Original Article

DIFFERENT METHODS OF ASSESSING SUSCEPTIBILITY OF SOYBEAN GENOTYPES TO WHITE MOLD

DIFERENTES MÉTODOS DE AVALIAÇÃO DA SUSCETIBILIDADE DE GENÓTIPOS DE SOJA AO MOFO BRANCO

Guilherme de Camargo HÜLLER[1]; David de Souza JACCOUD FILHO[2]; Marcelo Luiz Cunha PIERRE[3]; Hamilton Edemundo TULLIO[4]; Edilaine Mauricia Gelinski GRABICOSKI[5]; Fernando Cezar JULIATTI[6]

1. Engenheiro Agrônomo pela Universidade Estadual de Ponta Grossa – UEPG – Ponta Grossa, PR, Brasil.; 2. Biólogo, Engenheiro Agrônomo, Professor, Doutor, Departamento de Fitotecnia e Fitossanidade – DEFITO, Universidade Estadual de Ponta Grossa – UEPG – Ponta Grossa, PR, Brasil. dj1002@uepg.br; 3. Engenheiro Agrônomo pela UEPG, Ponta Grossa, PR, Brasil; 4. Engenheiro Agrônomo pela UEPG, Ponta Grossa, PR, Brasil; 5. Engenheiro Agrônoma, Mestre, Universidade Estadual de Ponta Grossa – UEPG – Ponta Grossa, PR, Brasil; 6. Engenheiro Agrônomo, Professor, Doutor, Instituto de Ciências Agrárias – ICIAG, Universidade Federal de Uberlândia - UFU, Uberlândia, MG, Brasil.

ABSTRACT: White mold caused by the fungus *Sclerotinia sclerotiorum* is an important disease in relation to soybean. The use of less susceptible genotypes can be a productive strategy in the management of this disease, and the development of an appropriate methodology for soybean inoculation is useful for the differentiation of disease-resistant genotypes. The present study aimed to assess the susceptibility of 77 soybean genotypes based on their reaction to oxalic acid, as well as to determine correlations between three traditional disease assay methods (detached leaf, non-wounded stem and straw tests) and the results of the oxalic acid assay. Oxalic acid susceptibility was assessed by using a wilting score scale. For the other methods, the severity of disease symptoms was assessed. To compare methodologies, the values obtained for the genotypes using each method were categorized into classes, and a severity index was used to represent individuals within each class. All the methods used were efficient for the differentiation of soybean genotypes in terms of susceptibility to *S. sclerotiorum*; however, the behavior of the genotypes depended on the inoculation method adopted. Even though no significant relationship was identified between the severities of the damage resulting from the methodologies, the rankings acquired from the methods strongly agreed. The oxalic acid method was the most rapid, the least laborious, and was the cheapest compared with the other methods that were used.

KEYWORDS: *Glycine max*. Pathogen. Resistance. *Sclerotinia sclerotiorum*.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most devastating and widespread fungal pathogens. It affects over 400 plant species worldwide, including important agronomic crops such as soybean, and causes the disease called white mold (BOLAND; HALL, 1994; SAHARAN; MEHTA, 2008). White mold epidemics in soybean may reduce yield by more than 40% when conditions are favorable, such as long rainy periods and cool temperatures (PELTIER et al., 2012; HENNEBERG et al., 2012; JACCOUD FILHO et al., 2014). The fungus produces a resistant structure (sclerotium), which allows the fungus to survive in soil for up to five years (ADAMS; AYERS, 1979; STEADMAN, 1983).

Due to the characteristics of *S. sclerotiorum*, one single strategy has not been sufficient to control the disease. An integrated management program is recommended, including the use of resistant or less susceptible genotypes, which has been cited as one of the most efficient and economic approaches (LU, 2003; PIERRE et al., 2011). It would be most ideal to analyze the susceptibility of genotypes under natural conditions, but *S. sclerotiorum* has an erratic distribution in the field and depends on specific weather conditions (ALEXOPOULOS et al., 1996). Therefore conducting assays in the field is unreliable and highly variable (SCHWARTZ; SINGH, 2013).

Several tests have been developed to analyze the susceptibility of genotypes to *S. sclerotiorum* in the laboratory or greenhouse. Among the available methods, the most common inoculation techniques use a PDA disc containing the fungal mycelium on different plant tissues. These tests include the detached leaf test (WEGULO et al., 1998), the cut stem test (straw test) (TERÁN et al., 2006) and the non-wounded stem test (GARCIA; JULIATTI, 2012).

Received: 24/08/15
Accepted: 20/01/16

S. sclerotiorum colonization is associated with many enzymes that are capable of digesting and degrading the cell wall of the host. In addition, several authors have associated the production of oxalic acid (OA) with virulence (HAREL et al., 2006; KIM et al., 2008; WALZ et al., 2008; LIANG et al., 2015). Therefore, some susceptibility screening assays are based on the responses of different genotypes to oxalic acid (GONÇALVES;SANTOS, 2010), however, the authors are not aware of any publications assessing the levels of resistance of soybean to *S. sclerotiorum* based on the wilting responses of OA, as has been efficiently used for common bean (KOLKMAN; KELLLY, 2000; ANTONIO et al., 2008).

The present study aimed to: 1) establish and test a methodology to assess the level of susceptibility of soybean genotypes to white mold based on the response to OA, 2) compare the results of OA sensitivity to three traditional disease screening methods that use agar plugs with actively growing mycelia as inoculum, 3) analyze the correlations between the methodologies to determine the susceptibility of the genotypes to *S. sclerotiorum* based on ranking.

MATERIAL AND METHODS

Study area

This study was conducted in the Laboratory of Applied Plant Pathology of the State University of Ponta Grossa (Universidade Estadual de Ponta Grossa – UEPG) in the State of Paraná, Brazil.

Experimental design

Four experiments were performed (one for each proposed method of inoculation) with 77 soybean genotypes grown in a greenhouse. The experiments were developed using a completely randomized design with four replicates each.

Inoculum source

The mycelia for the detached leaf method, straw test and non-wounded stem inoculation methods were obtained by culturing sclerotia that came from commercial fields in Jataí (GO-Brazil). The sclerotia were previously disinfected in 70% ethanol and 0.5% sodium hypochlorite (diluted in distilled water), isolated in Petri dishes on potato dextrose agar (PDA) medium, and incubated at 22 °C with a 12-h light and 12-h dark photoperiod for the formation of fungal mycelia. For the inoculation, PDA plugs approximately 8 mm in diameter, containing four day-old fungal mycelia, were used.

Wounded stem inoculation using micropipette tips (straw test)

Four plants from each genotype, at the V2 phenological stage (second trifoliate leaf expanded) according to the scale of Fehr and Caviness (1977) were assayed. The apical shoot tip was removed and a PDA plug was placed on top, mycelial side in contact with the plant surface. Micropipette plastic tips of 1 mL were placed on the plant to prevent the disk from falling or touching any part of the plant other than the injured area. The inoculated plants were misted with sterile distilled water, covered with plastic bags and incubated at 20 °C with a 12h light and 12h dark cycle. The disease severity was assessed 72 hours after inoculation based on the proportion of the lesion length on the stem in comparison with the total stem length (both measured with a ruler).

