VERSION: 1.0	**DATE:** 11/2014
PATHOGEN: Fusarium oxysporum f sp niveum	
HOST: Cucurbits	
COMMON NAME: Watermelon (Citrullus lanatus var. lanatus)	
METHOD: Cb 6.1 Freeze Blotter (Syngenta)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 500 seeds	

PROCEDURE:

1. Treat seeds with Thiram to a rate approximating the label rate. The method has only been validated using Thiram 42S, but other formulations may be equally effective.

2. Prepare a suspension of 500 ppm dichloran. The method has only been validated using Botran 5F, but other formulations may be effective.

3. Place blotter paper (or three layers of Whatman No. 1 filter paper if using Petri dishes) into the dichloran solution. The blotter should be thick enough to provide adequate moisture for the entire incubation period such that the container does not need to be disturbed to supplement with additional water.

4. Remove and briefly allow the excess water to drain.

5. Place blotter paper in the bottom of a sealable tray (or sterile 100mm Petri dish placed upside-down).

6. Place seed onto blotter paper at a density low enough to have clear separation between seeds. The recommendation for a Petri dish is 10 seeds which is equal to 7.8 cm^2 per seed.

7. Seal the dish with Parafilm laboratory film if needed to prevent drying and contamination. Petri dishes need to be sealed to prevent drying.

8. Incubate seeds on the bench top for 24 hours to allow imbibition.

9. Place plates into a -20 to -80°C freezer for 24 hours.

10. Remove from freezer and incubate for 7-10 days on the bench top in an area receiving natural light.

11. Visually examine the seeds.

12. Search for seeds with dense white fluffy mycelium near the hilum. Refer to **Figure 1** below. F. oxysporum produces macroconidia and microconidia when having colonized a seed.

13. Aseptically transfer suspects to individual acidified PDA+ agar plates and subculture if necessary to purify the culture.

14. The culture should be beige to pink on the underside of the medium and be relatively slow growing. Refer to **Figures 2 and 3** below. Cultures not meeting these criteria should be discarded.

15. Seeds of “Black Diamond” watermelon should be planted in vermiculite or similar plant growth media in preparation for a pathogenicity test. The method was validated using both Tri-X 313 and Black Diamond, but other cultivars may work well, too.

16. When the seedlings have reached the late cotyledon stage (approximately 7-10 days depending on conditions and cultivar), prepare a negative control for the pathogenicity test. Pinch off approximately 10 cm from the root tips and place the seedlings in a vessel containing deionized water. Upon removal, replant seedlings into potting soil making sure to pack the soil tightly around the roots.

17. Next, prepare a solution of each suspect Fusarium culture. With a clean microscope slide, scrap hyphae off the agar surface into approximately 25 ml of sterile tap water. Mix the suspension.

18. Prepare for the inoculation of 2-4 seedlings per suspect isolate. Remove the seedlings and shake off vermiculite. Pinch off approximately 10 cm from the root tips and place the seedlings in a vessel containing the spore suspension. Let the seedlings remain in the spore suspension for 10-20 seconds. Upon removal from the spore suspension, replant seedlings into potting soil making sure to pack the soil tightly around the roots.

19. Prepare a positive control using a known Fon isolate as described previously.

20. Plants should be incubated from 22-24°C. Water from the bottom as needed.

21. Observe the seedlings after a maximum of 14 days but not less than 6 days. Pathogenic reactions include seedling collapse, yellowing of leaves, vascular discoloration, wilt of seedlings,

and death of seedlings. Vascular discoloration is evident when the hypocotyl is cut lengthwise. Refer to **Figure 4** for a typical positive reaction.

FIGURES:

Figure 1. Fon suspect seed.

