Collaboration for Plant Pathogen Strain Identification

Guidelines for the Identification of Races of *Fusarium oxysporum* f. sp. *niveum* using Differential Watermelon Lines

Original Version 2021
Version 3.0: January 2024

Authors: Kimberly Webb, Marco Bello and Phyllis Himmel,
Revision: Phyllis Himmel, Kelley Clark

Host: Watermelon (*Citrullus lanatus*)
Pathogen: *Fusarium oxysporum* f. sp. *niveum*

Background

Fusarium wilt of watermelon is caused by *Fusarium oxysporum* f. sp. *niveum* (E. F. Sm.) W. C. Snyder & H. N. Han. This soilborne fungal pathogen was first described in the United States in Georgia and South Carolina in 1894 (Smith 1894) and only causes disease in watermelon. Today, the disease is found worldwide in most watermelon growing areas (Egel and Martyn, 2013). Primary infection occurs in the roots and movement of field soil on tools and equipment contributes to disease spread within and between fields. The formation of thick-walled chlamydospores enables pathogen survival in the soil for up to15 – 20 years, which makes disease management difficult. While the use of resistant cultivars and root stocks help to mitigate the impact of this disease, it can still be highly destructive and significantly limit production if more virulent strains or new pathogenic races appear. Disease symptoms will vary depending on the age of the plant at infection, environmental conditions, aggressiveness, and density of the pathogen population. Symptoms can also vary depending on host genotype (Kleczewski and Egel, 2011). Damping off may occur in young seedlings, while in older plants an initial overall graying of foliage is followed by chlorosis, wilt, and necrosis. Wilting of individual runners can also be seen in older plants (**Fig. 1**). Wilt occurs more rapidly when plants are stressed for water and at fruit set. When stems of affected plants are cut lengthwise the characteristic necrosis of the vascular tissue can be seen (**Fig. 2**) (Kleczewski and Egel 2011, Martyn 1996).

Figure 1. Wilting of individual runners that can be seen in older plants (Purdue University)

Figure 2. characteristic necrosis of the vascular tissue (Purdue University)

Races and resistance

In 1976, a standard method for the naming of races of the pathogen and the genes that confer resistance to the disease was proposed. Under that system, resistance genes are numbered according to their order of discovery and races are named according to the resistance genes they overcome (Risser et al., 1976). Using this system, four races of the pathogen are defined based on the response to resistance genes *Fon1* and *Fon2* present in watermelon cultivars Sugar Baby, Charleston Gray, Calhoun Gray and PI 296341 FR (**Table 1**; Cirulli 1972, Martyn and Netzer, 1991, Zhou et al., 2010).

Table 1. Classification of races of *Fusarium oxysporum* f. sp. *niveum* according to pathogenicity on differential host cultivars of *Citrullus lanatus* (Martyn and Netzer 1991).

Differential Hosts	Fon: 0	Fon: 1	Fon: 2	Fon: 3*
Black Diamond	S	S	S	S
Charleston Grey	R	S	S	S
Calhoun Grey	R	R	S	S
PI 296341 FR	R	R	R	S

S = Susceptible, R = Resistant

Note: The susceptible and resistance responses of Black Diamond, Charleston Grey, and Calhoun Grey are based on the percent wilt of inoculated seedlings, where ≥66% wilt is rated as a susceptible response, and ≤66% wilt is rated as a resistant response (Martyn and Bruton, 1989).

While race 0 can be problematic for some heirloom watermelon varieties, it is of little economic importance in commercial watermelon production areas (Engle and Martyn 2013, Keinath et al. 2020). Today, races 1 and 2 are widespread in many watermelon production areas, so prevention and management of this disease is critically important (Engle and Martyn 2013, Martyn 2014, Keinath et al. 2020). While there are now multiple reports of race 3 in the US (Amaradasa et al. 2018, Petkar 2019, Zhou et al. 2010), so far, distribution is limited.