Non-wounded stem inoculation method

Four plants from each genotype at the V2 phenological stage (FEHR; CAVINESS, 1977) were assessed. A PDA plug from a four day-old culture was placed mycelial side towards the plant, on the first trifoliate axillary bud, and fixed in place with adhesive tape to each plant. The inoculated plants were misted with sterile distilled water, covered with plastic bags and incubated at 20 °C with a 12h light and 12h dark cycle. The severity of the disease development was assessed 72 hours after inoculation, based on the proportion of the lesion length on the stem in comparison with the total stem length (both measured with a ruler).

Detached leaf method

The second trifoliate, from four plants at the V4 growth stage (FEHR; CAVINESS, 1977), was detached and placed separately in germination boxes (gerbox) containing two sheets of blotting paper saturated with sterile distilled water. Prior to inoculation, the leaflets were sprayed with water and one PDA disk, taken from a four day-old culture, was placed with the mycelial side facing the plant surface, on the center of the leaflets. The germination boxes containing the leaflets were incubated at 20 °C with a 12h light and 12h dark cycle. Four trifoliates (twelve leaflets) from each genotype were assessed. The severity was assessed every 24 hours after inoculation based on the size of the lesion in proportion to the total size of the leaflet (also measured with a ruler). Lesion size at 48 hours post-inoculation was used as a comparison with the other inoculation methods, as nearly all leaflets were completely necrotic by 72h.

Oxalic acid method

After 21 days of sowing, the plants were cut near the crown region, and placed through a polystyrene sheet into a 20 mM oxalic acid solution (Analytical Reagent on its acid form, LABSYNTH Ltda.) pH adjusted to 4.0 using 10 M sodium hydroxide), such that the stems were maintained submerged 2 cm for 20 hours at 20 °C in the dark (ANTONIO et al., 2008). Four plants from each genotype were assayed. The susceptibility to OA was assessed using a wilting score index designed by the authors (Figure 1), developed based on a common bean assay (ANTONIO et al., 2008).

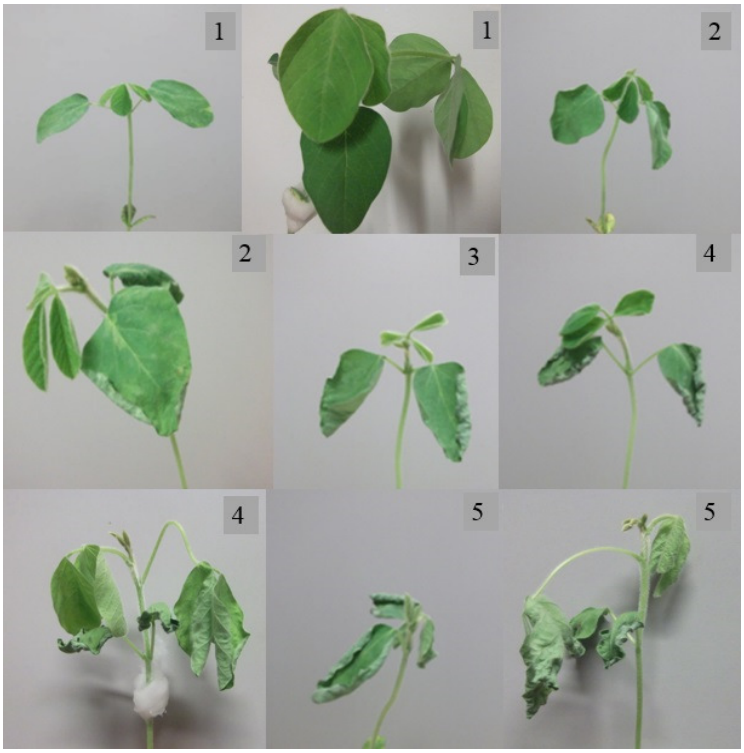

Figure 1. Examples of wilting scores assigned to soybean responses to the oxalic acid method.

Statistical analysis

The Scott-Knott test was used to make comparisons of the means for the response of the soybean genotypes to each method by using the Assistat® program.

To enable a comparison between the results obtained by the different methods, because the assessment of genotype susceptibility did not follow the same evaluation standards, the values obtained from the genotypes for each method were categorized into classes. The number of classes was defined by the square-root of the number of genotypes ($k = \sqrt{n}$, where k was the number of classes and n the number of genotypes analyzed). The data intervals for each class were defined based on the difference between the maximum and minimum values for each method, divided by the number of classes (SOARES et al., 1991), which obtained the severity index that was used to represent the individuals within each class, as shown in Table 1.

Table 1. Severity index assigned based on the assessments of oxalic acid (wilting scores) and the three traditional methods of inoculation of *Sclerotinia sclerotiorum* (%), according to the highest and lowest severities obtained from samples by each method.

Severity Index	Wilting scores for Oxalic Acid	Severities Stem method (%)	Severities Straw method (%)	Severities Detached Leaf method (%)
1	1 – 1.4	3.50 – 11.04	0.45 – 6.07	4.67 – 14.91
2	1.5 – 1.9	11.05 – 18.59	6.08 – 11.70	14.92 – 25.16
3	2.0 – 2.4	18.60 – 26.14	11.71 – 17.33	25.17 – 35.41
4	2.5 – 2.9	26.15 – 33.69	17.34 – 22.96	35.42 – 45.66

5	3.0 – 3.4	33.70 – 41.24	22.97 – 28.59	45.67 – 55.91
6	3.5 – 3.9	41.25 – 48.79	28.60 – 34.22	55.92 – 66.16
7	4.0 – 4.4	48.80 – 56.34	34.23 – 39.85	66.17 – 76.41
8	4.5 – 5.0	56.35 – 63.90	39.86 – 45.50	76.42 – 86.67

The combined analyses of the experiments were based on the homogeneity of variance, which was determined using Bartlett's test (STEEL et al., 1997). Spearman's coefficient of rank correlation (r_s) was used to measure the correspondence between (i) the results obtained for the susceptibility of the genotypes to the different methods and (ii) the ranking of the cultivars assessed by the applied methods (SOARES et al., 1991; LARSON; FARBER, 2010; RODRIGUES, 2010). Finally, the cultivars were ranked according to their average performance in the following four tests (stem inoculation method, detached leaf, response to oxalic acid and straw test).

RESULTS AND DISCUSSION

The genotypes presented different responses when exposed to all the methods (Table 2), demonstrating that all the methods used were efficient, either directly or indirectly, in differentiating between the soybean genotypes in terms of susceptibility to *S. sclerotiorum*. Regarding the OA assay, some genotypes were extremely susceptible, such as BRS 255 RR, CD 215 and FTS CAMPO MOURÃO RR, whereas others did not show high susceptibility (BMX APOLO RR and BMX FORÇA RR). A similar situation was observed in relation to the other methods, with many intermediate levels of susceptibility (Table 2).

Table 2. Results of the four inoculation methods, separated according to the differences among genotype wilting scores (oxalic acid method) and disease severities (detached leaf, non-wounded stem and straw tests).

