Scientific Note

Novel seed inoculation method for early selection of soybean genotypes resistant to target spot[1]

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ABSTRACT

Target spot, caused by *Corynespora cassiicola*, is a major soybean disease that may lead to early defoliation and yield loss. This study aimed to develop and validate a diagrammatic scale for assessing target spot on soybean cotyledons; compare seed and spray inoculation methods in V4 stage plants under greenhouse conditions; and correlate the severity of artificial inoculations with natural field conditions. Cotyledons with varying severity levels were photographed and analyzed using the Assess® software. The resulting scale included five severity levels based on the Weber-Fechner law: 0.3, 2.9, 20.8, 70.0, and 95.4 %. The use of this scale improved the precision and accuracy, even for inexperienced evaluators. Thirty-five genotypes (26 lines and nine cultivars) were tested under controlled conditions and later in field trials carried out in Lucas do Rio Verde and Campo Novo do Parecis (Mato Grosso state, Brazil). Both inoculation methods differentiated the genotype responses, with seed inoculation showing a higher repeatability. The trial in Lucas do Rio Verde was more informative because of the greater disease severity and showed a positive correlation with the greenhouse results.

KEYWORDS: *Corynespora cassiicola*, *Glycine max*, genetic resistance.

RESUMO

Novo método de inoculação de sementes para seleção precoce de genótipos de soja resistentes à mancha-alvo

A mancha-alvo, causada por *Corynespora cassiicola*, é uma das principais doenças da soja, podendo causar desfolha precoce e redução de produtividade. Objetivou-se desenvolver e validar uma escala diagramática para a avaliação da mancha-alvo em cotilédones de soja; comparar os métodos de inoculação por sementes e por pulverização em plantas no estádio V4 sob condições de casa-de-vegetação; e correlacionar a severidade em inoculações artificiais com as condições naturais de campo. Cotilédones com diferentes níveis de severidade foram fotografados e analisados utilizando-se o software Assess®. A escala resultante inclui cinco níveis de severidade baseados na lei de Weber-Fechner: 0,3; 2,9; 20,8; 70,0; e 95,4 %. O uso da escala aprimorou a precisão e a acurácia das avaliações, inclusive por avaliadores inexperientes. Trinta e cinco genótipos (26 linhagens e nove cultivares) foram testados sob condições controladas e posteriormente em ensaios de campo realizados em Lucas do Rio Verde e Campo Novo do Parecis (MT). Ambos os métodos de inoculação permitiram diferenciar as respostas dos genótipos, sendo que a inoculação por sementes apresentou maior repetibilidade. O ensaio em Lucas do Rio Verde foi o mais informativo, devido à maior severidade da doença, e apresentou correlação positiva com os resultados obtidos em casa-de-vegetação.

PALAVRAS-CHAVE: *Corynespora cassiicola*, *Glycine max*, resistência genética.

Target spot, caused by *Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei, is a major soybean disease present in nearly all soybean-producing regions of Brazil and worldwide (Lin et al. 2022). Its severity varies by region and season, and is influenced by the climate and genetic variability of both the pathogen and the host (Lin et al. 2022). Once considered minor, the target spot has re-emerged as a significant threat due to changes in agricultural practices, such as cultivar selection, crop rotation, and repeated fungicide use, leading to yield losses of 10-42 % (Molina et al. 2018, Molina et al. 2022).

In Brazil, the increasing incidence of this disease has highlighted the need for resistant cultivars. Resistance is quantitatively inherited, mainly additive, and has low heritability (Soares & Arias 2020). Although commercial cultivars have varying susceptibilities, their resistance to *C. cassiicola* remains underexplored.

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Effective genotype selection requires the use of reliable inoculation methods. The Brazilian Ministry of Agriculture (Brasil 2007) recommends spraying a spore suspension (1.0-1.5 × 10^4 conidia mL^{-1}) on V4-V5 stage plants, with severity assessed at 21-25 days post-inoculation. However, this method is time consuming. Seed inoculation, in which seeds contact fungal colonies, induces early symptoms in seedlings and cotyledons (Tanaka et al. 1996, Araújo et al. 2006, Puia et al. 2022), producing lesions such as those on leaflets (Goulart & Utiamada 2020). Soybean seedlings originating from infected seeds exhibit circular lesions with concentric rings, reddish-brown centers, and yellowish-green halos, which are characteristic symptoms of the target spot (Goulart & Utiamada 2020). Seed-borne infections enable an early establishment of the pathogen, representing an important pathway for disease dissemination and a potential primary inoculum source for crop development (Goulart & Utiamada 2020). However, no diagrammatic scales exist for cotyledon assessments.