Given the limited economic impact of race 0 in commercial watermelon production, race 0 and Charleston Grey were dropped as a watermelon Fon reference strain and differential host offered as CPPSI Reference Materials shown in **Table 2**.

Table 2. Classification of races of *Fusarium oxysporum* f. sp. *niveum* according to pathogenicity on differential host cultivars of *Citrullus lanatus*

Differential Hosts	Fon: 1	Fon: 2	Fon: 3*
Black Diamond	S	S	S
Calhoun Grey	R	S	S
PI 296341 FR	S	R	S

S = Susceptible, R = Resistant

**Fusarium oxysporum* f. sp. *niveum* race 3 is novel to the United States and we were unable to validate a reference isolate.

PI 296341 is reported resistant to races 1 and 2. However both traits segregate independently in this cultivar and one or the other can be lost during seed increases if subsequent generations are not selected carefully (Egel and Martyn 2013, Martyn and Netzer 1991). Resistance to race 1 was lost in the CPPSI differential host, PI 296341, but remains relevant as a differentiating race 2 host as Calhoun Gray is resistant to race 1 and susceptible to race 2. In the presence of a suspected race 3, both PI 296341 and Calhoun Gray are both susceptible. Additional inoculations and selections of PI 296341 are under way and an improved PI 296341 with resistance to races 1 and 2 should be ready for distribution by the end of 2022.

Preparation of host plants and inoculum:
Methods for inoculation of watermelon seedlings vary. Most protocols use either spore suspensions or a mycelium-agar slurry as inoculum. Other sources of method variation include the age of the seedlings at inoculation, the methodology for dipping roots into the inoculum solution, and the environmental conditions under which the seedlings are maintained after inoculation. The following is an inoculation procedure that has been demonstrated to give consistent results for this work.

Seeds are sown in a commercial potting mixture and grown in a greenhouse at 25-30^{o}C with a 16 hour photoperiod to germinate.

At the same time, inoculum is started by transferring the pathogen to ~10 plates ½ PDA grown at 25°C in an incubator or 10 flasks of cultured in potato dextrose broth (200 ml) and placed in a rotary shaker at 25°C and set fast enough to keep the culture aerated (Fig. 3). Czapek media may also be used instead of PDA.

Figure 3. Flask of inoculum in liquid culture on a rotary shaker at 25°C (Sakata)

Inoculation, incubation and evaluation for resistance and susceptibility:
Seedlings are inoculated when the first trifoliate leaf has fully expanded (~10 to 14 days after sowing). The inoculum is prepared by straining the culture through cheesecloth (Fig. 4) (or by scrapping ½ PDA plates and straining though cheesecloth) and collecting the spore suspension.

Depending on the virulence of the isolate used, the spore suspension is adjusted to ~100ml of 1 X 10^6 spores/ml to 2 X 10^6 spores/ml dilution.

Figure 4. Strain inoculum culture through cheese cloth (Sakata).

Figure 5. Removal of seedlings from the potting soil (Webb, USDA).

For inoculation, remove seedlings from their containers (Fig. 5) and wash the roots with water to remove as much potting soil as possible. The washed roots are then submerged in 100ml of inoculum for 5 minutes gently swirling the mixture every few minutes (Fig. 6). A mock inoculation (Fig. 7) is recommended to rule out the impact of the root dip and transplanting processes.

Figure 6. Root dip inoculation (Sakata).

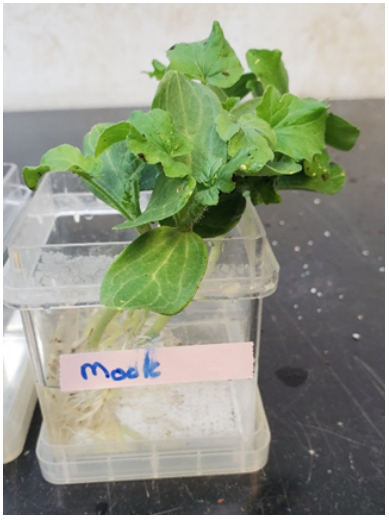

Figure 7. Root dip mock inoculation (Webb, USDA).