CULTIVAR	Assessed methods (original data collected)			
	Oxalic Acid[1]*	Straw[2]*	Detached leaf[2]*	Stem[2]*
BMX APOLO RR	1.10 e	12.00 d	52.92 a	51.06 a
BMX TITÂN RR	3.30 b	9.29 d	42.09 b	31.21 b
BMX ENERGIA RR	3.20 c	8.74 d	42.92 b	34.04 b
BMX POTÊNCIA RR	2.40 d	10.12 d	29.17 c	34.49 b
BMX MAGNA RR	2.10 d	17.02 c	37.50 b	5.31 e
BMX TURBO RR	2.20 d	17.56 c	24.43 c	19.50 d
BMX ATIVA RR	2.40 d	15.54 c	18.98 c	6.23 e
BMX FORÇA RR	1.10 e	22.84 b	18.55 c	17.83 d
FTS CASCAVEL RR	2.20 d	19.71 c	46.25 a	27.15 c
FTS CAMPO MOURÃO RR	4.30 a	22.95 b	31.25 c	13.50 d
FTS FÊNIX	3.00 c	23.67 b	32.25 c	12.03 d
FTS MAUÁ RR	3.00 c	17.18 c	19.00 c	13.31 d
FTS MAMBORÊ RR	1.25 e	26.98 b	15.05 c	9.35 e
CD 202	3.70 b	20.27 c	50.59 a	12.45 d
CD 206	2.24 d	13.41 d	59.17 a	6.41 e
CD 215	4.20 a	14.27 d	37.50 b	5.40 e
CD 216	1.40 e	21.39 b	33.09 c	10.09 e
CD 221	1.70 e	19.76 c	22.00 c	5.47 e
CD 224	2.60 c	12.27 d	53.34 a	16.02 d
CD 206 RR	2.30 d	14.66 d	22.65 c	5.48 e
CD 213 RR	2.00 d	33.76 a	39.58 b	47.98 a
CD 214 RR	1.30 e	21.00 c	54.00 a	14.76 d
CD 226 RR	1.50 e	20.86 b	47.50 a	16.45 d
CD 231 RR	3.70 b	11.24 d	41.67 b	34.95 b
CD 233 RR	4.20 a	10.77 d	25.30 c	8.97 e
CD 235 RR	2.90 c	17.24 c	34.33 b	7.60 e
CD 236 RR	3.30 b	25.36 b	53.34 a	9.16 e
CD 239 RR	2.80 c	25.29 b	24.45 c	12.45 d
CD 241 RR	2.80 c	30.04 a	18.00 c	24.83 c

CD 248 RR	3.90	a	27.45	b	16.05	c	6.83 e
CD 249 RR	2.70	c	29.17	a	24.28	c	8.05 e
CD 250 RR	2.50	d	20.71	c	16.25	c	10.39 e
EMBRAPA 48	2.60	c	16.75	c	41.42	b	5.11 e
BRS 184	2.00	d	13.04	d	18.28	c	5.91 e
BRS 214	2.60	c	11.77	d	30.34	c	7.40 e
BRS 232	1.40	e	13.02	d	33.92	b	7.60 e
BRS 258	2.60	c	22.06	b	35.83	b	13.37 d
BRS 282	1.90	d	35.85	a	52.08	a	44.39 a
BRS 283	1.34	e	24.81	b	28.75	c	6.52 e
BRS 284	2.40	d	21.88	b	34.42	b	6.00 e
BRS 242 RR	1.20	e	26.67	b	47.92	a	12.40 d
BRS 243 RR	1.60	e	20.21	c	43.17	b	7.87 e
BRS 245 RR	1.70	e	23.53	b	50.42	a	14.87 d
BRS 246 RR	2.60	c	31.39	a	52.92	a	23.34 c
BRS 255 RR	4.20	a	10.84	d	33.50	b	8.84 e
BRS 256 RR	1.60	e	15.98	c	52.17	a	19.13 d

Continues

Continuation

BRS 294 RR	2.80	c	15.80	c	25.93	c	5.15 e
BRS 295 RR	2.80	c	25.17	b	40.42	b	6.26 e
BRS 316 RR	1.30	e	17.28	c	19.40	c	5.87 e
SPRING	2.40	d	20.10	c	37.00	b	11.26 d
NK 3363	1.60	e	26.51	b	17.08	c	22.27 c
SYN 3358 RR	1.20	e	18.46	c	40.83	b	16.72 d
NK 7054 RR	3.40	b	31.98	a	47.50	a	7.02 e
NK 7059 RR	3.40	b	21.67	b	34.59	b	16.62 d
SYN 1059 RR	3.30	b	25.58	b	17.48	c	9.05 e
TMG 4001 RR	3.00	c	17.23	c	60.00	a	7.10 e
TMG 1066 RR	2.00	d	25.14	b	42.09	b	14.86 d
MSOY 5942	2.00	d	19.01	c	57.50	a	12.94 d
M 6009 RR	3.30	b	16.15	c	40.84	b	9.36 e
M 6707 RR	2.90	c	22.09	b	22.34	c	5.88 e
NS 4823 RR	1.30	e	26.52	b	42.08	b	23.30 c
NA 5909 RR	2.90	c	24.03	b	56.67	a	7.88 e
NA 6411 RR	3.60	b	22.14	b	43.75	b	8.77 e
NA 4725 RR	3.50	b	15.73	c	35.42	b	8.04 e
A 7321 RR	3.90	a	25.68	b	53.75	a	19.74 d
NA 4990 RR	1.50	e	21.74	b	20.70	c	37.65 a
NS 5858 RR	3.70	b	20.57	c	24.23	c	7.82 e
NS 6636 RR	2.90	c	22.97	b	17.73	c	12.38 d
NS 6262 RR	3.10	c	28.45	a	19.10	c	19.42 d
NS 7100 RR	3.50	b	20.53	c	26.30	c	7.88 e
EXP 11631 RR	2.00	d	26.09	b	15.73	c	47.54 a
EXP 11497 RR	2.30	d	32.19	a	18.63	c	23.79 c
EXP 11920 RR	2.40	d	26.88	b	16.85	c	44.53 a
EXP 11026 RR	2.00	d	28.64	a	34.00	b	16.73 d
EXP 11900 RR	1.20	e	32.27	a	29.35	c	25.10 c
FPS JUPITER RR	2.10	d	28.15	a	29.78	c	26.20 c
FPS URANO RR	3.40	b	21.14	b	14.23	c	18.12 d
Coefficient of variation (%)	24.82		20.46		22.59		23.00

[1] – Data analysed based on wilting score index; [2] – Data analysed based on the disease severity (%); * Means followed by the same letter do not differ according to the Scott-Knott test at 5% probability.

When the severity levels were transformed into the severity index, in order to make a comparison between the methods, there was a significant difference ($p < 0.01$) in the genotype susceptibility to *S. sclerotiorum* depending on the method utilized. Considering the significant interaction ($p < 0.01$) between both factors (genotypes x methods), the level of genotype susceptibility varied according to the method of inoculation that was applied.

Although the detached leaf method presented the highest level of severity (Table 2), the

straw test showed the largest mean value between the methods for the severity index (Table 3), followed by the OA method, detached leaf and finally, the non-wounded stem inoculation method.

