

Validation of Diagram Scale for Leaf Spot Caused by *Pestalotiopsis* sp. in Coconut Seedlings

Aline Figueiredo Cardoso

Plant Protection Laboratory, Institute of Agrarian Sciences, Federal Rural University of Amazon, CEP.066.077-830.Belém, Pará Brazil. E-mail: aline_f_cardoso@hotmail.com

Fernanda Sarmiento de Oliveira Louzano

Plant Protection Laboratory, Institute of Agrarian Sciences, Federal Rural University of Amazon, CEP.066.077-830.Belém, Pará Brazil. E-mail: feehsarmiento@gmail.com

Cássia Cristina Chaves Pinheiro

Plant Protection Laboratory, Institute of Agrarian Sciences, Federal Rural University of Amazon, CEP.066.077-830.Belém, Pará Brazil. E-mail: cassiapinheiro002@gmail.com

Paulo Manoel Pontes Lins

Sococo Agroindústria da Amazônia AS, Distrito Industrial Q1 setor A lote 6-10 - Cep: 67.033-310 Santa Isabel, Pará Brasil. E-mail: paulom@sococo.com.br

Gisele Barata da Silva

Plant Protection Laboratory, Institute of Agrarian Sciences, Federal Rural University of Amazon, CEP.066.077-830.Belém, Pará Brazil. E-mail: giselebaratasilva@gmail.com
(Corresponding Author)

Received: Oct. 15, 2019

Accepted: Nov. 18, 2019

Published: Nov. 26, 2019

doi:10.5296/jas.v8i2.15621

URL: <https://doi.org/10.5296/jas.v8i2.15621>

Abstract

The absence of a methodology to quantify *Pestalotiopsis* sp. in coconut plants (*Cocos nucifera* L.) justified the elaboration and validation of a diagrammatic scale containing seven values (2%, 5%, 10%, 15%, 20%, 40% and 60%) of disease severity, in a sampling under

natural conditions of occurrence in the field. To elaborate the diagrammatic scale, 100 leaves of *Cocos nucifera* L. were obtained from an experimental field of Fazenda Reunidas Sococo in Santa Isabel, Pará. The images of these leaves were obtained with the aid of a digital camera. Subsequently, with the aid of Assess 2.0 APS software, the actual disease severity values were obtained in percentage terms. The validation of the proposed diagrammatic scale was performed by ten inexperienced evaluators who evaluated the projected images without the aid of the suggested scale, and later, with the aid of the scale. From the data obtained from the evaluators with and without scale, linear regression analysis was performed, relating the real severity and the estimated severity. The accuracy of the estimates was evaluated by the regression determination coefficient (R^2) and the variance of the absolute errors. The accuracy of each evaluator was determined by the t-test applied to the angular coefficient of line (b) and the linear coefficient of line (a), both obtained by linear regression. Through the coefficient of determination values, It is possible to verify that the use of the scale gave greater accuracy to 100% of the evaluators with an average of 0.94 and 94% repeatability. The use of the diagrammatic spot scale for *Pestalotiopsis* sp. it enables future work with accuracy and precision, as well as optimizing disease control practices within a coconut tree nursery management program.

Keywords: *Cocos nucifera*, pathometry, disease

1. Introduction

Coconut palm (*Cocos nucifera* L.) is considered a rustic plant, grown in several countries, being the largest producers Indonesia, Philippines, India and Brazil. Brazil is the fourth largest producer, highlighting the states of Bahia (30, 3%), Sergipe (13.2%), Pará (11%) and Ceará (10.4%) with their respective contributions to national production. The state of Pará is the third national producer, with production of 197,387 thousand fruits (IBGE, 2017).

