A set of standard area diagrams to assess white mold severity on the leaflets of common beans

Inaia Rhavene Freire Fagundes-Nacarath, Daniel Debona, Maria Izabel Costa Novaes, Fabrício Ávila Rodrigues*

Universidade Federal de Viçosa, Departamento de Fitopatologia, Laboratório da Interação Planta-Patógeno, Viçosa, 36570-900, Minas Gerais State, Brazil

ABSTRACT

The fungus Sclerotinia sclerotiorum is the major pathogen affecting common beans yield worldwide and an adequate disease quantification is demanded in some trials. However, a set of standard area diagrams (SADs) to aid visual assessment of severity of white mold (SWM) is lacking. This study developed SADs consisting of eight color images of diseased leaflets with severity values that ranged from 0.4 to 53.7%. Twenty raters [10 experienced (ER) and 10 inexperienced (IR)] validated the SADs by assessing the same set of 50 images twice, the first without SADs and the second using it as an aid. The SADs significantly improved both accuracy and precision for IR as evidenced by increases from 0.86 to 0.98 in the coefficients of bias (C_b) from 0.93 to 0.98 in correlation coefficient (r) and from 0.86 to 0.96 in overall agreement [Lin's concordance correlation coefficients (ρ_c)] without and with SADs, respectively, whereas for ER only precision (r) was improved by SADs. The SWM estimates were also more reliable because inter-rater reliability (coefficient of determination, R^2) was significantly increased for both ER and IR by using SADs. Therefore, the SADs presented is thought to be a valuable tool to provide accurate, precise and reliable estimates of the SWM on common bean leaflets in epidemiological studies, evaluation of disease control methods, assessment of aggressiveness of pathogen isolates, disease resistance and other types of surveys regarding the common beans-S. sclerotiorum interaction.

1. Introduction

The fungus Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of white mold, is one of the most devastating and cosmopolitan pathogens worldwide (Bolton et al., 2006). The pathogen has a wide range of hosts, infecting over 400 plant species from several botanical families, including economically important crops such as common beans and soybeans (Boland and Hall, 1994). The annual losses caused by S. sclerotiorum reach millions of dollars, causing yield losses ranging from 20 to 100% (Steadman and Boland, 2005; Bolton et al., 2006; Schwartz and Singh, 2013).

In common beans, white mold symptoms begin as wilting; afterwards, leaflets, stems and pods display water-soaked spots that are light-brown in color and soft in consistency and quickly expands forming necrotic lesions, on which a cottony fungal mycelium can be observed (Purdy, 1979; Steadman, 1983). White mold epidemics are favored by high plant density, long rainy periods, mild temperatures (< 20 °C) and high relative humidity (> 70%) (Purdy, 1979). White mold can occur at any growth stage of common beans plants, but the flowering growth stage is the most critical period for disease epidemics; at this phase, the high foliar index makes the microclimate highly favorable to S. sclerotiorum (Steadman, 1983).

Some control methods including chemical (Mueller et al., 2002; Vieira et al., 2012), genetic (Lehner et al., 2015), cultural and biological control (Singh and Schwartz, 2010) have been adopted for white mold management. Disease quantification plays a critical role to assess the efficacy of methods for disease control (Madden et al., 2007) and must be accurate, precise and reliable (Nutter and Schultz, 1995; Madden et al., 2007). Accuracy measures the degree of closeness between the estimates and the actual severity (Nutter and Schultz, 1995; Madden et al., 2007); precision is a measure of similarity within estimates/measurements obtained by the same rater (intra-rater precision or reliability) (Madden et al., 2007) as well as under different conditions, raters or methods (inter-rater precision or reproductibility) (Nutter et al., 1993; Madden et al., 2007).

The standard area diagram (SAD) is a standardized method to disease quantification and it has been used as a tool to estimate disease severity, an essential variable in phytopathometry (Del Ponte et al.,

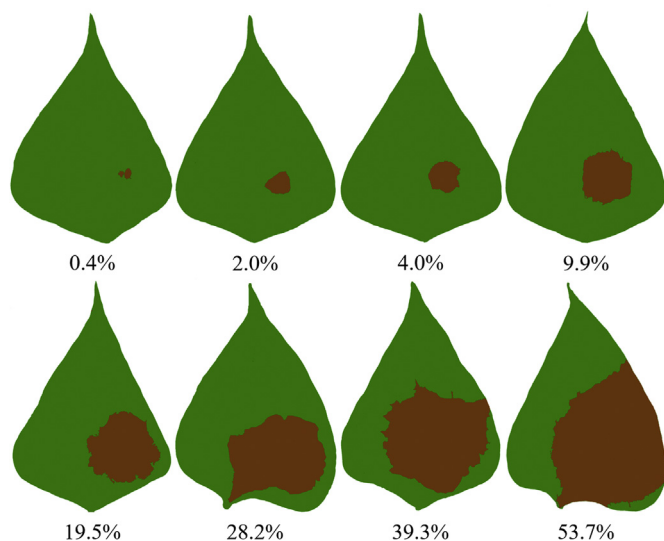**Fig. 1.** Symptoms of white mold on common bean leaflets inoculated with *Sclerotinia sclerotiorum*.

Fig. 2. Set of standard area diagrams (SADs) developed as an aid for assessment of white mold severity on common beans leaflets. Values represent the percentage (%) of the leaflet area showing white mold symptoms.

