Angular Leaf Spot

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Angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* (Sacc.) Ferr. is an important disease in both tropical and temperate regions where beans are produced. In Brazil, the fungal disease has been reported to cause significant loss of bean seed yield (de Jesus et al., 2001). The angular leaf spot pathogen has been reported to have a high degree of pathogenic variability (Guzmán et al., 1995; Sartorato, 2002; Pastor Corrales et al., 1998). Pastor-Corrales and Jara (1995) proposed a set of 12 differential lines to be used to characterize angular leaf spot pathotypes (Table 1). The first six differentials are Middle American (MA) origin and the second six are Andean origin. A unique binary value is assigned first to each susceptible MA differential followed by a different number for the susceptible Andean differentials. Numbers are separated by colon e.g. Race 6:27 attacks MA differentials G 2858 and Flor de Mayo and Andean differentials Don Timoteo, G 11796, Montcalm and Amendoin (Table 1).

Resistance to specific isolates of *P. griseola* has been reported to be simply inherited and molecular markers have been identified for some of these resistance genes (Mahuku et a., 2004; Ferreira et al., 2000;Carvalho et al., 1998, Miklas et al., 2005). Ragagnin et al. (2005) found the ALS resistance in AND 277 to race 63:23 to be conferred by a single dominant gene (*Pgh-1*). Cornell 49-242 has *Pgh-2* which confers resistance to *P. griseola* pathotype 31:17 (Nietsche et al., 2000). G10474 has a single dominant gene that confers resistance to pathotype 63:63 (Mahuku et a., 2004). A SCAR marker at 5 cM from the resistance gene was identified but the marker was found only to be effective in the Andean gene pool of common bean. CIAT (2003) reports that the RAPD marker OPE04 has been used in Uganda to select for an ALS resistance gene derived from Mexico 54. SCAR markers are also

available for ALS resistance genes from G 10474 and G 10909. In Honduras, the Andean lines G 05668 and G 06727 have been used as sources of resistance to ALS. G 06727 has resistance to pathotype 63:59 (J.C. Rosas, personal communication). Sartorato (2005) reported that 'Ouro Negro' had resistance to 8 pathotypes, including *P. griseola*. race 63:63 from Brazil.

Table 1. Bean differentials for angular leaf spot.

Gene Pool	Name	Seed type	Value	Resistance gene(s)	Binary system MA:A (6:27)
Middle American	PAN 72	1,S	1		-
	G 2858	2M,M	2		+
	Flor de Mayo	5M,S	4		+
	Mexico 54	5,M	8		-
	BAT 332	1,S	16		-
	Cornell 49242	9,S	32	*Pgh-2*	-
Sum of binary values for Middle American lines			2+4=6		
Andean	Don Timoteo	6,S	1		+
	G 11796	3, L	2		+
	Bolón Bayo	2, L	4		-
	Montcalm	6K,L	8		+
	Amendoin	5M,L	16		+
	G 5686	2,L	32		-
Sum of binary values for Andean lines			1+2+8+16=27		

Source: Pastor-Corrales and Jara (1995)

Single spore isolates of *P. griseola* can be cultured on Petri dished containingV-8 juice agar (Pastor Corrales et al. 1998). Ayala and Schwartz (1979) obtained abundant sporulation when isolates were grown in the laboratory on V-8 medium (200 ml V-8 juice, 3 g $CaCo_3$, 18 g Bacto agar and 800 ml distilled-deionized water. The culture was incubated at 19° C in darkness for 10 days. Conidia were harvested in a water suspension from the petri plates by rubbing the colony surface with a soft brush or thin wire. The inoculum was adjusted to a concentration of 2×10^4 conidia ml^{-1}. Spraying a conidial suspension onto the second or third trifoliate leaf was found to be a reliable and practical method to evaluate large nurseries.

In the greenhouse, Mahuku et al. (2004) inoculated the first trifoliate leaves of bean plants at 17d after planting with an aqueous suspension of conidia at a concentration of 2×10^4 conidia ml^{-1}. The inoculated plants were placed in a humid chamber for four days at a temperature of 22° C, a relative humidity > 95% and 12 h light/dark cycle. On the fifth day after inoculation, the plants were placed in a greenhouse where the temperature ranged from 24 to 30° C. The inoculated leaves were evaluated using the CIAT 1-9 scale at 17 d after inoculation. Plants with scores ≤ 3 were classified as resistant.

Ragagnin et al. (2005) described a detached-leaf inoculation technique that can be used to screen bean lines for resistance to ALS. Partially-expanded leaflets were immersed in a spore suspension (2 x 10^4 conidia/mL) and placed on moistened paper in a Petri dish. The Petri dishes were incubated for 18 days in a growth chamber at 19°C under a 12 h photoperiod. Reaction of bean lines to ALS were similar using detached-leaf and conventional techniques.