Severity, the main variable used for foliar disease quantification, is typically estimated visually using tools such as diagrammatic scales that reduce subjectivity (Amorim & Bergamin Filho 2018). These scales must reflect real-field symptoms and define severity limits.

Early resistance screening in controlled environments may accelerate breeding; however, a strong correlation with field conditions is essential. Thus, this study aimed to develop and validate a diagrammatic scale for cotyledon assessment; compare seed and spray inoculation methods in V4 plants under greenhouse conditions; and correlate artificial inoculation severity with natural field severity.

A diagrammatic scale was developed using 100 soybean cotyledons with varying disease severities collected from greenhouse trials involving different cultivars. Inoculation was performed using the infected seed method (Tanaka et al. 1996). Cotyledons were photographed and analyzed with the Assess® software (Lamari 2002) to quantify severity. The minimum and maximum severity values were determined, and intermediate severity levels were defined using a logarithmic scale based on the Weber-Fechner law.

Scale validation involved eight inexperienced evaluators estimating severity from 40 cotyledon images with and without the scale. The accuracy and precision of the evaluators' visual estimates were determined using linear regression analysis with regression slope of 1. The mathematical model for the regression analysis was $Yi = a + bx + \mathcal{E}i$, where Yi is the estimated severity, a the intercept or linear coefficient, b the slope of the regression line or angular coefficient, x the actual target spot severity or independent variable, and Ɛi the errors with normal distribution ($N \approx 0, \sigma^2$). The accuracy of estimates was assessed using a t-test ($p \leq 0.05$) applied to the linear regression coefficients. Precision was evaluated using the coefficient of determination (R^2) and the variance of absolute errors, calculated by subtracting the actual severity from the estimated severity (Nutter & Schultz 1995). Analyses were performed using ExpDes in R (v4.2.1; R Core Team 2022).

The experiments used the *C. cassiicola* isolate nº 403 from GDM Genética do Brasil, originally collected in Lucas do Rio Verde (Mato Grosso state, Brazil), in 2020, and cultured in V8 medium. Thirty-five genotypes (26 lines and nine cultivars) were tested during the 2021/2022 season, using two inoculation methods: conidial spray and infected seeds.

Spray inoculation was performed according to Brasil (2007), with minor adjustments. Seeds treated with Standak® Top (pyraclostrobin + thiophanate-methyl + fipronil) were sown in sterilized soil in 1-L pots. Inoculation was carried out at the V4 stage by foliar spraying until runoff using a 1.0 × 10^4 conidia mL^{-1} suspension, and the plants were maintained at 28-30 ºC in a greenhouse. After inoculation, the pots were covered with transparent plastic sheets, for 48 h, to promote optimal conditions for pathogen infection.

The seed infection method was adapted from Tanaka et al. (1996). Seeds were surface-disinfested, placed in a single layer on fungal colonies grown in V8 medium for 48 h (28 ºC and 12/12 h photoperiod), and then sown in 250-mL pots filled with autoclaved sand. The conditions were maintained at 28-30 ºC, with > 80 % of humidity. Each method was tested in two randomized experiments, with three replicates. Each experimental unit consisted of a pot containing five plants.

Severity was assessed at 15 days post-inoculation (spray method) (Soares et al. 2009) and 12 days after sowing (seed method) using the newly developed cotyledon scale. Data were analyzed with the Kruskal-Wallis test in RStudio (v4.2.1) (R Core Team 2022), with the Agricolae package.

Field trials were conducted during the 2021/2022 growing season in Lucas do Rio Verde and Campo Novo do Parecis (Mato Grosso state, Brazil), both of which have a known history of high target spot incidence. Sowing occurred on November 14 and December 3, respectively. The meteorological data from these locations are presented in Figure 1.

A randomized complete block design with three replicates was used. Natural infection was monitored, and disease severity was assessed using the Soares et al. (2009) diagrammatic scale, based on observations from the two central rows of each plot (4 rows per plot, spaced 0.45 m apart and 10 m in length).

