Guidelines for the Identification of Races of *Fusarium oxysporum* f. sp. *melonis* using Differential Melon Lines

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Host: Melon (*Cucumis melo* L.*)*
Pathogen: *Fusarium oxysporum* f. sp. *melonis*

Background

Fusarium wilt of melon is caused by *Fusarium oxysporum* f. sp. *melonis* (Leach and Currence) W. C. Snyder and H. N. Hans. Four races of the pathogen have been described to date: 0, 1, 2, and 1.2 (see Table 1). While the disease was first reported in New York in 1930, and is now widespread globally, the four races of the pathogen are not uniformly distributed throughout all melon producing regions.

In 1976, Risser et al. proposed a standard method for the naming of races of the pathogen, and the genes that confer resistance to the disease (Risser et al., 1976). Under that system, resistance genes are numbered according to their order of discovery, and races are named according to the resistance genes they overcome. Using this system, four races of the pathogen are defined based on the resistance genes *Fom1* and *Fom2* (see Table 1). A third resistance gene, *Fom3*, was later described in the cultivar Perlita FR (Zink et al., 1985). While there are some differences in the phenotypes of resistance, *Fom3* confers resistance to the same set of races as *Fom1.*

Two forms of race 1.2 have been described, based on symptoms caused: wilting (race 1.2w), or yellowing (race 1.2y). Polygenic partial or intermediate resistance to race 1.2 has been described (Perchepied and Pitrat, 2004).

Table 1. Classification of races of *Fusarium oxysporum* f. sp. *melonis* according to pathogenicity on differential host cultivars of *Cucumis melo* (adapted from Risser et al., 1976).

	Differential hosts and their genes for resistance			
Race	Charantais T	Doublon *(Fom1)* Perlita FR *(Fom3)*	CM17187*(Fom2)*	Isabelle
Race 0	S	R	R	R
Race 1	S	S	R	R
Race 2	S	R	S	R
Race 1.2*	S	S	S	IR

S = Susceptible, R = Resistant
* *Fusarium oxysporum* f. sp *melonis*, race 1.2 does not occur in the US. Isolates can be ordered from GEVE- MATREF: Valerie.GRIMAULT@geves.fr

Guidelines for differentiating races of *Fusarium oxysporum* f. sp. *melonis* using the melon differential lines. Methods for inoculation of melon seedlings vary. Most protocols use

either spore suspensions or a mycelium-agar slurry as inoculum. Other variations include the age of the seedlings at inoculation, and the conditions under which the seedlings are maintained after inoculation. The following is an inoculation procedure that has been demonstrated to give consistent results:

1. Seeds are sown in vermiculite, geminated at 25-30^{o}C, and grown in a greenhouse at 25-30^{o}C with 16 hour photoperiod. Plants should not be fertilized prior to inoculation.

2. Six days before inoculation of the plants, the inoculum is started by transferring the pathogen to V-8 broth (200 ml V-8 juice, 800 ml water, 3 g $CaCO_3$). The culture is then incubated at 30^{o}C on a rotary shaker set fast enough to keep the culture aerated.

3. Inoculation is done when cotyledons of the seedlings are fully expanded (10 to 14 days after sowing). The inoculum is prepared by straining the culture through cheesecloth, and adjusting the concentration of the spore suspension to 1 X 10^6 spores/ml. The seedlings are gently lifted from the vermiculite, and the roots washed. The washed roots are then submerged the inoculum for 5 minutes. The seedlings are then transplanted into potting mix, and allowed to recuperate in a cool, dark, humid environment overnight.

4. The day after inoculation the plants are moved to a growth room, and maintained for three weeks at 24^{o}C with 12 hours of light/day. The soil should be kept moist, but not saturated. The plants are fertilized 10 days after inoculation.

