inoculation. A rolled towel assay as described by Ellis et al. (2011, 2014) is a quick 10-day assay that could be used to assess seedling pathogenicity of an Fov isolate or be used for the initial screening of germplasm for resistance to Fov races. Another method that might be utilized is to infest soil with a carrier, such as Fov-infested grain seed, and directly plant the cotton seeds into the infested soil. This method would reduce the work and time needed to inoculate and transplant cotton seedlings and would also be more similar to actual conditions out in the field in comparison with the root dip inoculation. Becerra Lopez-Lavalle et al. (2012), developed a similar assay using a Fov-infested wheat for screening of cotton germplasm in Australia. Efforts are ongoing to compare the utilization of these different inoculation assays for the screening of US cotton germplasm.

The last Fov survey in California was conducted in 2012 and 2013 by Cianchetta et al. (2015). In the survey, only Fov race 4 was surveyed and identified from California, therefore the identification of other Fov races was excluded. Also, the race specific primers utilized in the survey to identify Fov race 4 (Yang et al., 2006), were later found to be unable to differentiate race 4 from races 3 and 7 (Crutcher et al., 2016). Since the last survey, a new set of primers that can separate Fov race 4 from other Fov races has been developed by Ortiz et al. (2017) based on the Tfo1 transposon insertion in the phosphate: H+ symporter gene unique to some Fov race 4 isolates. Based on the previous surveys, it was believed that Fov race 4 was a clonal population in California (Kim et al., 2005), however more recent data may suggest that this not the case for current Fov race 4 populations in California. During the 2017 and 2018 growing seasons, Fov isolates were collected from symptomatic plants from seven commercial or grower fields in the San Joaquin Valley. All Fov isolates collected produced the 208 bp fragment unique to Fov races 3, 4 and 7 (Yang et al. 2006). The isolates were also genotyped using the primers designed by Ortiz et al. (2017). Of the 138 Fov isolates tested with the primers by Ortiz et al. (2017), 85 isolates produced the 583 bp amplicon unique to Fov race 4, while 53 isolates produced the 396 bp that is produced by other Fov races. These results suggest that another Fov race or race 4 variant is present and could be contributing to disease losses in cotton fields within California. There may also be an interaction between the two Fov variants that may be able to break the resistance found in some varieties with high levels of resistance to Fov race 4 (Diaz and Ellis, *unpublished data*). Further genotyping of these Fov isolates is ongoing.

In summary, current ongoing research objectives include to (1) survey and molecularly identify Fov races and other seedling and wilt pathogens in commercial and grower cotton fields in California and Texas; (2) use representative identified Fov races for phenotypic evaluation of selected US and Uzbekistan germplasm; (3) and evaluate seedling and wilt capabilities of Fov races with different inoculation methods using susceptible and resistant Pima and Upland germplasm.

Acknowledgements

Funding for our research has been provided by the California Cotton Alliance and California Cotton Ginners and Growers Association. We also thank Vivian Maier, Tari Lee Frigulti-Schramm, and Mark Keeley for their technical assistance and cooperation.

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