

<b>VERSION:</b> 1.0	<b>DATE:</b> 2001
<b>PATHOGEN:</b> <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i>	
<b>HOST:</b> Maize ( <i>Zea mays</i> )	
<b>COMMON NAME:</b> Goss' bacterial wilt and leaf blight	
<b>METHOD:</b> Mz 9.1 sCNS Culture plate Method (Shepherd, 1999) (formerly Cb 1.1)	
<b>METHOD CLASS:</b> STANDARD (A)	
<b>SAMPLE:</b> 400 seeds	

**PROCEDURE:**

1. Divide a representative sample of 400 corn seeds into 4 sub-lots of 100.
2. Surface sterilize seeds with 0.5% sodium hypochlorite (NaOCl) for 3 minutes. Rinse thoroughly with sterile water.
3. Grind each sub-sample until finely pulverized and add to 100ml phosphate buffer saline solution (seeds may be ground dry or soaked overnight at 6°C before grinding).
4. Shake sub-samples at room temperature for 1 hour.
5. Prepare a dilution series comprising the original suspension and two 10-fold dilutions.
6. Plate each of the three dilutions onto sCNS in triplicate.
7. Incubate at 28°C for 5 to 7 days.
8. Any orange colonies that appear in the first 1 to 3 days should be disregarded.
9. Round and peach colored colonies appear after 3 days and by 9 days are putative colonies of *C. michiganensis* subsp. *nebraskensis* (Cmn).

10. Cmn can be confirmed by plant injections of culture suspensions into seedlings of Goss' wilt susceptible corn varieties (such as A632). Seedlings are then examined for typical Goss' wilt symptoms on leaves.

11. Alternatively, Cmn can be confirmed by CORYNE strip tests, performed according to manufacturer's (bioMeriux-Vitek Inc., Hazelwood, MO) directions on colonies plated on TSA agar. The positive code for Cmn is 6550004 or 6150004. ELISA kits (Agdia, CMM kit) can also be used to eliminate negatives, but are known to give false positives, so any positives should undergo further testing.

**MEDIA PREPARATION:**

sCNS:

Distilled water 1 liter

Nutrient broth 8.0g

Yeast extract 2.0g

K<sub>2</sub>HPO<sub>4</sub> (dibasic) 2.0g

KH<sub>2</sub>PO<sub>4</sub> (monobasic) 0.5g

Dextrose 5.0g

MgSO<sub>4</sub>\* 7H<sub>2</sub>O 0.25g

Agar 15g

Bravo 720 (Chlorothalonil) 0.2ml diluted 1:50 in water

Mertect 340F (Thiabendazole) 50µl (1 drop)

Potassium dichromate 0.02g

After autoclaving filter sterilize the following adding to media just prior to pouring:

Naladixic acid 40mg

Cycloheximide 100mg

**REFERENCES:**

Shepherd, L. M. 1999. Detection and transmission of *Clavibacter michiganensis* subsp. *nebraskensis* of corn. Ms Thesis, Iowa State University, Ames, IA.