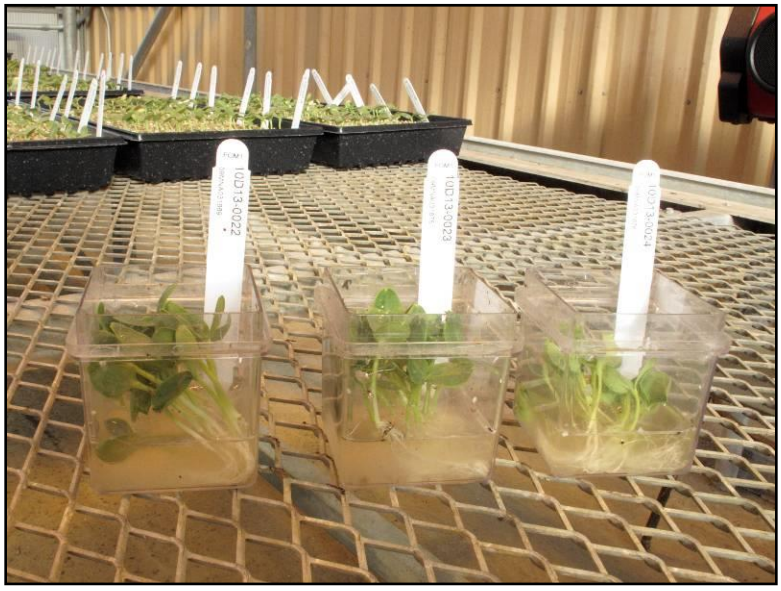
5. The seedlings will typically regain turgor after inoculation, and then the susceptible plants will start to wilt 5 to 7 days after inoculation. By three weeks after inoculation the results should be clear, with resistant plants remaining asymptomatic, and susceptible plants developing symptoms including wilt, stunting, vascular discoloration, and death. It is sometimes necessary to cut the stems of stunted plants to look for vascular necrosis.

Liquid cultures of *Fusarium oxysporum* f. sp. *melonis* grown in V-8 broth.

Melon seedlings grown in vermiculite.

Removal of seedlings from the vermiculite.

Washing off the roots.



Seedlings soaking in the inoculum.

Melon seedlings transplanted into potting mix (one day after inoculation).

Inoculated melon seedlings in a growth room.

Melon seedlings three weeks after inoculation with Fom. Resistant seedlings (last row on the right) are asymptomatic, but susceptible seedlings are dead or dying.

Ordering seeds of melon differential lines:

Seeds of each of the differential lines listed in Table 1 can be ordered from the USDA GRIN (Germplasm Resources Information Network). 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.

To set up an account, go to https://npgsweb.ars-grin.gov/gringlobal/search and select 'New user' at the top of the opening page and follow instructions to create a profile and establish an account.

To order seeds, go to https://npgsweb.ars-grin.gov/gringlobal/search and log in to your USDA GRIN account. Type in 'melon Fusarium wilt differentials' 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.

A limited supply of seeds per differential 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.

Note: A limited supply of 50 seeds per differential can be ordered at no charge, as long as there is adequate seed in supply. The NPGS may not always have adequate seed of all the differentials listed above to provide a full set of differentials.

If you have difficulties ordering seeds, contact us at cppsi@ucdavis.edu for assistance.

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

Reference isolates of races 0, 1 and 2 of *Fusarium oxysporum* f. sp. *melonis* can be ordered from the National Center for Genetic Resources Preservation.

The isolates were provided by the laboratory of Dr. Tom Gordon, UC Davis. They are:

Race 0: Isolate TX388, Rio Grande, Texas, VCG 0134, R. Martyn.
Race 1: Isolate GL174, Fresno County, CA, VCG 0134.
Race 2: P2 Fresno County, CA, VCG 0130, D. Jacobson.

To request the isolates, contact:

Dr. Stephanie Greene
Seed Curator
USDA-ARS-NCGRP, Plant and Animal Genetic Resources Preservation Unit
1111 South Mason St.
Fort Collins, CO 80521
970-495-3227 (tel)
Stephanie.greene@ars.usda.gov

Please note that isolates of race 1.2 are not available from CPPSI because the race does not occur in the USA). With the proper permit, isolates can be ordered from GEVE- MATREF: Valerie.GRIMAULT@geves.fr

Contacting CPPSI

Inquiries on how to participate and support CPPSI, provide feedback on new strains identified, views on the inoculation protocols, differential hosts, or any related matter are welcomed. Please contact:

Phyllis Himmel: pthimmel@ucdavis.edu
Office: + 530 752 5874
www.cppsi.org

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 *Fusarium oxysporum* f. sp. *melonis,* and associated resistance in melon plants.

References

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.

Perchepied, L., and Pitrat, M. 2004. Polygenic inheritance of partial resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in melon. Phytopathology 94(12):1331-336.

Zink, F.W. and Gubler, W.D. 1985. Inheritance of resistance in muskmelon to Fusarium wilt. J. Amer. Soc. Hort. Sci. 110(5):600-604.

Zitter, T. 1999. Fusarium wilt of melon, a worldwide problem in temperate and tropical regions. Proc. 1st Int. Symp. Cucurbits. Eds. K. Abak & S. Büyükalaca. Acta Hort. 492, ISHS.