

<b>VERSION:</b> 1.0	<b>DATE:</b> 2001
<b>PATHOGEN:</b> <i>Cochliobolus carbonum</i> (syn: <i>Bipolaris zeicola</i> )	
<b>HOST:</b> Maize ( <i>Zea mays</i> )	
<b>COMMON NAME:</b> Northern corn leaf spot	
<b>METHOD:</b> Mz 2.1 ISU Freezing Blotter Method (McGee 1994) (formerly Cf 2.1)	
<b>METHOD CLASS:</b> STANDARD (A)	
<b>SAMPLE:</b> 400 seeds	

**PROCEDURE:**

1. Randomly select 400 seeds.
2. Wash thoroughly in running water to remove chemical seed treatment.
3. Immerse seeds in 0.5 % (v/v) sodium hypochlorite (NaOCl) for 3 minutes; then immerse in distilled water containing 200 mg/liter of benomyl (Benlate 50w) and 100mg/liter of streptomycin sulfate (735 units/gram) for 30 minutes.
4. Place two autoclaved (sterile) blotters in each polystyrene box (25cm x 15cm x 4cm deep).
5. Moisten the blotters with 70 ml of sterile distilled water containing 0.035 grams of Botran fungicide 75W (dicloran).
6. Aseptically place seeds on blotters.
7. Incubate samples at 25°C for 2 days.
8. Transfer to -20°C for at least 15 hours.
9. Transfer to 25°C for 7 additional days with 8 hours of light per day.
10. Examine seeds for the presence of *Cochliobolus* species.

11. Suspect black to brown mycelium with fruiting bodies should be examined with a light microscope to confirm species, as described by Ellis & Holliday (1972).

**REFERENCES:**

Ellis, M. B. and Holliday, P. 1972. *Cochliobolus carbonum*. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 349.

McGee, D. C. 1994. Seed assays for Stewart's wilt and other seed-borne diseases of corn. Pages 161 – 168 in: Proc. Annu. Corn Sorghum Res. Conf. 48. American Seed Trade Association, Washington D. C.