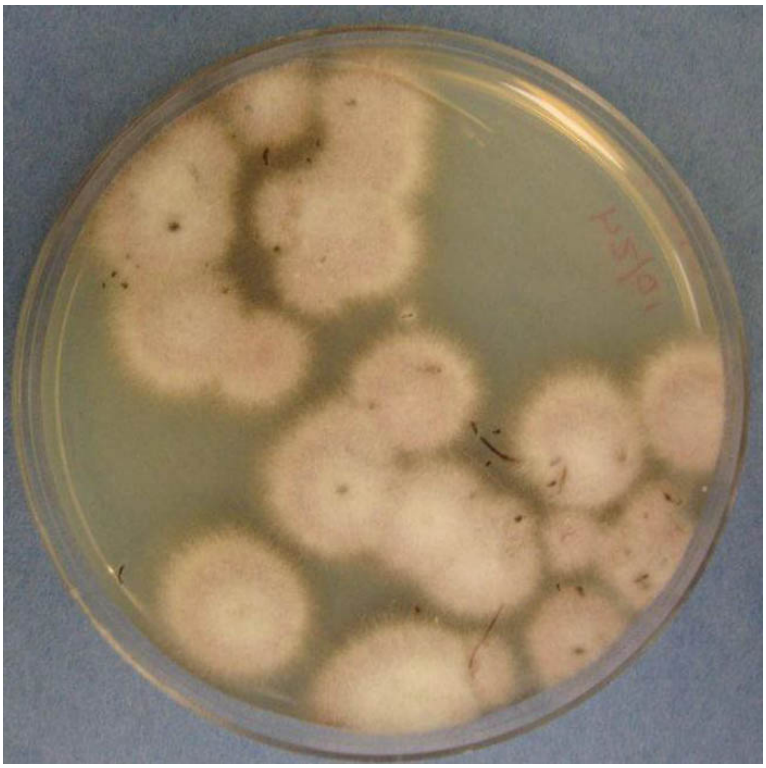

Figure 2. Typical culture of Fon on acidified PDA from top of plate

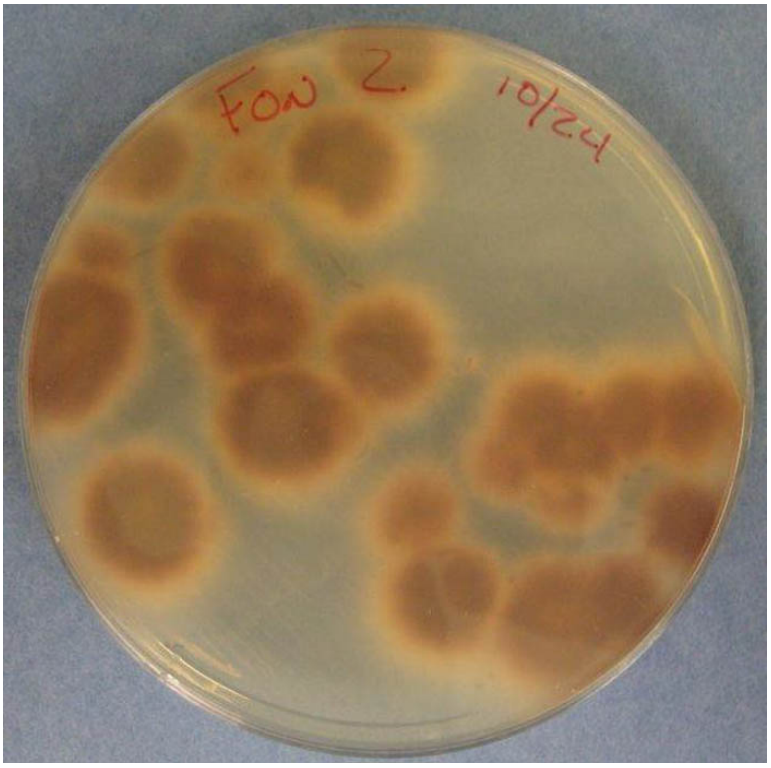

Figure 3. Typical culture of Fon on acidified PDA from bottom of plate

Figure 4. Typical reaction in Fon pathogenicity testing

MEDIA:

aPDA+ recipe		
Reagent	**Amount/Liter**	**Comments**
potato dextrose agar	29 g	
agar	5 g	Provides additional hardening to help with scraping off mycelium for the pathogenicity test
deionized water	1000 ml	
85% lactic acid	1.25 ml	Reduces pH to prevent bacterial growth

REFERENCES:

Haire, B. 2013. Old disease continues assault on Georgia melons. Southeast Farm Press. http://southeastfarmpress.com/vegetables/old-disease-continues-assault-georgia-melons For Print: http://www.southeastfarmpress.com/print/19475

Tran-Nguyen, L., Conde, B., Smith, S. and Ulyatt, L. 2013. Outbreak of Fusarium wilt in seedless watermelon seedlings in the Northern Territory, Australia. Australasian Plant Disease Notes. 8:5- 8.

Osman, A., Sherif, A., Elhussein, A. and Mohamed, A. 2012. Sensitivity of some nitrogen fixers and the target pest Fusarium oxysporum in the fungicide Thiram. Interdisciplinary Toxicology. 5(1):25-29.