Coconut cultivation has significant importance in the contribution of the Brazilian economy, through the generation of jobs along its entire production chain to industry (CUENCA, 2001). One of the main advantages in its cultivation is that it can be originated several products of high economic value focused on food, cosmetics, crafts and others (SIQUEIRA, 2002). In the coconut production system there are factors that can hinder its development, we highlight the phytosanitary responsible for damage and significant economic loss (WARWICK, 1989). Leaf spotting occurs mainly in adult plants, such as leaf burning caused by *Lasiodiplodia theobromae* (Pat.) (Griffo; Maubl), small sandpaper caused by *Phyllachora torrendiella* (Batista) Subileau, and large sandpaper whose etiological agent is *Sphaerodothis acrocomiae* (Montagne) von Arx; Muller (WARWICK; LEAL, 2002).

In the production of seedlings we highlight the leaf spots caused by the fungus *Pestalotiopsis* sp., This genus can have three central cells of dark color and two cells of the light colored extremities and can have simple or branched appendages in the apical cell and a simple appendix in the basal cell (KARAKAYA, 2001).

In the leaves, symptoms are evident in the form of rounded elliptic lesions with defined edges, dark in color and between 3 and 5mm in size. With the expansion, the lesions showed reddish

brown discolourations and, subsequently, the drying of the rachis areas (CARDOSO et al., 2003).

For leaf disease severity studies, one of the proposed tools is the use of a diagrammatic scale, which consists of illustrated representations of parts of a plant with symptoms at different levels of severity (BERGAMIN FILHO; AMORIM, 1996). The use of scale may result in a better understanding of epidemiological studies and may propose effective control strategies giving subsidies for quantification (HALFELD-VIEIRA; NECHET, 2006; TROJAN; PRIA, 2018). The climatic conditions of the Amazon region, with temperatures high and high relative air units may favor the evolution of disease symptoms, resulting in the need to obtain tools that support the management of this disease. The diagrammatic scale assists in decision making (MORAES et al., 2011).

The objective was to elaborate and validate a diagrammatic scale to estimate the severity of *Pestalotiopsis* sp. in leaves of coconut palm seedlings to standardize the assessment of disease severity.

2. Methods

Location and sample collection

The experiment was conducted at the Reunidas Sococo farm located in Santa Izabel - PA ($1^{\circ} 13'26''$ S and $48^{\circ} 02'29''$ W), in partnership with the LPP plant protection laboratory of the Federal Rural University of Amazonia. -UFRA ($1^{\circ} 27'31''$ S and $48^{\circ} 26'04.5''$ O).

One hundred leaves of coconut seedlings were collected, with different values of area affected by leaf spots, randomly (Figure 2). These sheets were taken to the laboratory, where they were selected and photographed. Then, each leaf was analyzed according to the injured area, using the Assess 2.0 APS program (LAMARI, 2008), where the actual disease severity values were obtained in percentage terms. From this, the coconut leaves with the lowest number of lesions and the highest number were determined, thus establishing the lower and upper limits of the diagrammatic scale, respectively. Intermediate levels of the scale must respect the limitations of human visual acuity defined by the Weber-Fechner stimulus-response law, in which visual acuity is proportional to the stimulus intensity logarithm (HORSFALL; COWLING, 1978). From the values obtained, the diagrammatic scale was established (MARTINS et al., 2004).

Scale Validation

For the scale to be validated, leaf images with different values of affected area were presented to different evaluators. In the first phase, 10 inexperienced evaluators evaluated the projected images without the aid of the suggested scale, in the second phase inexperienced evaluators evaluated the images with the aid of the scale. The data estimated by the evaluators were tabulated and subsequently compared with the actual severity data obtained with the aid of the Assess 2.0 program.

Statistical analysis

From the data obtained from the evaluators with and without scale, linear regression analysis was performed, considering the real severity as an independent variable and the estimated severity as the dependent variable. The accuracy of the estimates will be assessed by the regression determination coefficient (R^2) and the variance of the absolute errors (estimated severity minus actual severity). The accuracy of each evaluator will be determined by the t-test applied to the angular coefficient of line (b) and the linear coefficient of line (a), both obtained by linear regression (real severity x estimated severity) (MARTINS et al., 2004; LAMARI, 2008).