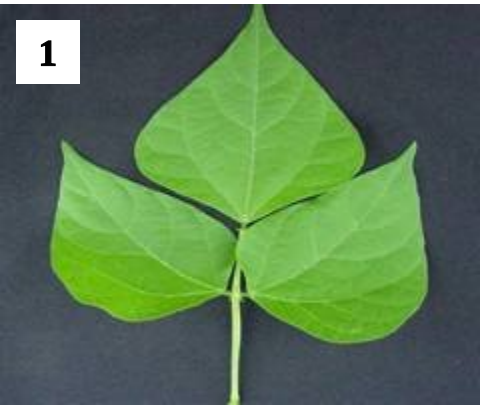
Inglis et. al (1988) inoculated bean plants in the field using dry inoculum. The inoculum was prepared from diseased bean leaves collected from the field or from diseased leaves from greenhouse-inoculated plants. Inglis et al. (1988) dried diseased leaves without heat using an electric fan. The dried leaves were pulverized in a Wiley Mill and sifted in an 18-mesh sieve to < 1 mm. The inoculum can be stored in double plastic bags at 5-10° C. The concentration of conidia per gram of dried inoculum can be estimated by diluting the inoculum 1:100 with water and counting the number of conidia per milliliter with a hemacytometer. The conidial suspension ranged from 1.1 x 10^4 to 5.8 x 10^4 conidia ml^{-1}. The application rates were adjusted so that 1.0 x 10^5 conidia were applied to each plant. The field plots were inoculated 3 weeks after planting when the plants had two sets of fully-expanded trifoliolate leaves. The plots were inoculated early in the evening after overhead irrigation to provide moist conditions during the period of infection. Leaves of the plants receiving the dry inoculum were first wetted with an aqueous solution containing a sticking agent (12% potassium resinate and 2.5% potassium oleate a.i.) at a concentration of 250 mg/ml. Each 5-m row received 0.5 L of the solution with the sticking agent. The dry inoculum was shaken over the leaves so that the distribution would be as uniform as possible. Beginning at two weeks after inoculation and at two-week intervals, full-expanded leaflets from six plants were evaluated using a 1-5 scale to calculate a mean disease rating (Table 1).

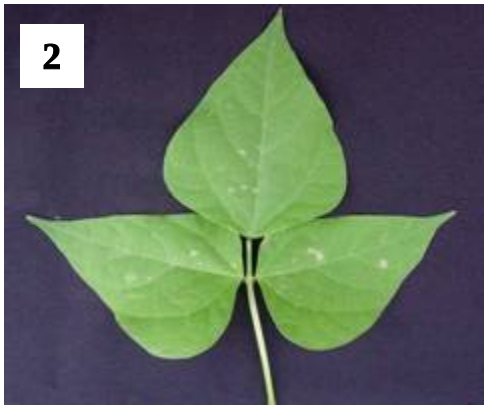
Muhuku et al. (2003) evaluated bean lines in the field with a mixture of isolates collected from the site of evaluation. Plants were inoculated 4 times at weekly intervals starting at 21 days after planting (second or third leaf stage) to ensure a high and uniform level of infection. Inoculation consisted of spraying an aqueous suspension of *P. griseola* spores suspended in tap water to a final concentration of 2 x 10^4 spores ml^{-1}. Plants were inoculated after sunset to benefit from darkness and higher humidity during the night. Disease evaluations were initiated at 14 days after inoculation using the CIAT (1-9) scale where 1 = plants with no symptoms, 3 = plants with 5-10% of the leaf area with lesions, 5 = plants with ≥ 20% leaf area infected and sporulation, 7 = plants with up to 60% of the leaf area with lesions and sporulation associated with chlorosis and necrosis and 9 = 90% of the leaf area with lesions frequently associated with early defoliation and plant death. Plants with scores ≤ 3 were considered resistant.

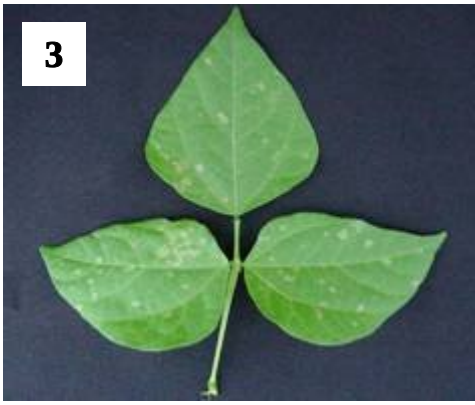
Table 2. Evaluation scale for screening for angular leaf spot reaction.

