

Use of an Insulin Syringe for Fusarium Wilt Inoculation of Watermelon Germplasm

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Sources of resistance to Fusarium wilt (*Fusarium oxysporum* Schlecht. (emend. Snyder & Hans.) f. sp. *niveum* (E.F. Sm.)) in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) has been an ongoing endeavor of many breeding programs. Several sources of resistance have been found, but this has generally been to races 0 and 1 (1, 4, 5). Netzer and Martyn (6) have identified a source of resistance to race 2, but it has not been generally incorporated into commercial sources. In addition, Fusarium wilt resistance can break down in the presence of root-knot nematode, which can further complicate breeding for resistance (7).

In 1998, we received a grant from the U.S. Dept. Agriculture to screen the available USDA watermelon germplasm collection for resistance to both Fusarium wilt race 2 and root-knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood). At the time the grant was awarded, there were approximately 1400 available accessions in the collection. This presented the problem of having reasonably reproducible inoculation techniques for Fusarium wilt and root-knot nematode.

The Fusarium wilt inoculation proved to be troublesome. Initially, we planned on using the tray dip method used by Martyn and Netzer (5). Immediately, it was recognized that this would be impossible with our limited resources. As the trays are held in the inoculum, the trays absorb the solution so that very quickly the inoculum is used up. We lacked the facilities to produce the large volumes of inoculum the technique would require.

The plant inoculum technique (5) was also rejected because of the difficulty of removing plants from the soil, inoculating, and replanting. Finally, the infested

soil technique could not be used because Fusarium wilt race 2 has not been isolated in Georgia and we were prohibited from inoculating under field conditions.

This study was undertaken to determine if a small quantity of inoculum could be injected with a syringe into each plant with acceptable development of disease.

Methods. Plants were seeded in the greenhouse into 28 x 56 cm flats with 809 inserts (8 packs of 9 cells, 3.8x3.8x6.4 cm) filled with Metromix 300 soil mix (Scotts-Sierra Products Co., Marysville, OH). The experiment design was a 5 x 4 factorial arranged in a randomized complete block with 8 replications and 9 plants per treatment combination. Plants were seeded on 2 March 1998 and inoculated 2 weeks later. Plants were evaluated 4 weeks after inoculation on a 0 to 9 scale (0=no signs of disease progressing, 9=death of the plant).

The inoculum was prepared with potato dextrose broth (Fisher Scientific, Norcross, GA) according to manufacture's directions. This broth was inoculated with Fusarium wilt race 2, which was obtained from Dr. Fenny Dane (Auburn University). This was the same culture as stored with American Type Culture Collection (Manassas, VA), culture number 62939. The inoculated broth was kept agitated at 20 C for 2 weeks. At this time, the inoculum was adjusted to 1.5×10^6 microspores per ml with a hemacytometer.

Five inoculation treatments were used: a control (nothing), insulin syringe with 50 μ l of water, insulin syringe with 50 μ l of inoculum, tray dip in water for 10 minutes, tray dip in inoculum for 10 minutes (5). The insulin syringe (Becton Dickinson Co., Franklin

Lakes, NJ) was a 0.5 cc (50 unit) syringe graduated in 50 µl (5 unit) increments. Each plant inoculated with a syringe had 50 µl of solution injected at the base of the stem just above the soil line. Plants were not placed back into the flats after inoculation to prevent contamination of adjacent packs.

The second factor tested was cultivar. The four cultivars tested were AU-Producer (Hollar Seed Co., Rocky Ford, CO), Starbrite (Asgrow Seed Co., Gonzales, CA), PI 296341-FR, and a self-pollinated selection of PI 296341-FR. AU-Producer is an open-pollinated, Fusarium wilt race 2 susceptible cultivar. Starbrite is an F₁ hybrid susceptible cultivar. PIs 296341-FR and 296341-FR self are a resistant selection and its progeny, respectively.

Results. There were no differences among the cultivars tested and there was no interaction effect between cultivars and inoculation technique (Table 1). However, there were differences among the

inoculation techniques. Untreated plants had the lowest rating of 1.1 and differed significantly from all other inoculation techniques. The insulin syringe with Fusarium wilt inoculum had a more severe incidence of Fusarium wilt compared to the syringe with water. However, both of these techniques had lower disease severity ratings compared to the tray dip with Fusarium wilt or tray dip with water (Table 1).

The lack of differences among the cultivars is surprising but not unexpected. Fusarium wilt reaction among resistant and susceptible cultivars is highly variable and under some conditions can cause soil suppression of the pathogen (2). This high degree of variability of results may also be due to mutability of the pathogen. Different reactions even under the most stringent controls can be particularly troublesome. Interactions between cultivars, races of the pathogen, and soil microbes are apparent but not completely understood (3).

Table 1. Cultivar and inoculation technique effects on mean rating for Fusarium wilt race 2 reaction in watermelon.

Treatment	Rating ^z
Cultivar	
AU-Producer (susceptible)	3.6a
Starbrite (susceptible)	3.5a
PI 296341-FR (resistant)	4.3a
PI 296341-FR Self (resistant)	3.7a
Inoculation Technique	
Nothing	1.1c ^y
Syringe with water	2.4c
Syringe with Fusarium	3.4b
Tray dip with water	5.6a
Tray dip with Fusarium	6.3a
Probability > F	
Cultivar	0.418
Inoculation Technique	0.000
Cultivar x Inoculation Technique	0.974

^z0-9 rating scale: 0-no disease present, 9-severe disease symptoms.

^yMeans followed by the same letter in a column are not different by Fisher's Protected LSD ($p \geq 0.05$).

The fact that there was no difference between the tray dip with *Fusarium* wilt and the tray dip with water is also not completely unexpected. Similar observations have been seen with uninoculated watermelon plants placed in high humidity/high temperature incubation chambers where these plants have reacted in a similar fashion to those that have been inoculated with a foliar pathogen prior to placement in the incubation chamber.

Although the syringe with *Fusarium* wilt inoculum had a lower disease reaction compared to the tray dip with or without inoculum, it was better than the syringe with water alone. Based on these results and the logistics of having to deal with such a large collection with a limited supply of inoculum, it was decided the syringe method would be better for our needs.

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