Table 3. Test result applied to separate the means of severity index for the four methods of inoculation used.

Inoculation methods	Severity index
Oxalic acid	3.87 b*
Straw	4.14 a*
Detached leaf	3.38 c*
Stem inoculation	2.19 d*

* Means followed by the same letter do not differ according to the Scott-Knot test at 5% probability.

The severity index for each genotype (Table 4), comparing the results of the OA method with the non-wounded stem inoculation method, revealed a higher susceptibility to the OA method for many genotypes. For example, the genotypes BMX APOLO RR, CD 213 RR, BRS 282, NA 4990 RR, EXP 11631 RR, EXP 11920 RR and EXP 11900 RR were extremely susceptible to the stem inoculation method, but did not react strongly to the OA. Both methods yielded similar results for the other genotypes.

Table 4. Results of the four inoculation methods separated according to the differences among genotypes and methods based on the severity index.

CULTIVAR	Assessed methods (severity index)											
	Oxalic acid*			Straw*			Detached leaf*			Stem*		
BMX APOLO RR	1.25	d	B	2.75	d	B	5.25	a	A	6.75	a	A
BMX TITÂN RR	5.75	b	A	2.00	d	B	4.25	b	A	4.25	b	A
BMX ENERGIA RR	5.50	b	A	2.00	d	B	4.25	b	A	4.50	b	A
BMX POTÊNCIA RR	4.50	b	A	2.25	d	B	2.75	c	B	4.75	b	A
BMX MAGNA RR	2.75	c	A	3.50	c	A	3.75	b	A	1.00	d	B
BMX TURBO RR	3.50	c	A	3.75	c	A	2.50	d	A	3.00	c	A
BMX ATIVA RR	3.25	c	A	3.50	c	A	2.00	d	B	1.00	d	B
BMX FORÇA RR	1.25	d	B	4.50	b	A	2.00	d	B	2.50	c	B
FTS CASCAVEL RR	3.00	c	A	4.00	c	A	4.50	b	A	3.50	b	A
FTS CAMPO MOURÃO RR	7.75	a	A	4.50	b	B	3.25	c	C	2.00	c	C
FTS FÊNIX	4.75	b	A	4.50	b	A	3.25	c	A	1.25	d	B
FTS MAUÁ RR	5.00	b	A	3.50	c	A	2.00	d	B	1.50	d	B
FTS MAMBORÊ RR	1.50	d	B	5.00	b	A	1.50	d	B	1.25	d	B
CD 202	5.75	b	A	4.00	c	A	5.00	a	A	1.50	d	B
CD 206	3.00	c	B	3.00	d	B	5.75	a	A	1.00	d	C
CD 215	7.50	a	A	3.00	d	B	3.50	c	B	1.00	d	C
CD 216	1.75	d	B	4.25	b	A	3.00	c	A	1.50	d	B
CD 221	2.25	d	B	3.75	c	A	2.00	d	B	1.00	d	B
CD 224	3.75	c	A	2.50	d	B	5.25	a	A	2.00	c	B
CD 206 RR	3.50	c	A	3.00	d	A	2.25	d	A	1.00	d	B
CD 213 RR	2.75	c	B	6.25	a	A	4.00	b	B	6.00	a	A
CD 214 RR	1.50	d	B	4.00	c	A	5.25	a	A	2.25	c	B
CD 226 RR	1.75	d	B	4.25	b	A	4.50	b	A	2.00	c	B
CD 231 RR	6.50	a	A	2.25	d	C	4.00	b	B	4.50	b	B

Continues

Continuation

CD 233 RR	7.25	a	A	2.25	d	B	2.75	c	B	1.25	d	B
CD 235 RR	4.75	b	A	3.50	c	A	3.25	c	A	1.00	d	B
CD 236 RR	5.50	b	A	5.00	b	A	5.50	a	A	1.25	d	B
CD 239 RR	4.25	b	A	4.75	b	A	2.50	d	B	1.50	d	B
CD 241 RR	4.25	b	A	5.50	b	B	1.75	d	B	3.25	b	B
CD 248 RR	6.50	a	A	5.25	b	A	1.50	d	B	1.00	d	B
CD 249 RR	4.00	c	A	5.50	a	A	2.25	d	B	1.25	d	B
CD 250 RR	3.75	c	A	4.00	c	A	1.75	d	B	1.50	d	B
EMBRAPA 48	3.25	c	A	3.25	c	A	4.00	b	A	1.00	d	B
BRS 184	3.50	c	A	2.75	d	A	2.00	d	B	1.00	d	B

BRS 214	2.50	d	A	2.75	d	A	3.00	c	A	1.25	d	A
BRS 232	3.50	c	A	2.50	d	A	3.25	c	A	1.00	d	B
BRS 258	1.75	d	B	4.50	b	A	3.25	c	A	1.75	d	B
BRS 282	2.25	d	B	6.75	a	A	5.00	a	A	6.00	a	A
BRS 283	1.50	d	C	5.00	b	A	3.00	c	B	1.00	d	C
BRS 284	3.25	c	A	4.25	b	A	3.50	c	A	1.00	d	B
BRS 242 RR	1.25	d	B	5.25	b	A	5.00	a	A	1.75	d	B
BRS 243 RR	2.25	d	B	4.00	c	A	4.50	b	A	1.00	d	B
BRS 245 RR	1.75	d	B	4.50	b	A	4.75	a	A	2.00	c	B
BRS 246 RR	4.25	b	B	6.00	a	A	4.75	a	B	3.25	b	B
BRS 255 RR	7.00	a	A	2.50	d	B	3.25	c	B	1.25	d	C
BRS 256 RR	1.75	d	B	3.25	c	A	4.25	b	A	2.75	c	B
BRS 294 RR	3.75	c	A	3.00	d	A	2.50	b	A	1.00	d	B
BRS 295 RR	4.75	b	A	4.75	b	A	3.75	b	A	1.00	d	B
BRS 316 RR	4.75	b	A	3.50	c	A	2.00	d	B	1.00	d	B
SPRING	1.75	d	B	3.75	c	A	3.75	b	A	1.75	d	B
NK 3363	3.50	c	B	5.00	b	A	1.75	d	B	3.00	c	B
SYN 3358 RR	1.50	d	B	3.50	c	A	3.75	b	A	2.25	c	B
NK 7054 RR	5.50	b	A	6.00	a	A	4.75	a	A	1.00	d	B
NK 7059 RR	6.00	a	A	4.25	b	B	3.25	c	B	2.25	c	B
SYN 1059 RR	5.50	b	A	4.75	b	A	2.00	d	B	1.50	d	B
TMG 4001 RR	5.00	b	A	3.50	c	B	5.75	a	A	1.00	d	C
TMG 1066 RR	2.50	d	B	4.75	b	A	4.50	b	A	2.25	c	B
MSOY 5942	3.00	c	C	4.00	c	B	5.75	a	A	1.75	d	C
M 6009 RR	5.25	b	A	3.25	c	B	4.00	b	B	1.25	d	C
M 6707 RR	4.50	b	A	4.25	b	A	2.25	d	B	1.00	d	B
NS 4823 RR	1.75	d	B	5.00	b	A	4.00	b	A	3.00	c	B
NA 5909 RR	4.25	b	A	4.50	b	A	5.75	a	A	1.00	d	B
NA 6411 RR	6.25	a	A	4.25	b	B	4.25	b	B	1.25	d	C
NA 4725 RR	5.75	b	A	3.25	c	B	3.50	c	B	1.25	d	C
A 7321 RR	6.75	a	A	4.75	b	B	5.25	a	B	2.50	c	C
NA 4990 RR	1.75	d	B	4.00	c	A	2.00	d	B	5.00	a	A
NS 5858 RR	6.25	a	A	4.00	c	B	2.50	d	C	1.25	d	C
NS 6636 RR	5.00	b	A	4.50	b	A	2.00	d	B	1.50	d	B
NS 6262 RR	4.75	b	A	5.25	b	A	2.00	d	B	2.75	c	B
NS 7100 RR	5.75	b	A	4.00	c	B	2.75	c	C	1.25	d	C
EXP 11631 RR	2.75	c	B	5.00	b	A	1.50	d	B	6.25	a	A
EXP 11497 RR	3.25	c	B	6.50	a	A	1.75	d	B	3.00	c	B
EXP 11920 RR	3.75	c	B	5.25	b	A	2.00	d	C	5.75	a	A
EXP 11026 RR	2.25	d	B	5.50	a	A	3.25	c	B	2.50	c	B
EXP 11900 RR	1.50	d	C	6.25	a	A	3.00	c	B	3.50	b	B
FPS JUPITER RR	3.00	c	B	5.50	a	A	2.75	c	B	3.50	b	B
FPS URANO RR	5.75	b	A	4.00	c	B	2.00	d	C	2.25	c	C
Coefficient of variation (%)							33.61 %					