3. Results and Discussion

The minimum and maximum values of *Pestalotiopsis* sp. found in the samples were 2% and 60% respectively (Figure 3). The scale was developed with seven severity values to represent the highest frequency ranges according to the values found in the field. Symptom representation includes only necrotic tissue or chlorotic spots. Symptoms are observed in fully expanded leaves, with the occurrence of brown spots on the edge of the leaf, which, when the disease progresses in the tissue, is blackened without a certain shape, with rounded shape defined. In the elaborated diagrammatic scale the values that corresponded to the necrotic leaf area were 2, 5, 10, 15, 20, 40 and 60% (Figure 1).

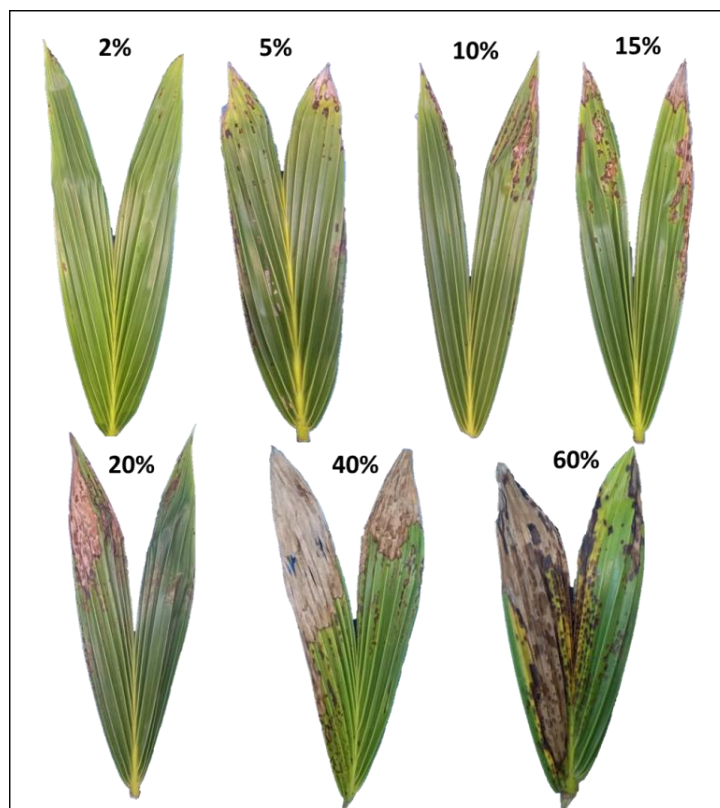


Figure 1. Diagrammatic scale for spot on leaves of coconut palm seedlings caused by *Pestalotiopsis* sp. according to the Assess 2.0 program, Santa Isabel – PA

Accuracy is defined as the accuracy of a systematic error-free measurement (BERGAMIN FILHO; AMORIM, 1996), evaluating the angular coefficient (b) and intercept of linear regression (a) between actual and estimated severity, and it is expected that the slope. The linear regression between real and estimated values must be equal to 1, without systematic deviations, and the intercept must be equal to 0 (NUTTER et al, 1993).

When the severity of leaf spot caused by *Pestalotiopsis* sp. in coconut palm seedlings was estimated without the aid of the scale, seven evaluators were poorly accurate, and the evaluators 1, 2, 5, 6, 7 and 8 presented intercept values significantly different from zero ($P \leq 0.01$) and Evaluator 9 presented an angular coefficient significantly different from 1 ($P < 0.01$) (Table 1).