The area under the disease progress curve (AUDPC) was calculated using the Shaner & Finney's (1977) formula and normalized according to Fry (1978). The data were tested for homogeneity and normality, and the means grouped using the Scott-Knott test (5 % of significance). If the assumptions failed, the Friedman's test was applied to the randomized complete block design (RCBD) analysis using the RStudio (version 4.2.1) (R Core Team 2022), with the Agricolae package.

A diagrammatic scale with five severity levels (0.3-95.4 % of the affected cotyledon area) was developed (Figure 2).

Its use improved the evaluator precision, with determination coefficients (R^2) ranging from 0.70 to 0.97 (mean = 0.86; Table 1). Precision, defined as the closeness of the estimated severity to actual values (Nutter & Schultz 1995), increased significantly, especially among inexperienced evaluators, as shown by reduced intercept values (Juliatti et al. 2013). Without the scale, the accuracy was low (R^2 = 0.24-0.86; mean = 0.62), and evaluators tended to overestimate severity. For example, the evaluator 4 overestimated 75 % of the cases without the scale, but only 47.5 % with it, showing an improved balance in estimates (Table 2). Similar overestimation patterns have been reported by Soares et al. (2009). The error variance also decreased significantly with the scale; for example, the evaluator 1's error variance decreased from 502.01 to 38.50 (Table 2), being consistent with the findings of Soares et al. (2009) and Acco et al. (2020).

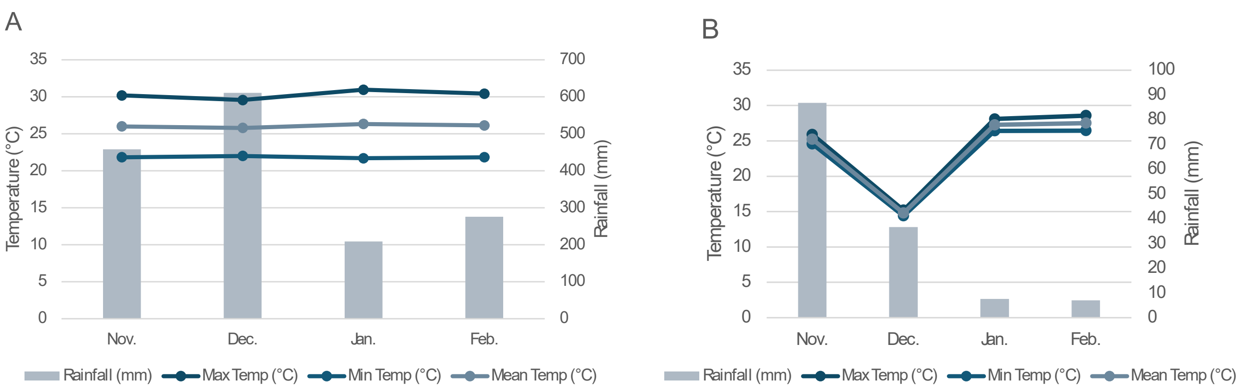

Figure 1. Meteorological data from the municipalities of Lucas do Rio Verde (A) and Campo Novo do Parecis (B) (Mato Grosso state, Brazil), during the experiments conducted in the 2021/2022 growing season.

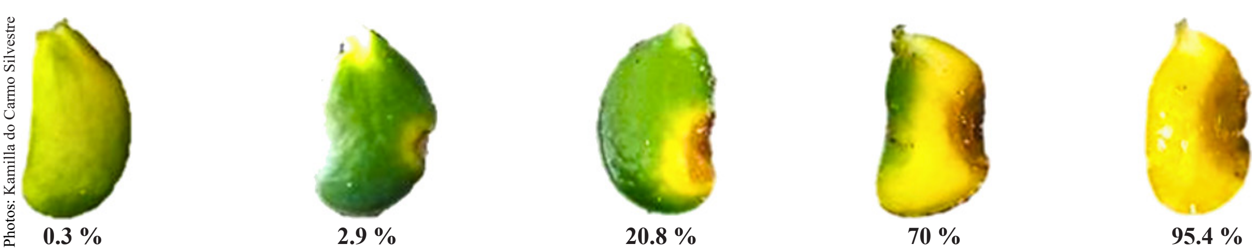

Figure 2. Diagrammatic scale for target spot assessment in soybean cotyledons.