* Means followed by the same letter do not differ according to the Scott-Knox test at 5% probability (A, B, C, D – rows; a, c, b, d – columns).

The comparison of the results of the OA method with the straw test revealed that many genotypes exhibited similar behavior for both methods (BMX APOLO RR, BMX MAGNA RR, BMX ATIVA RR, BMX TURBO RR, FTS CASCAVEL RR, FTS FÊNIX, FTS MAUÁ RR, CD 202, CD 206, CD 206 RR, CD 235 RR, CD 236 RR, CD 239 RR, CD 248 RR, CD 249 RR, CD 250 RR, EMBRAPA 48, BRS 214, BRS 184, BRS 232, BRS 284, BRS 294 RR, BRS 295 RR, BRS 316 RR, NK 7054 RR, SYN 1059 RR, M 6707 RR, NA 5909 RR, NS 6636 RR and NS 6262 RR). However, some genotypes (BMX TITAN RR, BMX ENERGIA RR, BMX POTÊNCIA RR, FTS CAMPO MOURÃO, CD 215, CD 224, CD 231 RR, CD 233 RR, CD 241 RR, BRS 255 RR, NK 7059 RR, TMG 4001 RR, M 6009 RR, NA 6411 RR, NA 4725 RR, A 7321 RR, NS 5858 RR, NS 7100 RR and FPS URANO RR) showed more susceptibility in relation to the OA method, and many other genotypes (BMX FORÇA RR, FTS MAMBORÊ RR, CD 216, CD 221, CD 213 RR, CD 214 RR, CD 226 RR, BRS 258, BRS 282, BRS 283, BRS 242 RR, BRS 243 RR, BRS 245 RR, BRS 246 RR, BRS

256 RR, SPRING, NK 3363, SYN 3358 RR, TMG 1066 RR, MSOY 5942, NS 4823 RR, NA 4990 RR, EXP 11631 RR, EXP 11497 RR, EXP 11920 RR, EXP 11026 RR, EXP 11900 RR and FPS JUPITER RR) were more susceptible in relation to the straw test.

Similarly, the comparison of the results of the OA method with the detached leaf method revealed that some genotypes exhibited similar susceptibility (BMX TITAN RR, BMX ENERGIA RR, BMX MAGNA RR, BMX FORÇA RR, BMX TURBO RR, FTS CASCAVEL RR, FTS FÊNIX, FTS MAMBORÊ RR, CD 202, CD 221, CD 224, CD 206 RR, CD 213 RR, CD 235 RR, CD 236 RR, EMBRAPA 48, BRS 214, BRS 232, BRS 284, BRS 246 RR, BRS 294 RR, BRS 295 RR, NK 3363, NK 7054 RR, TMG 4001 RR, NA 5909 RR, NA 4990 RR, EXP 11631 RR, EXP 11497 RR, EXP 11026 RR and FPS JUPITER RR). However, in relation to the OA method, many genotypes were more susceptible (BMX POTÊNCIA RR, BMX ATIVA RR, FTS CAMPO MOURÃO RR, FTS MAUÁ RR, CD 215, CD 231 RR, CD 233 RR, CD 239 RR, CD 241 RR, CD 248 RR, CD 249 RR, CD 250 RR, BRS 184, BRS 255 RR, BRS 316 RR, NK 7059 RR, SYN 1059 RR, M 6009 RR, M 6707 RR, NA 6411 RR, NA 4725 RR, A 7321 RR, NS 5858 RR, NS 6636 RR, NS 6262 RR, NS 7100 RR, EXP 11900 RR and FPS URANO RR), and many were less susceptible (BMX APOLO RR, CD 206, CD 216, CD 214 RR, CD 226 RR, BRS 258, BRS 282, BRS 283, BRS 242 RR, BRS 243 RR, BRS 245 RR, BRS 256 RR, SPRING, SYN 3358 RR, TMG 1066 RR, MSOY 5942 RR, NS 4823 and EXP 11900) than to the detached leaf method.

It was noted that the genotype response varied according to the inoculation method used, with the exception of the BMX TURBO RR, FTS CASCAVEL RR and BRS 214 genotypes, which exhibited constant levels of susceptibility (mid-low, high and low respectively) regardless of the inoculation method that was used.

In many cases the similarity between the susceptibility of the methods was observed for different genotypes, while the susceptibility of some genotypes depended on the method that was used. The inconsistence of the results obtained from the different methods of *S. sclerotiorum* inoculation has been shown in similar studies. Wegulo et al. (1998) used four different techniques to assess the susceptibility of soybean genotypes to *S. sclerotiorum* in controlled conditions (detached leaf, incidence of stem rot following mycelial inoculation of foliage, lesion lengths on stems discolored by oxalic acid, and levels of soluble pigment in stems) and they observed significant differences between the methods for the 12 cultivars that were assessed. Chen and Wang (2005) compared the results of controlled environment assays with assays conducted under field conditions and they observed the same variability between the susceptibility of the genotypes.

Comparing inoculation methodologies to field condition assays, Vuong et al. (2004) showed that the stem inoculation method using a mycelium disc (stems severed with a sterile razor blade) had a strong relationship with the field results, both in soybean, beans and sunflower. Wegulo et al. (1998) suggested that methods based on the determination of levels of soluble pigments in stems and the measurement of lesion lengths on stems discolored by OA, were better and more reliable than the mycelial inoculation of stems or foliage in evaluating soybean cultivars because of their repeatability and correlation with field results regarding resistance to *S. sclerotiorum*.