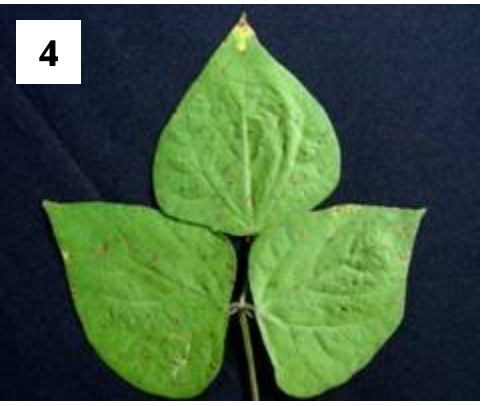
1-9 scale	1-5 scale	% leaflet area with lesions
1	1	1-10
3	2	11-25
5	3	26-50
7	4	> 50
9	5	Defoliation

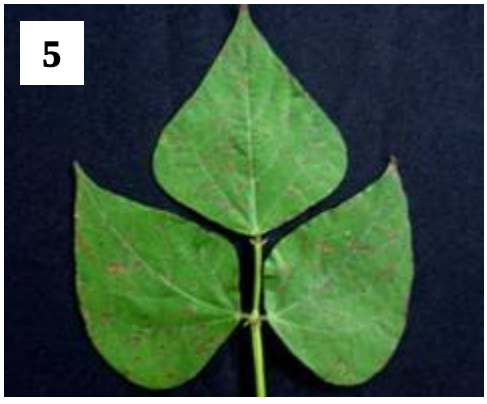
Source: Inglis et al. (1988).

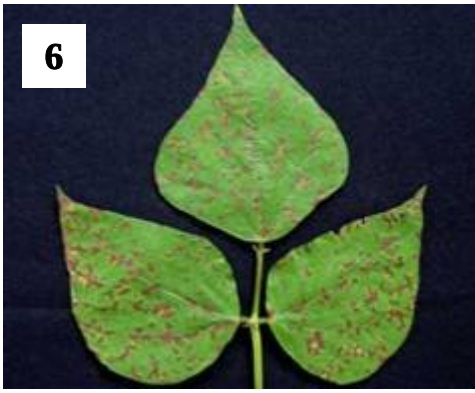


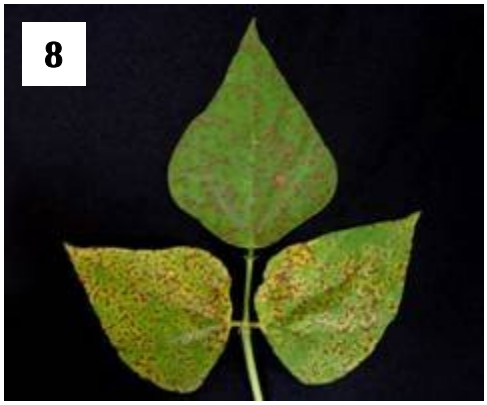


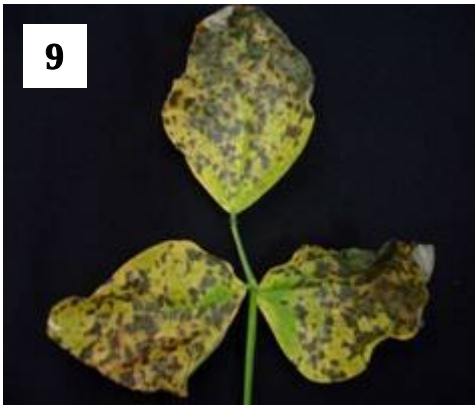












Phaeoisariopsis griseola was the first bean pathogen in which coevolution was clearly demonstrated between pathogen and host gene pool (Guzmán et al., 1995). Molecular data on pathogen variability continues to support the separation into two major groups (Andean and Middle American; Guzmán et al., 1999). The implications of these findings to bean breeders is discussed in detail by Miklas et al. (2005).

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Table 3. Sources of resistance to angular leaf spot in different bean seed classes.

Name or number	Seed color / type	Resistance genes	Reference
Ouro Negro	9 / Black	Resistance to 8 *P. griseola* pathotypes, including 63:63, from Brazil. SCARM02 is the SCAR marker linked to *Pgh-ON* (Queiroz et al., 2004)	Sartorato (2005)
Cornell 49-242		*Pgh-2* confers resistance to *P. griseola* pathotype 31:17. Two RAPD markers, OPN 02_{890c} and OPE 04_{650c}, were linked in the coupling phase, at 3.2 and 12.5 cM of the resistance gene, respectively. SN02 is the SCAR marker linked to *Pgh-2* (Sartorato et al., 2000)	Nietsche et al. (2000)
	1 / White		
	2M / Pinto		
	1 / Great Northern		
	7 / Red Mexican		
G 10474	6 / Small red	Resistance to *P. griseola* pathotype 63-63 conferred by single dominant gene	Mahuku et al., 2004
	5 / Pink		
	2R / Cranberry		
	3 / Yellow (Andean)	Jalo EEP 558 and ESAL 550. The SSR marker PV-actc 001 was linked in coupling phase to a resistance allele at a distance of 7.6 cM.	Ferreira da Silva (2003)
AND 277	6M / Red mottled	*Phg-1 confers resistance to P. griseola* pathotype 63:23. SH13 is the SCAR marker linked to *Phg-1* (Queiroz et al., (2004)	Carvalho et al. (1998) Ragagnin et al. (2005)
	6K / Dark red kidney		
	1 / Snap		

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