Table 1. Estimated values and t-test for the intercept or linear coefficient (a), angular coefficient (b), and determination coefficients (R^2) for the linear regression model, comparing the actual and estimated target spot severity in cotyledons, with and without the use of the diagrammatic scale.

Evaluator	Without scale					With scale				
	Estimate		t-test values for the parameter		Determination coefficients	Estimate		t-test values for the parameter		Determination coefficients
	a	b	a	b	R^2	a	b	a	b	R^2
1	17.94**	0.85***	3.12	7.94	0.61	0.17^{ns}	1.01***	0.11	34.04	0.97
2	19.83***	0.88***	4.03	9.60	0.70	3.88^{ns}	0.99***	1.20	16.51	0.87
3	33.53***	0.52***	5.43	4.58	0.34	11.61*	0.81***	2.56	9.59	0.70
4	7.66*	0.88***	2.29	14.12	0.84	1.78^{ns}	0.99***	0.83	24.82	0.94
5	9.08**	0.95***	2.75	15.48	0.86	4.96^{ns}	0.94***	1.58	16.10	0.87
6	37.95***	0.16^{ns}	5.35	1.91	0.24	4.62^{ns}	0.82***	1.15	10.95	0.75
7	30.40***	0.68***	7.42	8.99	0.67	7.26*	0.88***	2.55	16.70	0.88
8	11.72*	0.84***	2.33	8.98	0.67	2.44^{ns}	0.94***	0.90	18.69	0.90
Mean	21.01	0.72			0.62	4.59	0.92			0.86

***, **, * and ns: t-test values significant at 0.1 % ($p \leq 0.001$), 1 % ($p \leq 0.01$), 5 % ($p \leq 0.05$), and not significant, respectively. The critical values for the t-test at the 1 % and 5 % levels with 38 degrees of freedom were $t_{(0.01;\ 38\ df)} = 2.712$ and $t_{(0.05;\ 38\ df)} = 2.024$.

Table 2. Range of error variation ($[\ ]_{Errors}$), parameter estimates obtained through linear regression residuals (σ^2_{Errors}, σ_{Errors}, and $\boldsymbol{X}_{Errors}$), number of overestimated and underestimated evaluations, with and without the aid of the diagrammatic scale, for assessing the target spot severity in soybean cotyledons.

Evaluator		$[\ ]_{Errors}$	Errors			Over	Under
			σ^2_{Errors}	σ_{Errors}	$\boldsymbol{X}_{Errors}$	Estimates	
1	Without scale	-68.35 ; 71.81	502.01	22.41	4.80 e-15	30	10
	With scale	-15.66 ; 13.84	38.50	6.20	1.61 e-15	17	19
2	Without scale	-63.06 ; 68.68	368.12	19.19	-1.42 e-15	35	4
	With scale	-63.04 ; 15.18	159.15	12.62	8.08 e-15	26	9
3	Without scale	-58.76 ; 49.95	578.52	24.05	-2.91 e-14	33	6
	With scale	-58.18 ; 52.02	312.22	17.67	-1.77 e-14	23	13
4	Without scale	-58.04 ; 17.48	170.62	13.06	-1.41 e-14	30	9
	With scale	-25.49 ; 13.64	69.77	8.35	-2.18 e-14	19	16
5	Without scale	-62.24 ; 17.73	166.05	12.89	-1.04 e-14	36	3
	With scale	-61.07 ; 12.69	149.94	12.24	2.00 e-15	24	11
6	Without scale	-47.93 ; 421.29	763.81	27.64	2.75 e-14	24	16
	With scale	-61.62 ; 32.90	247.84	15.74	6.41 e-15	17	18
7	Without scale	-44.82 ; 65.50	254.99	15.97	-1.51 e-14	37	3
	With scale	-41.13 ; 22.98	123.45	11.11	-1.17 e-14	20	15
8	Without scale	-61.22 ; 23.28	383.34	19.58	7.46 e-15	31	9
	With scale	-46.55 ; 20.30	111.30	10.55	-3.28 e-15	18	20

Severity data from the spray and seed inoculation methods did not meet the normality and homogeneity assumptions, justifying the use of non-parametric tests (Mutapi & Roddam 2002). Table 3 shows the severity (% of affected leaf area) across the soybean genotypes in two experiments per method. Spray inoculation reached a maximum severity of 52 % with low repeatability between the experiments. Genotypes such as the lines 13, 22, and 7 and M7739 IPRO showed a consistent resistance.