Although some studies have compared results obtained in the field with results obtained under laboratory conditions (WEGULO et al, 1998; VUONG et al, 2004; CHEN; WANG, 2005), only Wegulo et al. (1998) conducted analyses in order to show the relationship between the techniques of inoculation to distinguish genotypes. They concluded that the OA based method and detached leaf method were better than the others.

The ranking of the cultivars varied depending of the method (Table 5). It was noted that some genotypes (CD 221, BRS 284, BRS 214, FTS MAMBORÊ RR, BMX ATIVA RR, CD 206 RR, BRS 232, BMX FORÇA RR, BRS 283, CD 216 and BRS 232) demonstrated the lowest levels of susceptibility when compared to the mean ranking mean of the other cultivars. By contrast, some genotypes (NA 6411 RR, CD 202, CD 231 RR, NK 7054 RR, CD 236 RR, FTS CAMPO MOURÃO RR, CD 213 RR, BRS 246 RR, BRS 282 and A 7321 RR) presented high levels of susceptibility when compared to the other genotypes that were assessed.

According to Spearman's coefficients of rank correlation (r_s) (Table 6), which was used to measure the correspondence between the results obtained from the severity index of the genotypes in relation to the different methods, it was noticeable that there was only a small significant correlation between the straw test and the non- wounded stem inoculation method. When comparing the other methods, no correlation was observed, confirming that the severities of the genotypes varied between the methods.

Table 5. Ranks of 77 soybean cultivars (1.00 = least susceptible, 26.00 = most susceptible) by four methods of evaluation for susceptibility to *Sclerotinia sclerotiorum* (elaborated based on severity index).

Cultivars	Rank				Rank's mean
	Oxalic acid	Straw	Detached leaf	Stem	
CD 221	4	8	3	1	4.00
BRS 184	9	4	3	1	4.25
BRS 214	5	4	7	2	4.50
FTS MAMBORÊ RR	2	13	1	2	4.50
BMX ATIVA RR	8	7	3	1	4.75
CD 206 RR	9	5	4	1	4.75
BRS 232	9	3	8	1	5.25
BRS 294 RR	10	5	5	1	5.25
BMX FORÇA RR	1	11	3	7	5.50
BRS 283	2	13	7	1	5.75
					Continues
Continuation					
CD 216	3	10	7	3	5.75
BMX MAGNA RR	6	7	10	1	6.00
CD 250 RR	10	9	2	3	6.00
BRS 316 RR	14	7	3	1	6.25
SPRING	3	8	10	4	6.25
SYN 3358 RR	2	7	10	6	6.25
BRS 258	3	11	8	4	6.50
EMBRAPA 48	8	6	11	1	6.50
BRS 243 RR	4	9	13	1	6.75
BRS 284	8	10	9	1	7.00
FTS MAUÁ RR	15	7	3	3	7.00
M 6707 RR	13	10	4	1	7.00
BRS 256 RR	3	6	12	8	7.25
CD 235 RR	14	7	8	1	7.50
NA 4990 RR	3	9	3	15	7.50
BMX TURBO RR	9	8	5	9	7.75
CD 206	7	5	18	1	7.75
CD 226 RR	3	10	13	5	7.75
CD 239 RR	12	12	5	3	8.00
CD 249 RR	11	15	4	2	8.00
NS 6636 RR	15	11	3	3	8.00
BRS 245 RR	3	11	14	5	8.25
CD 214 RR	2	9	16	6	8.25
NK 3363	9	13	2	9	8.25
BRS 242 RR	1	14	15	4	8.50
CD 224	10	3	16	5	8.50
CD 233 RR	24	2	6	2	8.50
EXP 11026 RR	4	15	8	7	8.50
BMX POTÊNCIA RR	13	2	6	14	8.75
FTS FÊNIX	14	11	8	2	8.75
M 6009 RR	16	6	11	2	8.75
NA 4725 RR	18	6	9	2	8.75
NS 7100 RR	18	9	6	2	8.75
SYN 1059 RR	17	12	3	3	8.75
BRS 255 RR	23	3	8	2	9.00
FPS URANO RR	18	9	3	6	9.00
NS 4823 RR	3	13	11	9	9.00
NS 5858 RR	20	9	5	2	9.00
TMG 1066 RR	5	12	13	6	9.00
BRS 295 RR	14	12	10	1	9.25
CD 248 RR	21	14	1	1	9.25
EXP 11497 RR	8	18	2	9	9.25
EXP 11900 RR	2	17	7	11	9.25
EXP 11631 RR	6	13	1	18	9.50

MSOY 5942	7	9	18	4	9.50
CD 241 RR	12	15	2	10	9.75
FPS JUPITER RR	7	15	6	11	9.75
NS 6262 RR	14	14	3	8	9.75
BMX APOLO RR	1	4	16	19	10.00
CD 215	25	5	9	1	10.00
FTS CASCAVEL RR	7	9	13	11	10.00
TMG 4001 RR	15	7	18	1	10.25
NA 5909 RR	12	11	18	1	10.50
BMX ENERGIA RR	17	1	12	13	10.75
BMX TITÂN RR	18	1	12	12	10.75
EXP 11920 RR	10	14	3	16	10.75
NK 7059 RR	19	10	8	6	10.75
NA 6411 RR	20	10	12	2	11.00

Continues

Continuation

CD 202	18	9	15	3	11.25
CD 231 RR	21	2	11	13	11.75
NK 7054 RR	17	16	14	1	12.00
CD 236 RR	17	13	17	2	12.25
FTS C. MOURÃO RR	26	11	8	5	12.50
CD 213 RR	6	17	11	17	12.75
BRS 246 RR	12	16	14	10	13.00
BRS 282	4	19	15	17	13.75
A 7321 RR	22	12	16	7	14.25

Table 6. Spearman's rank correlation between the susceptibility of genotypes to the four different methods of inoculation (severity index).

	Oxalic Acid	**Straw**	**Detached leaf**	**Stem**
Oxalic acid	-	-0.20	-0.06	-0.20
Straw		-	-0.14	0.27*
Detached leaf			-	0.04
Stem				-

* Significant according to the Spearman's rank correlation coefficient (r_s) at 5% probability.

Although the ranking of cultivars varied among the methods, significant and higher Spearman's coefficient of rank correlation (r_s) were observed between all the methods that were utilized, demonstrating that these four methods, including the OA method, were efficient in determining differences between the genotypes to *S. sclerotiorum*, achieving very similar ranking (Table 7). Wegulo et al. (1998) used t ranking correlation analyses but did not find significant results.

Table 7. Spearman's rank correlation between the ranks of 77 cultivars assessed in four different methods of inoculation (severity index).

	Oxalic Acid	**Straw**	**Detached leaf**	**Stem**
Oxalic acid	-	0.92*	0.92*	0.89*
Straw		-	0.95*	0.95*
Detached leaf			-	0.95*
Stem				-

* Significant according to the Spearman's rank correlation coefficient (r_s) at 5% probability.

