

## Halo Blight



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Halo blight, caused by *Pseudomonas syringae* pv. *phaseolica* (Burkh.) Dows, is an important seed-borne disease of common bean. Re-classification of the pathogen as *Pseudomonas savastanoi* pv. *phaseolicola* has been proposed after DNA studies revealed relatedness amongst *P. syringae* pathovars (Gardan *et al.* 1992). Management of halo blight includes the use of disease-free seed, crop rotation and resistant varieties (Coyne and Schuster, 1983; Webster *et al.*, 1983a).



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Nine races of the pathogen have been reported based on their reactions on eight differential cultivars and lines (Taylor *et al.*, 1996a) (Table 1). Combination of race-specific and race non-specific resistance, enhance the chances in developing cultivars with durable resistance (Taylor *et al.*, 1996b).

Table 1. Race differentiation of *P. syringae* pv. *phaseolicola* on 8 differential cultivars and lines (Taylor *et al.*, 1996).

Differential	R-genes	Races								
		1	2	3	4	5	6	7	8	9
Canadian Wonder	-	+	+	+	+	+	+	+	+	+
A52 (ZAA 54)	4	+	+	+	+	-	+	+	+	+
Tendergreen	3	+	+	-	-	+	+	+	+	+
Red Mexican UI 3	1,4	-	+	+	+	-	+	-	+	-
1072	2	+	-	+	-	-	+	-	+	+
A53 (ZAA 55)	3,4	+	+	-	-	-	+	+	+	+
A43 (ZAA 12)	2,3,4,5	+	-	-	-	-	+	-	-	-
Guatemala 196-B	3,4	-	+	-	-	-	+	-	+	-

+, compatible (susceptible); -, incompatible (resistant)

Bean seedlings with fully expanded primary leaves can be used for resistance screening to the halo blight pathogen. Inoculum is prepared by suspending 24 to 48-hr-old cultures, grown on King's B agar (King *et al.*, 1954) at 25°C, in sterile tap water and adjusting it to contain approximately 10<sup>8</sup> CFU/ml using a spectrophotometer. Plants are inoculated with a DeVilbiss atomiser or painter's airbrush (15 p.s.i = 103 kPa) by spraying the bacterial suspension in two small areas (0.5 mm diameter) either side of the mid rib onto the abaxial surface of the leaves, thereby forcing the bacteria into the leaf tissue (Taylor *et al.* 1996a). The whole leaf area is then sprayed with the bacterial suspension until completely wet. Inoculated plants are kept in a humidity chamber (19°C±1°C, RH=100%) for 48 hr before being transferred to normal greenhouse conditions (Taylor *et al.* 1996a). Plants are rated for infection 10 days after inoculation on a 1 to 5 scale (Innes *et al.*, 1984) with 1 being highly resistant and 5 being highly susceptible (Table 2).

Table 2. Rating scale (1-5) used to evaluate beans for reaction to halo blight after inoculation of seedlings with fully expanded primary leaves (Innes *et al.*, 1984)

Halo blight score	Leaf inoculation	
	Water-soaking at the inoculation point	Reaction
1	Red brown necrotic reaction in area of maximum inoculation either side of the leaf mid rib	Highly resistant
2	Red brown necrotic reaction with trace of water-soaking	Resistant
3	Some necrosis but more extensive water-soaking confined to the area of maximum inoculation	Slightly susceptible
4	Small water-soaked lesions (<1mm diameter) distributed at random over the leaf undersurface	Susceptible
5	Larger-soaked lesions (1-3 mm diameter) distributed at random over the leaf undersurface	Fully susceptible

Because halo blight resistance has been found to be controlled by different genes (Hill *et al.*, 1972), plants need to be inoculated and evaluated at different stages of development to identify these different forms of resistance.

Mills and Silbernagel (1992), therefore, proposed a rapid screening method for screening for halo blight resistance in stems, leaves and pods. Inoculum was produced at 22 ° C on yeast dextrose calcium agar (Hotink *et al.*, 1966). Primary inoculum was prepared from a composite of four isolates of the halo blight pathogen by suspending 24 h cultures in sterile 0.01 M MgSO<sub>4</sub> at 10<sup>6</sup> cells/ml. Cell counts were determined using a hemocytometer and spectrometer.

- Plants were inoculated at emergence ('crook neck' stage) by placing a droplet of inoculum on the hypocotyl between the cotyledons. The stem was then punctured 2-3 times through the inoculum droplet using a hypodermic needle.
- Leaf halo reactions were studied by inoculating  $\frac{3}{4}$  expanded trifoliolate leaves with a multiple-needled florist frog (2 cm square metal base supporting rows of needles 3 mm apart and 12 mm in length) dipped in inoculum.
- Pods were inoculated with a florist frog at when they reached between  $\frac{1}{2}$  to  $\frac{3}{4}$  of maximum length. Pods were excised, inoculated and incubated in a pan lined with moist paper towels and sealed with a clear paper wrap.