Seed inoculation produced similar severity patterns, with values ranging from 1.17 to 95.4 %. This method showed a greater repeatability, with consistent rankings of susceptible genotypes across the experiments. The M7739 IPRO genotype and the lines 16, 21, and 22 showed a lower severity in both trials.

The Spearman's correlation analysis (Table 4) confirmed a higher repeatability for seed inoculation (r = 0.82) than for spray inoculation (r = 0.71), supporting its reliability for resistance screening.

Table 3. Severity of target spot (% of affected leaf area) in soybean genotypes inoculated by spraying and infected seeds under controlled environment conditions.

Genotypes	Inoculation method			
	Spraying[1]		Seed[2]	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Line 12	52.00 a*	51.33 abc	95.40 a	78.47 abc
Line 24	52.00 a	52.00 a	36.93 abcd	47.63 abcd
Line 25	52.00 a	51.33 abc	53.47 abcd	53.47 abcd
Line 26	52.00 a	52.00 a	53.47 abcd	36.93 abcd
BMX Olimpo IPRO	52.00 a	32.50 bcdef	31.10 abcd	30.97 bcd
BMX Auge E	52.00 a	48.67 ab	55.23 abcd	53.47 abcd
DM75I74 IPRO	52.00 a	42.00 abcd	95.40 a	78.47 abc
NEO750 IPRO	48.67 ab	42.00 abcd	86.93 ab	78.47 abc
Line 1	45.33 abc	42.00 abcd	86.93 ab	95.40 a
BMX Voraz IPRO	45.33 abc	45.33 abc	70.00 abcd	53.47 abcd
Line 2	42.00 bc	42.00 abcd	86.93 ab	70.00 abcd
Line 18	42.00 bc	45.33 abc	36.93 abcd	70.00 abcd
BMX Ciclone CE	42.00 bc	36.50 bcde	70.00 abcd	53.47 abcd
BMX Bônus IPRO	42.00 bc	31.00 cdefg	86.93 ab	70.00 abcd
BMX Extra IPRO	42.00 bc	42.00 abcd	53.47 abcd	61.93 abcd
Line 23	36.50 cd	11.17 fghi	61.93 abcd	86.93 ab
Line 17	31.00 cde	25.50 defgh	14.57 bcd	36.93 abcd
Line 8	21.50 de	42.00 abcd	86.93 ab	78.47 abc
Line 19	21.50 de	11.67 fghi	70.00 abcd	78.47 abc
Line 11	17.50 def	42.00 abcd	70.00 abcd	86.93 ab
Line 3	13.50 efg	21.50 defghi	78.47 abc	70.00 abcd
Line 5	13.50 efg	45.33 abc	78.47 abc	70.00 abcd
Line 6	13.50 efg	11.17 fghi	70.00 abcd	78.47 abc
Line 16	13.50 efg	45.33 abc	8.73 cd	7.00 cd
Line 15	8.83 fgh	42.00 abcd	31.10 abcd	31.10 abcd
Line 9	6.50 ghi	42.00 abcd	78.47 abc	53.47 abcd
Line 10	6.50 ghi	42.00 abcd	53.47 abcd	70.00 abcd
Line 14	6.50 ghi	8.83 ghi	36.93 abcd	36.93 abcd
Line 20	6.50 ghi	8.83 ghi	36.93 abcd	53.47 abcd
Line 4	5.33 hi	17.50 efghi	36.93 abcd	36.93 abcd
Line 13	5.33 hi	6.50 hi	20.40 bcd	53.47 abcd
Line 22	5.33 hi	6.50 hi	8.73 cd	8.73 cd
Line 7	3.00 i	8.33 ghi	36.93 abcd	53.47 abcd
Line 21	3.00 i	17.50 efghi	2.03 d	2.90 cd
M7739 IPRO	2.33 i	3.00 i	1.17 d	1.17 d

[1] Severity assessed at 15 days after inoculation, according to the scale proposed by Soares et al. (2009). [2] Severity assessed at 12 days after sowing, according to the diagrammatic scale for evaluating target spot in soybean cotyledons. * Means in the column followed by the same letters do not differ statistically from each other according to the Kruskal-Wallis analysis ($p < 0.05$).