Several techniques have been developed to distinguish the susceptibility of soybean genotypes to *S. sclerotiorum*. Some of them used ascospores as inoculum source (GARCIA; JULIATTI, 2012), and many used mycelia as inoculum (AUCLAIR et al., 2004; CHEN; WANG, 2005; SAGATA, 2010; BASTIEN et al., 2012), as were three of the methods analyzed here. These methodologies require a process to obtain the inoculum to be used, which is laborious and time-consuming, especially for the methodologies that use ascospores as inoculum (KULL et al., 2003). OA based methods have been used by some authors to obtain a positive correlation with at least one traditional method that

used fungal inoculation, and they were efficient in comparing soybean genotypes (WEGULO et al., 1998), as well as many common bean genotypes (KOLKMAN; KELLY, 2000; ANTONIO et al., 2008; GONÇALVES; SANTOS, 2010).

The OA method used in the present study was efficient to compare the susceptibility of the soybean genotypes and it can be considered adequate to determine soybean genotype differences because it achieved equivalent ranking results compared to traditional methods. In addition, the OA method does not use a fungal inoculum, does not require fungal isolation, does not require medium preparation or fungal incubation, and is independent of the difference in the pathogenicity of isolates (KULL et al., 2003; LI et al., 2003). Furthermore, this method evaluated the susceptibility at 20 hours after incubation, much earlier than the other methods. These advantages make the OA method time-saving, less laborious and, consequently, less expensive than the others methods analyzed here. Thus, this study presented the potential of the OA method for pre-screening of soybean breeding material for susceptibility to white mold.

ACKNOWLEDGEMENTS

To National Council of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) and to the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA) for the scholarship and financial support (Edital 064/2008). Thanks to the companies that provided the seed samples that were used in this study.The authors would like to thank Prof. Dr. João Bosco dos Santos (UFLA) for the assistance and contribution to this study.

RESUMO: O mofo branco causado pelo fungo *Sclerotinia sclerotiorum* é uma doença importante na cultura da soja. A utilização de genótipos menos suscetíveis pode ser uma estratégia interessante no manejo desta doença, e o desenvolvimento de um método de inoculação adequado para a soja é de grande valia para a discriminação de genótipos resistentes. O presente estudo objetivou avaliar a suscetibilidade de 77 genótipos de soja baseado na reação destes ao ácido oxálico, bem como através de três métodos tradicionais (folha destacada, inoculação na haste sem ferimento e utilizando ponteiras de micropipeta), a fim de verificar os resultados obtidos com o teste do ácido oxállico e correlações entre métodos. A suscetibilidade dos genótipos ao ácido oxálico foi avaliada através de uma chave de escores de murchamento. Para os demais métodos, a severidade de ataque do patógeno foi avaliada. Para permitir a comparação entre metodologias, os resultados obtidos para os genótipos foram organizados em classes, e um índice de severidade foi utilizado para representar os individuos de cada classe. Todos os métodos foram eficientes na diferenciação de genótipos de soja quanto à suscetibilidade à *S. sclerotiorum*, entretanto, o comportamento dos genótipos variou conforme o método empregado. Não foi observada relação entre as severidades e escores de murchamento obtidos com as metodologias, porém, os ranqueamentos dos genótipos obtidos dos diferentes métodos apresentaram forte correlação. O método baseado na reação ao ácido oxálico foi o mais rápido, menos trabalhoso e de menor custo dentre os métodos utilizandos neste trabalho.

PALAVRAS-CHAVE: *Glycine max*. Patógeno. Resistência. *Sclerotinia sclerotiorum*.

REFERENCES

ADAMS, P. B.; AYERS, W. A. Ecology of *Sclerotinia* Species. **Phytopathology**, v. 69, n. 8, p. 896-899, 1979. http://dx.doi.org/10.1094/Phyto-69-896

ALEXOPOULOS, C. J.; MIMS, C. W.; BLACKWELL, M. Introductory Mycology. 4ed. John Wiley & Sons, New York, NY, USA, 1996. 868 p.

ANTÔNIO, R. P.; SANTOS, J. B.; SOUZA, T. P.; CARNEIRO, F. F. Genetic control of the resistance of common bean to white mold using the reaction to oxalic acid. **Genetics and Molecular Research**, v. 7, p. 733-740, 2008. http://dx.doi.org/10.4238/vol7-3gmr466

AUCLAIR, J.; BOLAND, G. J.; KOHN, L. M.; RAJCAN, I. Genetic interactions between *Glycine max* and *Sclerotinia sclerotiorum* using a straw inoculation method. **Plant Disease**, v. 88, p. 891-895, 2004. http://dx.doi.org/10.1094/PDIS.2004.88.8.891

BASTIEN, M.; HUYNH, T. T.; GIROUX, G.; IQUIRA, E.; RIOUX, S.; BELZILE, F. A reproducible assay for measuring partial resistance to *Sclerotinia sclerotiorum* in soybean. **Canadian Journal of Plant Sciences**, v. 92, p. 279-288, 2012. http://dx.doi.org/10.4141/cjps2011-101

BATEMAN, D. F.; BEER, S. V. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. **Phytopathology**, v. 55, p. 204-211, 1965.

BOLAND, G. J.; HALL, R. Index of plant hosts to *Sclerotinia sclerotiorum*. **Canadian Journal of Plant Pathology**, v. 16, p. 93-108, 1994. http://dx.doi.org/10.1080/07060669409500766

CHEN, Y.; WANG, D. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum*. **Plant Disease**, v. 89, p. 1268-1272, 2005. http://dx.doi.org/10.1094/PD-89-1268

FAVARON, F.; SELLA, L.; D´OVIDIO, R. Relationships among endo-polygaracturonase, oxalate, pH, and plant polygaracturonase-inhibiting protein (PGIP) in the interaction between *Sclerotinia sclerotiorum* and soybean. **Molecular Plant-Microbe Interactions**, v. 17, p. 1402-1409, 2004. http://dx.doi.org/10.1094/MPMI.2004.17.12.1402

FEHR, W. R.; C. E. CAVINESS. Stages of soybean development. Spec. Rep. 80. Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames, 1977.

GARCIA, R. A.; JULIATTI, F. C. Ranking of soybean genotypes with partial resistance from different inoculation methods of *Sclerotinia sclerotiorum* and correlation analysis between procedures. **Tropical Plant Pathology**, v. 37, p. 196-203, 2012. http://dx.doi.org/10.1590/S1982-56762012000300006

GONÇALVES, P. R. C.; SANTOS, J. B. Physiological resistance of common bean cultivars and lines to white mold based on oxalic acid reaction. **Annual Report of the Bean Improvement Cooperative**, v. 53, p. 236-237, 2010.