Canadian Wonder can be used as susceptible check and. PI150414, GN #1 Sel 27 or Edmund can be included as race non-specific resistance check. Because the susceptible check is susceptible at all three stages of development, separate sets of checks for each stage of development should be included. Halo blight symptoms are noted for stem, trifoliolate leaf and pod reaction at 7-10 days after inoculation using a 1-9 scale (Tables 3,4 and 5).

Table 3. Rating scale (1-9) used to evaluate beans for reaction to halo blight after stem inoculations<sup>1</sup> at emergence.

Halo blight score	Stem inoculation <sup>2</sup>	
	Water-soaking at the inoculation point	Stem collapse
1	None	None
2	Trace (< 1mm)	None
3	Slight (1-2 mm)	None
4	Slight (1-2 mm)	Slight stem constriction above or below the inoculation point.
5	Moderate (2-3 mm)	Slight stem constriction above or below the inoculation point.
6	Moderate (2-3 mm)	Moderate stem constriction (<1/2 diameter of the stem).
7	Moderate to severe (3-4 mm)	Moderate stem constriction (<1/2 diameter of the stem).
8	Moderate to severe (3-4 mm)	Severe stem constriction (>1/2 diameter of the stem).
9	Severe (> 4mm)	Severe, Top dead or collapsed

<sup>1</sup> Cell suspension of  $10^6$ /ml from 24 h in 0.01 M MgSO<sub>4</sub>

<sup>2</sup> Syringe injection of the stem at 'crook neck' stage.

Source: Mills and Silbernagel (1992).

Table 4. Rating scale (1-9) used to evaluate beans for reaction to halo blight after trifoliolate leaf inoculations<sup>1</sup>.

Halo blight score	Trifoliolate leaf inoculation <sup>2</sup>	
	Water-soaking at the inoculation point	Halo development
1	None	None
2	Trace (< 1mm)	None
3	Slight (1-2 mm)	None
4	Slight (1-2 mm)	Slight (up to 1 mm beyond inoculation point)
5	Moderate (2-3 mm)	Slight (up to 1 mm beyond inoculation point)
6	Moderate (2-3 mm)	Moderate (up to 1-2 mm beyond inoculation point)
7	Moderate to severe (3-4 mm)	Moderate (up to 1-2 mm beyond inoculation point)
8	Moderate to severe (3-4 mm)	Moderate to severe (up to 2-3 mm beyond inoculation point)
9	Severe (> 4mm)	Severe (> 3 mm beyond inoculation point)

<sup>1</sup> Cell suspension of  $10^6$ /ml from 24 h in 0.01 M  $MgSO_4$

<sup>2</sup> Multiple needle (florist frog) inoculation of  $\frac{3}{4}$  expanded leaves.

Source: Mills and Silbernagel (1992).

Table 5. Rating scale (1-9) used to evaluate beans for reaction to halo blight after stem, leaf and pod inoculations inoculations<sup>1</sup>.

Halo blight score	Systemic chlorosis <sup>2</sup>	Water-soaking at the point of inoculation of the pod <sup>3</sup>
1	None	None
2	None	None with trace of necrosis
3	None	Slight (1-2 mm) turns necrotic
4	Transitory	Slight (1-2 mm) turns necrotic
5	Transitory	Moderate (2-4 mm) strong necrosis
6	Transitory	Moderate (2-4 mm) trace necrosis
7	Slight permanent (< $\frac{1}{4}$ of the leaflet affected)	Moderate (2-4 mm) trace necrosis
8	Moderate permanent (< $\frac{1}{4}$ - $\frac{1}{2}$ of the leaflet affected)	Severe (> 4 mm) no necrosis
9	Severe permanent (> $\frac{1}{2}$ of the leaflet affected)	Severe (> 4 mm) no necrosis

<sup>1</sup> Cell suspension of  $10^6$ /ml from 24 h in 0.01 M  $MgSO_4$

<sup>2</sup> Chlorosis after stem or leaf chlorosis.

<sup>3</sup> Multiple needle (florist frog) inoculation of  $\frac{3}{4}$  mature pods.

Source: Mills and Silbernagel (1992).

Table 6. Sources of resistance to halo blight in different seed classes.

Name or number	Seed color / type	Resistance genes	Reference
Domino, Black Magic	9 / Black		Kelly et al. (1987)
Edmund	1 / White		
Chase Sierra <sup>1</sup>	2M / Pinto		Coyne et al. (1994) Kelly et al. (1990)
Weihing Jules	1 / Great Northern	Race non-specific	Coyne et al. (2000) Taylor et al., 1996b
	7 / Purple		
	6 / Small red		
	5 / Pink		
	2R / Cranberry		
	6M / Red mottled		
	5K / Light red kidney		
	1 / Snap		
PI 150414	6 / Small red	Race non-specific	Taylor et al., 1996b
Wisconsin HBR 72	1 / White	Race non-specific	Taylor et al., 1996b
Nebraska #1 Sel. 27	1 / White	Race non-specific	Taylor et al., 1996b
Edmund	1 / White	Race non-specific	Taylor et al., 1996b
Jules			

<sup>1</sup> Resistant to Michigan races of halo blight.



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