NSHS
National Seed Health System

VERSION: 1.0	**DATE:** 2001
PATHOGEN: Didymella bryoniae (syns: Phoma cucurbitacearum; Stagonosporosis cucurbitacearum)	
HOST: Cucurbits	
COMMON NAME: gummy stem blight	
METHOD: Cb 2.2 Vegetables Blotter Assay (Originally ISHI test Version 0.4.2000, no longer listed by ISHI)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 1,000 seeds	

PROCEDURE:

1. Incubation vessels (2 layers of blotter paper on the bottom of a transparent plastic container, with lid) are prepared. Allow approx. 4cm^2 per seed.

2. 1000 seeds are surface sterilized by treating in 0.5% bleach solution for 10 min. (optional step)

3. Seeds are rinsed with water and dipped in a solution of 50ppm Dicloran (Botran) for 10 seconds. Seeds are then placed on a paper towel to remove excess liquid.

4. The blotter paper is saturated with sterile water (distilled or deionized) to water holding capacity.

5. Distribute the seeds onto to the wet blotter paper, and spread manually.

6. Containers are either closed using a clear plastic lid or enclosed in a clear plastic bag to ensure a humid environment. Containers are incubated at 78-80°F in the dark for 10 days.

7. Seedlings are examined visually. Suspect seedlings (root discoloration; stem collapse) are removed and placed in another container prepared as described above.

8. The suspect seedlings are incubated under 12 hour lights for 4-5 days to induce pycnidia formation.

9. Seedlings are examined under the stereo microscope for presence of pycnidia or pseudothecia. The identity is confirmed by observing spore morphology and size using the transmission microscope.