The field trials in Lucas do Rio Verde and Campo Novo do Parecis were evaluated using the final disease severity and normalized AUDPC (Table 5). Owing to later sowing, the disease severity was lower than that in earlier trials. Campo Novo do Parecis, where sowing took place at 19 days after Lucas do Rio Verde, showed a maximum leaf area of 20 %, likely due to unfavorable environmental conditions acting as a disease escape strategy. Thus, Lucas do Rio Verde provided more informative data. Ellis et al. (2014) emphasized the importance of selecting field sites that favor natural infections or epidemics. Environmental factors, such as temperature, humidity, and light, are critical for disease development (Liu & He 2019), and field trials are more variable than greenhouse trials (Webster et al. 2021).

Meteorological data from 2021/2022 revealed significant differences between the locations (Figure 1). Lucas do Rio Verde experienced more than 600 mm of rainfall in December and > 80 % of relative humidity throughout the crop cycle (Figure 1A), whereas Campo Novo do Parecis received less than

Table 4. Correlation coefficient (r_s) and p-value of the Spearman's correlations between severity data from the two experiments using the spraying inoculation method (spraying 1 and 2) and severity data from the two experiments using the infected seed method (seeds 1 and 2), under controlled growing conditions.

	r_s (p-value)			
	Spraying 1	Spraying 2	Seed 1	Seed 2
Spraying 1	-	0.71 (0.000)	0.90 (0.003)	0.34 (0.047)
Spraying 2	0.71 (0.000)	-	0.36 (0.035)	0.16 (0.354)
Seed 1	0.90 (0.003)	0.36 (0.035)	-	0.82 (0.000)
Seed 2	0.34 (0.047)	0.16 (0.354)	0.82 (0.000)	-

Table 5. Final severity (% of affected leaf area) and normalized area under the disease progress curve (nAUDPC) for target spot in soybean genotypes in naturally infected fields in Lucas do Rio Verde and Campo Novo do Parecis (Mato Grosso state, Brazil), during the 2021/2022 growing season.

Genotypes	Lucas do Rio Verde		Campo Novo do Parecis	
	Final severity[1]	nAUDPC	Final severity	nAUDPC
BMX Auge E	52.00 a*	49.86 a	19.33 a	5.86 fghij
NEO 750 IPRO	52.00 a	47.24 ab	15.00 c	4.49 lmn
Line 18	52.00 a	48.90 ab	15.00 c	4.92 klmn
Line 7	52.00 a	47.71 ab	15.33 c	5.59 jkl
BMX Extra IPRO	50.00 b	47.00 ab	18.33 a	6.67 defgh
Line 1	47.33 bc	36.83 abc	20.00 a	11.43 a
BMX Bônus IPRO	46.67 bc	31.77 bcd	17.67 b	6.14 ghij
Line 6	42.00 cd	37.68 abc	16.33 c	6.29 efghi
Line 5	40.00 de	27.14 efgh	18.67 a	8.85 ab
DM 75I74 IPRO	35.00 e	27.61 cdef	13.67 d	3.83 n
Line 8	35.00 e	28.25 cde	15.67 c	6.31 efghij
BMX Voraz IPRO	28.33 f	26.51 defg	14.33 d	4.50 mn
Line 19	25.33 f	20.90 hijk	17.00 b	6.29 efghij
BMX Olimpo IPRO	25.00 fg	25.32 efgh	15.67 c	7.00 abcde
Line 17	25.00 fg	24.68 fghi	13.67 d	4.96 klmn
Line 15	25.00 fg	25.32 efgh	17.00 b	7.26 abcd
Line 24	25.00 fg	24.04 ghij	16.33 c	6.44 defgh
Line 3	25.00 fg	25.32 efgh	13.00 d	3.96 n
Line 26	24.00 hi	24.32 ghijk	15.00 c	6.30 defgh
Line 25	23.33 gh	21.83 ghij	17.33 b	6.47 efghij
Line 4	21.67 hi	19.95 jkl	15.00 c	4.07 bcdef
BMX Ciclone CE	20.00 i	19.64 ijkl	13.67 d	4.07 mn
Line 10	20.00 i	19.36 jkl	19.33 a	8.73 abc
Line 16	18.00 j	19.29 kl	15.67 c	5.62 ijkl
Line 11	18.00 j	18.86 kl	17.67 b	8.36 abc
Line 20	17.00 jk	17.86 lm	15.67 c	4.91 klmn
Line 21	16.00 jkl	16.32 lm	15.67 c	5.11 klm
Line 22	14.67 kl	15.85 lm	13.67 d	4.23 mn
Line 2	14.00 lm	17.86 lm	14.33 d	5.76 hijk
Line 12	12.00 m	12.00 mn	15.67 c	4.77 lmn
Line 14	12.00 m	12.00 mn	16.33 c	6.23 efghij
Line 23	12.00 m	12.00 mn	17.33 b	6.64 cdefg
Line 9	12.00 m	11.14 n	15.67 c	6.67 bcdefg
Line 13	10.00 n	10.00 n	15.67 c	5.05 klm
M 7739 IPRO	10.00 n	11.29 n	13.00 d	4.72 lmn