GUIMARÃES, R. L.; STOTZ, H. U. Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. **Plant Physiology**, v. 136, p. 3703-3711, 2004. http://dx.doi.org/10.1104/pp.104.049650

HAREL, A.; BERCOVICH, S.; YARDEN, O. Calcineurin Is Required for Sclerotial Development and Pathogenicity of *Sclerotinia sclerotiorum* in an Oxalic Acid–Independent Manner. **Molecular Plant-Microbe Interactions**, v. 19, p. 682-693, 2006. http://dx.doi.org/10.1094/MPMI-19-0682

HENNEBERG, L.; GRABICOSKI, E. M. G.; JACCOUD FILHO, D. S.; PANOBIANCO, M. Efficiency of methods to detect *Sclerotinia sclerotiorum* in commercial soybean seed lots. **Pesquisa Agropecuária Brasileira**, v. 47, p. 763-768, 2012. http://dx.doi.org/10.1590/S0100-204X2012000600005

JACCOUD FILHO D. S.; HENNEBERG, L.; GRABICOSKI, E. M. G.; WUTZKI, C. R.; PIERRE, M. L. C.; BERGUER-NETO, A.; HULLER, G. C.; TULLIO, H. E.; BARAN, C. L.; SARTORI, F. F.; NADAL, V. G.; CASTRO, R. R.; VRISMAN, C. M.; CANTELE, M. A.; MANOSSO-NETO, M. O.; JUSTINO, A.; PEREIRA, A. B.; BERUSKI, G. C.; ZADRA, M. M.; FONSECA, A. F. Strategies to management and control of the white mold (*Sclerotinia sclerotiorum*) in soybean crops. **Tropical Plant Pathology**, v. 39 (Supplement), p. 15-17, 2014.

KIM, K. S.; DICKMAN, M. B.; MIN, J. Y. Oxalic acid is an elicitor of plant programmed cell death during *Sclerotinia sclerotiorum* disease development. **Molecular Plant-Microbe Interactions**, v. 21, p. 605-612, 2008. http://dx.doi.org/10.1094/MPMI-21-5-0605

KOLKMAN, J. M.; KELLY, J. D. An indirect test using oxalate to determine physiological resistance to white mold in common bean. **Crop Science**, v. 40, p. 281–285, 2000. http://dx.doi.org/10.2135/cropsci2000.401281x

KULL, L. S.; VUONG, T. D.; POWERS, K. S.; ESKRIDGE, K. M.; STEADMAN, J. R.; HARTMAN, G. L. Evaluation of resistance screening methods for Sclerotinia stem rot of soybean and dry bean. **Plant Disease**, v. 87, p. 1471-1476, 2003. http://dx.doi.org/10.1094/PDIS.2003.87.12.1471

LARSON, R.; FARBER, B. Applied statistics. = Estatística Aplicada. 4th ed. São Paulo: Pearson Prentice Hall (in Portuguese), 2010.

LI, G. Q.; HUANG, H. C.; LAROCHE, A.; ACHARYA, S. N. Occurrence and characterization of hypovirulence in the tan sclerotial isolate S10 of *Sclerotinia sclerotiorum*. **Mycology Research**, v. 107, n. 11, p. 1350–1360, 2003. http://dx.doi.org/10.1017/S0953756203008591

LIANG, X.; LIBERTI, D.; LI, M.; KIM, Y.; HUTCHENS, A.; WILSON, R.; ROLLINS, J. A. Oxaloacetate acetylhydrolase gene mutants of *Sclerotinia sclerotiorum* do not accumulate oxalic acid, but do produce limited lesions on host plants. **Molecular Plant Biology**, v. 16, n. 6, p. 559-571, 2015.

LU, G. Engineering *Sclerotinia Sclerotiorum* Resistance in Oilseed Crops. **African Journal of Biotechnology**, v. 2, n. 12, p. 509-516, 2003. http://dx.doi.org/10.5897/AJB2003.000-1101

MARCIANO, P.; DI LENNA, P.; MAGRO, P. Oxalic acid, cell-wall degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. **Physiological Plant Pathology**, v. 22, p. 339-345, 1983. http://dx.doi.org/10.1016/S0048-4059(83)81021-2

PELTIER, A. J.; BRADLEY, C. A.; CHILVERS, M. I.; MALVICK, D. K.; MUELLER, D. S.; WISE, K. A.; ESKER, P. D. Biology, yield loss and control of Sclerotinia stem rot of soybean. **Journal of Integrated Pest Management**, v. 3, n. 2, p. 1-7, 2012. http://dx.doi.org/10.1603/IPM11033

PIERRE, M. L. C.; JACCOUD FILHO, D. S.; VRISMAN, C. M.; BERGER NETO, A.; GRABICOSKI, E. M. G.; SARTORI, F. F.; HENNEBERG, L.; CANTELE, M. A.; CANTELE, L. E.; TULIO, H. E.; HULLER, G. C. Susceptibility evaluation of different soybean's genotypes inoculated with *Sclerotinia sclerotiorum* in laboratory. **Tropical Plant Pathology**, v. 3, p. 1456, 2011.

RODRIGUES, W. C. Applied statistics. = Estatística Aplicada. 8th ed. Rio de Janeiro: Creative Common License (in Portuguese), 2010, 61p.

SAGATA, E.; JULIATTI, F. C.; SILVA, C. L.; REZENDE, A. A.; CAIRES, A. M. Reaction of soybean genotypes for resistance to *Sclerotinia sclerotiorum*. **Tropical Plant Pathology**, v. 35, p. 205, 2010.

SAHARAN, G. S.; MEHTA, N. Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management. Springer, 2008, 486p. http://dx.doi.org/10.1007/978-1-4020-8408-9

SCHWARTZ, H. F.; SINGH, S. P. Breeding Common Bean for Resistance to White Mold: A Review. **Crop Science**, v. 53, p. 1832-1844, 2013. http://dx.doi.org/10.2135/cropsci2013.02.0081

SOARES, J. F.; FARIAS, A. A.; CESAR, C. C. Introduction to Statistics. = Introdução à Estatística. 1st ed. Guanabara Koogan S. A. Rio de Janeiro (in Portuguese), 1991, 378p.

STEADMAN, J. R. White mold: a serious yield-limiting disease of bean. **Plant Disease**, v. 67, p. 346 – 350, 1983. http://dx.doi.org/10.1094/PD-67-346

STEEL, R. G. D.; TORRIE, J. H.; DICKEY, D. A. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill, New York, 1997, 666p.

TERÁN, H.; LEMA, M.; SCHWARTZ, H. F.; DUNCAN, R.; GILBERTSON, R.; SINGH, S. P. Modified Petzoldt and Dickson scale for white mold rating of common bean. **Annual Reports - Bean Improvement Cooperative**, v. 49, p. 115–116, 2006.

VUONG, T. D.; HOFFMAN, D. D.; DIERS, B. W.; MILLER, J. F.; STEADMAN, J. R.; HARTMAN, G. L. Evaluation of Soybean, Dry Bean, and Sunflower for Resistance to *Sclerotinia sclerotiorum*. **Crop Science**, v. 44, p. 777-783, 2004. http://dx.doi.org/10.2135/cropsci2004.0777

WALZ, A.; ZINGEN-SELL, I.; THIESEN, S.; KORTEKAMP, A. Reactive oxygen intermediates and oxalic acid in the pathogenesis of the necrotrophic fungus *Sclerotinia sclerotiorum*. **European Journal of Plant Pathology**, v. 120, p. 317-330, 2008. http://dx.doi.org/10.1007/s10658-007-9218-5

WEGULO, S. N.; YANG, X. B.; MARTINSON, C. A. Soybean cultivar responses to *Sclerotinia sclerotiorum* in field and controlled environment studies. **Plant Disease**, v. 82, p. 1264–1270, 1998. http://dx.doi.org/10.1094/PDIS.1998.82.11.1264