After inoculation, the seedlings are immediately transplanted into new ‘cone-tainers’ containing pre-moistened potting mix, and allowed to recuperate in a cool, dark, humid environment overnight (Fig. 8).

Figure 8. Seedlings are transplanted into potting mix right after inoculation (Webb, USDA).

The day after inoculation the plants are moved to a growth room or greenhouse, and maintained for three weeks at 23-26°C with 16 hours of light/day. The soil should be kept moist, but not saturated. The seedlings will typically regain turgor after inoculation. The susceptible plants will start to wilt 5 to 7 days after inoculation. Three weeks after inoculation the results should be clear, with resistant plants remaining asymptomatic (Fig. 9), and susceptible plants developing symptoms including wilt, stunting, vascular discoloration, and/or complete death (Fig. 10).

Figure 9. Resistant response of PI 296341 - no visible symptoms 3 weeks after inoculation (Webb, USDA)

Figure 10. Susceptible seedlings are dead or dying 3 weeks after inoculation (Webb, USDA)

Differentiation of races 1 and 2 is made by the pattern of responses in these hosts to each tested race. Charleston Gray was dropped as a differential host due to the lack of a clear susceptible response to race 1 (Fig. 11) and race 2 (Fig. 12).

Figure 11. Race 1 inoculation of L to R Black Diamond, Charleston Gray, and Calhoun Gray (R) (BASF)

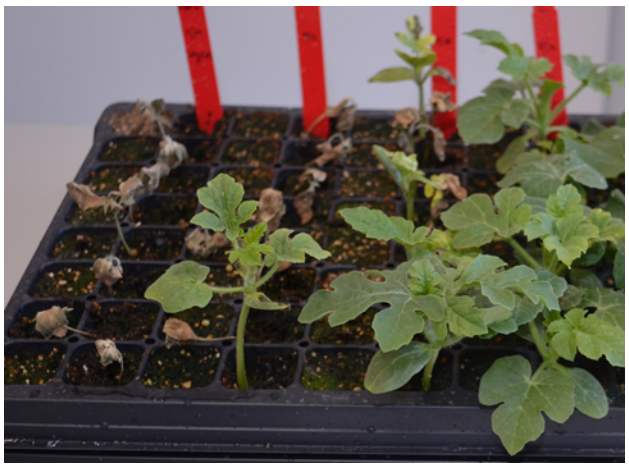

Figure 12. Race 2 inoculation of L to R, Black Diamond, Charleston Gray, Calhoun Gray and PI 296341 (BASF)

Ordering seeds of watermelon differential lines:

Seeds of each of the differential lines listed in Table 2 can be ordered from the USDA GRIN (Germplasm Resources Information Network: https://www.ars-grin.gov/). You may search the USDA GRIN database without logging in, but cannot order seeds until you create an account and log in to the database.

Type in '**CPPSI***' in the search window. Select the differential hosts to order. Select the cart button at the top of the page to generate an order form. Select 'submit' to place your order.

Fifty seeds of each of the differential lines can be ordered at no charge, as long as there is adequate seed in supply. The USDA National Plant Germplasm System in which the GRIN database is housed may not always have adequate seed of all the differentials listed to provide a full set of differentials.

If you have difficulties ordering seeds, contact Kelley Clark at kjclark@ucdavis.edu for assistance.

Ordering isolates of *Fusarium oxysporum* f. sp. *niveum*:

Reference isolates of races 1 and 2 of *Fusarium oxysporum* f. sp. *niveum* can also be ordered from the National Center for Genetic Resources Preservation via the online GRIN system. Follow the same GRIN access instructions for ordering seeds.

For questions regarding these reference races, contact:

amy.gurza@usda.gov
andy.hagan@usda.gov
Amy phone: 970-492-7554
Andy phone: 970-492-7555

National Lab for Genetic Resources Preservation Unit
1111 South Mason St.
Fort Collins, CO 80521

Feedback
Inquiries on how to participate and support CPPSI, provide feedback on new races identified, views on the inoculation protocols, differential hosts, or any related matter is welcomed. Please contact: Kelley Clark at kjclark@ucdavis.edu.

