

VERSION: 1.0	DATE: 12/2012
PATHOGEN: <i>Stemphylium botryosum</i> f. sp. <i>spinacia</i> (sexual: <i>Pleospora herbarum</i>)	
HOST: spinach (<i>Spinacia oleracea</i>)	
COMMON NAME: leaf spot	
METHOD: Lcb 6.1 Freeze blotter method (Hernandez-Perez and du Toit, 2006)	
METHOD CLASS: STANDARD (A)	
SAMPLE: 400 seeds	

PROCEDURE:

1. Place seed into a mesh tea strainer (Model 101, Venalicia Tea, Neuss, Germany).
2. Put the tea strainer in 150 ml of 1.2% NaOCl and shake manually for 60 seconds.
3. Triple-rinse seed in sterile deionized water and dry on a sterile paper towel in a laminar flow hood.
4. Flame-sterilize forceps, then plate 20 seed onto a sterile steel blue germination blotter (8.25 cm diameter; Anchor Paper Co., St. Paul, MN) that has been moistened with 4 ml of sterile deionized water in a 10-cm-diameter petri dish.
5. Seal the petri dishes with parafilm (Pechiney Plastic Packaging, Menasha, WI).
6. Incubate in the dark at 24°C for 25 h to imbibe moisture.
7. Freeze at –20°C for 22 to 25 h to prevent further germination.
8. Place in an incubator (Model I30BLL, Percival Scientific, Perry, IA) set at 24°C for 12 to 14 days with a 12h/12h day/night cycle, with cool-white fluorescent light and near-ultraviolet light by day.
9. Examine seeds approximately 4, 7, and 14 days after plating, at 8X to 100X magnification using a dissecting microscope for *S. botryosum*.

REFERENCES:

Hernandez-Perez, P. and du Toit, L. J. 2006. Seedborne *Cladosporium variabile* and *Stemphylium botryosum* in spinach. *Plant Dis.* 90:137-145.