VERSION: 1.0	**DATE:** 12/2012
PATHOGEN: Didymella bryoniae (syns: Phoma cucurbitacearum; Stagonosporosis cucurbitacearum)	
HOST: Cucurbits	
COMMON NAME: gummy stem blight	
METHOD: Cb 2.1 PCR (Ling et al., 2010)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 10,000 to 30,000 seeds	

PROCEDURE:

I. DNA Extraction

Extract total plant DNA from infected seeds using DNeasy plant kit (Qiagen, USA).

II. Real Time PCR

The authors used a SmartCycler II (Cepheid, USA) with a commercial PCR master-mix (iQ Supermix; Bio-Rad, USA) using primer and probe concentrations determined previously (Ha et al., 2009).

To determine the standard curve

1. Adjust a sample of fungal genomic DNA to 500 pg/µl

2. Dilute at 10-fold intervals to 10^{-6} (50 pg/µl – 0.5 fg/µl)

3. Use the mean Ct values to generate a standard curve

PCR conditions: initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 15 s and a combined step for annealing and elongation at 60°C for 40 s.

Forward primer: GTCCAGAGA TGAGGA TGGAGT

Reverse primer: GCTTGTAGGCGAATAATGAGCC

Probe: Texas Red-CGAAGGATATTGATCTA GACCGCACTTTC-BHQ2

REFERENCES:

Ling, K. S., Wechter, W. P., Somai, B. M., Walcott, R. R. and Keinath, A. P. 2010. An improved realtime PCR system for broad-spectrum detection of Didymella bryoniae, the causal agent of gummy stem blight of cucurbits. Seed Sci. & Technol. 38:692-703.

Ha, Y., Fessehaie, A., Ling, K. S., Wechter, W. P., Keinath, A. P. and Walcott, R. R. 2009. Simultaneous detection of Acidovorax avenae subsp. citrulli and Didymella bryoniae in cucurbit seedlots using magnetic capture hybridization and real-time polymerase chain reaction. Phytopathology. 99(6): 666-678