2017). The use of the SADs has demonstrated that inexperienced raters are more benefited in terms of accuracy and precision when compared with estimates made without SADs (Duarte et al., 2013; Rios et al., 2013; Braido et al., 2014; González-Domínguez et al., 2014; Vieira et al., 2014; Debona et al., 2015; Dolinski et al., 2017). Data analysis of SADs efficacy can be performed by linear regression or Lin's concordant correlation coefficient (LCCC) method (Lin, 1989; Madden et al., 2007; Bock et al., 2010). The meta-analysis of 105 studies (127 SADs) over the last 25 years of the scientific impact of the SADs demonstrated that there is a current trend to use LCCC analysis (Del Ponte et al., 2017), which was suggested to be a more robust statistic than the linear regression method (Bock et al., 2010; Del Ponte et al., 2017).

The white mold intensity has been estimated mostly through incidence (Hoffman et al., 1998; Yang et al., 1999; Del Río et al., 2007; Lehner et al., 2017), but severity is assessed in some cases (Madden et al., 2007). Although severity is commonly estimated using an ordinal rating scale (Hall and Phillips, 1996; Kolkman and Kelly, 2002), this method is little informative and can have a major impact on data analysis (Madden et al., 2007). Some studies regarding the efficacy of measures for white mold control, aggressiveness of *S. sclerotiorum* isolates and other aspects of the disease in different hosts have been performed through pathogen inoculation on the leaflets under controlled conditions (Lehner et al., 2016; Arfaoui et al., 2016). SADs have been employed to assess the resistance of soybean cultivars to white mold, but such SADs were not validated (Garcia and Juliatti, 2012). The lack of a standardized and validated method for disease quantification may result in inaccurate disease assessment, thus leading to mistaken conclusions and recommendations, with catastrophic consequences for the growers (Madden et al., 2007).

Considering that some experiments have been carried out under controlled conditions by inoculating common beans leaflets with *S.*

Table 1

Effect of the use of a set of standard area diagrams (SADs) as an assessment aid on bias, precision and agreement of estimates of severity of white mold (SWM) on common bean leaflets made by raters with or without experience in SWM assessment.

Experience	LCC statistic	Means		95% CI[a] of the difference between means
		No SADs	With SADs	
Inexperienced	Scale shift (v)[b]	1.08	1.05	−0.159, 0.120
	Location shift (μ)[c]	0.30	0.15	−0.363, 0.100
	Bias correction factor (C_b)[d]	0.86	0.98	**0.062, 0.181**
	Correlation coefficient (r)[e]	0.93	0.98	**0.022, 0.080**
	Concordance coefficient (ρ_c)[f]	0.81	0.96	**0.088, 0.231**
	Inter-rater coefficient of determination (R^2)[g]	0.77	0.92	**0.117, 0.182**
Experienced	Scale shift (v)	1.03	1.04	−0.046, 0.073
	Location shift (μ)	0.12	0.18	−0.038, 0.148
	Bias correction factor (C_b)	0.97	0.98	−0.008, 0.031
	Correlation coefficient (r)	0.96	0.98	**0.011, 0.026**
	Concordance coefficient (ρ_c)	0.94	0.96	−0.008, 0.031
	Inter-rater coefficient of determination (R^2)	0.83	0.95	**0.005, 0.053**

[a] Bootstrap calculated difference between means and confidence intervals (CIs). If the CIs embrace zero, difference is not significant at the 5% level. Bold numbers represent significance of the difference.

[b] Scale or slope shift (systematic bias) relative to the perfect relationship (1 = perfect relation between x and y).

[c] Location or height shift (constant bias) relative to the perfect relationship (0 = perfect relation between x and y).

[d] Bias correction factor that measures how far the best-fit line deviates from a line at 45°. No deviation from the 45° line occurs when $C_b = 1$. C_b is calculated from v and μ and is a measure of accuracy.

[e] Correlation coefficient (r) that measures precision.

[f] Lin's concordance correlation coefficient (ρ_c) combines both precision (r) and accuracy (C_b) ($\rho_c = r$. C_b) to measure agreement with the actual value (Lin, 1989).

[g] Mean coefficients of determination estimated from pairwise comparisons of assessments by visual raters.

sclerotiorum, and the lack of SADs to assess the severity of white mold (SWM), the present study aimed: i) to develop SADs to quantify SWM in common bean leaflets and ii) to determine the effect of SADs and rater experience in accuracy, precision and reliability of the estimates of SWM.

2. Materials and methods

2.1. Plant growth

A total of five seeds of common beans from cultivar Pérola, susceptible to *S. sclerotiorum*, were sown in plastic pots of 2 L containing 2 kg of Tropstrato® substrate (Vida Verde, Mogi Mirim, São Paulo, Brazil) composed of a mixture of pine bark, peat and expanded vermiculite (1:1:1). Each pot was thinned to three seedlings seven days after emergence. Plants were kept in a greenhouse (30 ± 5 °C, 65 ± 5% relative humidity and natural photosynthetically active radiation of 900 ± 15 µmol photons m^{-2} s^{-1}). Plants were fertilized weekly with 100 ml of a modified nutrient solution based on Clark (1975) as it follows: 0.8 mM KNO$_3$, 0.069 mM NH$_4$H$_2$PO$_4$, 1 mM NH$_4$NO$_3$, 1 mM Ca(NO$_3$).4H$_2$O, 0.9 mM KCl, 0.6 mM MgSO$_4$ 7H$_2$O, 0.5 µM CuSO$_4$ 5H$_2$O, 2 µM ZnSO$_4$ 7H$_2$O, 19 µM H$_3$BO$_3$, 7 µM MnCl$_2$