[1] Severity assessed using the diagrammatic scale proposed by Soares et al. (2009). * Means followed by the same letters in a column do not differ statistically from each other according to the Friedman's analysis ($p < 0.05$).

20 mm of rainfall after sowing (Figure 1B). These conditions favored the disease development in Lucas do Rio Verde, but not in Campo Novo do Parecis.

In Lucas do Rio Verde, the most susceptible genotypes were BMX Auge E, NEO 750 IPRO, and the lines 7 and 18, whereas the most resistant genotypes were M7739 IPRO and the lines 9 and 13 (Table 5). M7739 IPRO, classified as moderately resistant (brand holder), consistently showed a low severity across all trials. BMX Auge E was consistently susceptible, confirming the previous findings. This suggests that, even under conditions of low disease pressure, the standards used in this study accurately reflected their previously determined behavior.

The yield data (Table 6) showed that the line 13 had the highest yield at both locations, which was correlated with low disease severity. BMX Auge E, BMX Extra IPRO, and the lines 7 and 1 had a low yield and high severity, respectively. Line 8 showed a good yield, despite susceptibility. In Lucas do Rio Verde, BMX Bônus IPRO had a high severity (46.67 %), but an unaffected yield. Campo Novo do Parecis had lower overall yields, likely because of late sowing and low rainfall.

Disease severity, yield, and seed quality vary by region and year, and are influenced by climate, management, and genetic variability (Lin et al. 2022). The Spearman's correlations between greenhouse inoculation methods (spray and seed) and field data showed a significant correlation only for Lucas do Rio Verde. Campo Novo do Parecis showed significance in one experiment (Table 7).

Ishikawa (2018) studied the responses of soybean cultivars to *Sclerotinia sclerotiorum* in field and greenhouse conditions. The white mold severities obtained through four artificial inoculation methods in the greenhouse showed a significant and positive correlation with the white mold severity data obtained from naturally infested field conditions, ranging from r_s = 0.45 to 0.62 (depending on the inoculation method used).

On the other hand, Moellers et al. (2017) reported correlation coefficients between greenhouse and field experiments ranging from 0.12 to 0.17, in evaluations of soybean white mold. The authors state that, although informative, greenhouse assays may not correlate with field results.

Field experiments, even those artificially inoculated, do not ensure homogeneous inoculum pressure owing to uncertain environmental conditions and the overlapping natural infection of the pathogen, which may vary among genotypes. Therefore, studies conducted in controlled environments are more precise in assessing the actual physiological resistance of materials (Kandel et al. 2018). The moderate correlation values found between the controlled condition and the field results reinforce evidence from a previous study on other pathosystems (Ishikawa 2018). This suggests that greenhouse

Table 6. Yield of soybean lines and commercial cultivars in Lucas do Rio Verde and Campo Novo do Parecis (Mato Grosso state, Brazil), during the 2021/2022 growing season.