Liability waiver
The CPPSI Collaboration for Plant Pathogen Strain Identification, USDA NPGS/GRIN, APS, ASTA, and all other associated members and participating organizations or companies have done their best to provide information that is up-to-date and published in refereed journals and, therefore, no liability for the use of this information is accepted. The inoculation protocol described in this document has been demonstrated to be effective at identifying races of Fon and resistance in the above watermelon cultivars.

Selected References
Amaradasa, B.; Beckham, K.; Dufault, N.; Sanchez, T.; Ertek, T.; Iriarte, F.; Paret, M.; Ji, P. 2018. First report of *Fusarium oxysporum* f. sp. *niveum* race 3 causing wilt of watermelon in Florida, USA. Plant Dis. 102, 1029.

Bruton, B. D., Fish, W. W. 2010. On the reliability of Fusarium oxysporum f. sp. niveum Research: Do We Need Standardized Testing Methods? *in* Cucurbitaceae 2010 Proceedings. 320 pages. American Society for Horticultural Science.

Cirulli, M. 1972. Variation of pathogenicity in *Fusarium oxysporum* f. sp. *niveum* and resistance in watermelon cultivars. Pages 491-500 in: Actas III Congr. Un. Fitopatol. Mediterr. Oeiras.

Egel, D. S. and Martyn, R. D. 2007. Fusarium wilt of watermelon and other cucurbits. The Plant Health Instructor. DOI: 1094/PHI-I-2007-0122-01. Updated 2013

Keinath, A. P., DuBose, V. B., Katawczik, M. M., Wechter, W. P. 2020. Identifying Races of *Fusarium oxysporum* f. sp. *niveum* in South Carolina Recovered from Watermelon Seedlings, Plants and Field Soil. Plant Dis. 104:2481-2488.

Kleczewski, N. M. and Egel, D. S., 2011. A Diagnostic Guide for Fusarium Wilt of Watermelon. Plant Health Progress. DOI:10.1094/PHP-2011-1129-01DG.

Martyn, R. D., Netzer, D. 1991. Resistance to Races 0, 1 and 2 of Fusarium Wilt of Watermelon in *Citrullus* sp. PI 296341 – *FR*. HortScience 26(4) 429-432.

Martyn, R. D. 1996. Fusarium wilt of Watermelon 1996. Pages 13-14 in Compendium of Cucurbit Diseases. Eds. Zitter, T. A., Hopkins, D. L., Thomas, C. E. APS Press.

Martyn, R. D., 2014. Fusarium Wilt of Watermelon: 120 Years of Research. Pages 349-442 in Horticultural Reviews, Vol 42, First Edition. Ed. Jules Janick, Wiley- Blackwell.

Martyn, R. D., and Bruton, B. D. 1989. An initial survey of the United States for races of *Fusarium oxysporum* f. sp. *niveum*. HortScience 24:696-698

Petkar, A.; Harris-Shultz, K.; Wang, H.; Brewer, M.T.; Sumabat, L.; Ji, P. 2019. Genetic and phenotypic diversity of *Fusarium oxysporum* f. sp. *niveum* populations from watermelon in the southeastern United States. PLoS ONE 14, e0219821.

Risser, G., Banihashemi, Z., and Davis, D.W. 1976. A proposed nomenclature of *Fusarium oxysporum* f.sp. *melonis* races and resistance genes in *Cucumis melo*. Phytopathology 66:1105-1106.

Smith, E. F. 1894. The watermelon disease of the South. Proc. Am. Adv. Sci. 43:289-290.

Zhou, X. G., Everts, K. L., Bruton, B. D. 2010. Race 3, a new and highly virulent race of *Fusarium oxysporum* f. sp. *niveum* causing Fusarium wilt in watermelon. Plant Dis. 94, 92–98.