Lucas do Rio Verde		Campo Novo do Parecis	
Genotype	Yield[1]	Genotype	Yield[1]
Line 13	2,842 a*	Line 13	2,834 a
Line 26	2,763 a	Line 4	2,808 a
Line 22	2,715 a	Line 3	1,770 b
Line 25	2,657 a	Line 8	1,734 b
Line 8	2,650 a	Line 9	1,616 b
BMX Bônus IPRO	2,623 a	Line 16	1,520 b
M7739IPRO	2,582 a	Line 5	1,446 b
BMX Ciclone CE	2,457 b	Line 14	1,390 c
BMX Olimpo IPRO	2,430 b	Line 10	1,370 c
Line 3	2,370 b	Line 26	1,290 c
Line 20	2,278 b	Line 15	1,268 c
BMX Voraz IPRO	2,242 b	Line 17	1,266 c
Line 16	2,220 b	BMX Ciclone CE	1,260 c
Line 10	2,193 b	Line 7	1,248 c
Line 19	2,137 c	Line 24	1,220 c
Line 14	2,095 c	BMX Olimpo IPRO	1,200 c
Line 21	2,095 c	Line 2	1,162 c
75I74RSF IPRO	2,077 c	Line 19	1,142 c
Line 23	2,057 c	Line 6	1,128 c
Line 17	2,045 c	Line 12	1,074 c
Line 15	2,017 c	Line 11	1,044 d
Line 4	1,965 c	Line 23	1,026 d
Line 5	1,938 c	75I74RSF IPRO	1,022 d
NEO750 IPRO	1,872 c	Line 20	1,012 d
Line 12	1,837 c	Line 22	990 d
Line 11	1,812 c	Line 18	928 d
Line 24	1,772 c	BMX Bônus IPRO	870 d
Line 6	1,695 d	Line 21	802 d
Line 18	1,657 d	Line 25	800 d
Line 9	1,647 d	BMX Auge E	628 e
Line 2	1,603 d	M7739IPRO	610 e
BMX Auge E	1,522 d	Line 1	554 e
BMX Extra IPRO	1,490 d	BMX Voraz IPRO	544 e
Line 7	1,478 d	NEO750 IPRO	524 e
Line 1	988 e	BMX Extra IPRO	468 e

[1] Yield expressed in kg ha^{-1} and representative of a harvested area of 5 x 0.9 m.
* Means followed by the same letter in the column do not differ statistically according to the Scott-Knott test ($p < 0.05$).

Table 7. Spearman's correlation coefficient (r_s) values and p-values between inoculation methods (spraying and infected seeds) in a greenhouse, and data obtained under natural infection conditions in Lucas do Rio Verde and Campo Novo do Parecis (Mato Grosso state, Brazil), during the 2021/2022 growing season.

Inoculation method	Experiment	r_s (p-value)	
		Lucas do Rio Verde	Campo Novo do Parecis
Spraying	1	0.40 (0.017)	0.12 (0.479)
	2	0.31 (0.066)	0.24 (0.159)
Seed	1	0.31 (0.068)	0.15 (0.389)
	2	0.33 (0.050)	0.33 (0.049)
Spraying	1 and 2	0.41 (0.016)	0.14 (0.422)
Seed	1 and 2	0.32 (0.065)	0.24 (0.161)

and laboratory evaluations should be extensively conducted before they can be considered reliable for the correct identification and characterization of field resistance (Ishikawa 2018).

In general, target spot resistance carried out by soybean cultivars is quantitatively inherited, and a low heritability indicates that the effect of the environment on the resistance phenotype is high (Soares & Arias 2020). As in other pathosystems (Kandel 2011), because of the polygenic nature of inheritance and low heritability of the trait, achieving highly positive correlation coefficients between greenhouse and field trials is difficult, which could explain the moderate-to-low correlation observed in the results of the present study. It should also be considered that the similarity of rankings between field disease scores and disease in cotyledons in greenhouses may be affected, as resistance at the cotyledon stage may differ from that in adult plants (Ellis et al. 2014).

Thus, this study developed a rapid protocol for identifying soybean genotypic responses to *C. cassiicola* under controlled conditions suitable for large-scale resistance screening in breeding programs. Using inoculated seeds, disease symptoms in the cotyledons were assessed at 12 days after sowing, allowing each evaluation cycle to be completed within approximately 15 days. This protocol enables the efficient processing of multiple genotypes and is a practical tool for early resistance screening against target spot in soybean.

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