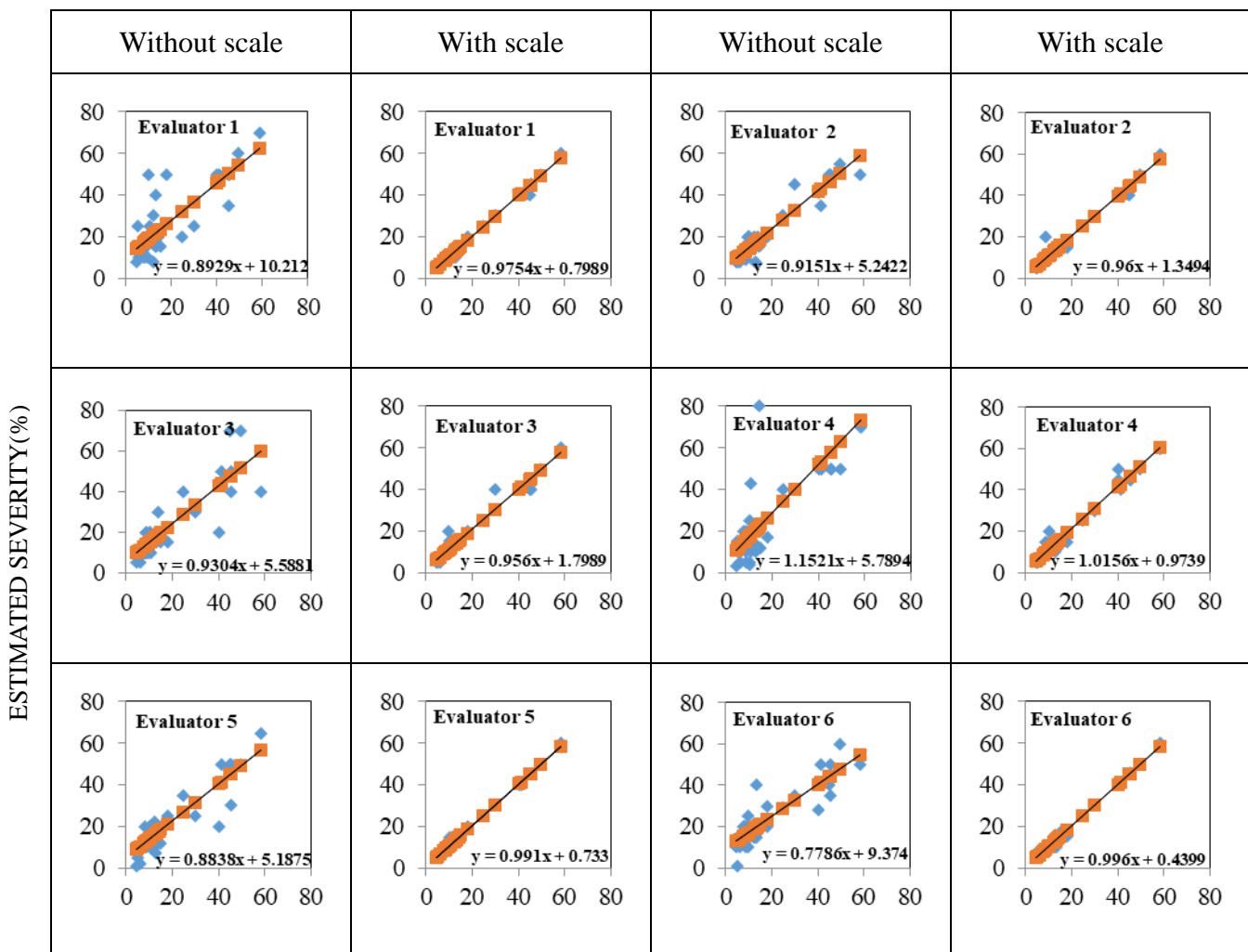
For the validation of diagrammatic scales it is reported that there is a tendency to overestimate values, which may have occurred with non-scale evaluators (SPÓSITO et al., 2004; HALFELD-VIEIRA; NECHET, 2006), this fact is proven. when the evaluators used the proposed scale, because the values of the intercept (a) did not differ from zero and neither did the angular coefficient values differ from 1 for any of the evaluators ($P \leq 0.01$) (Table 1).

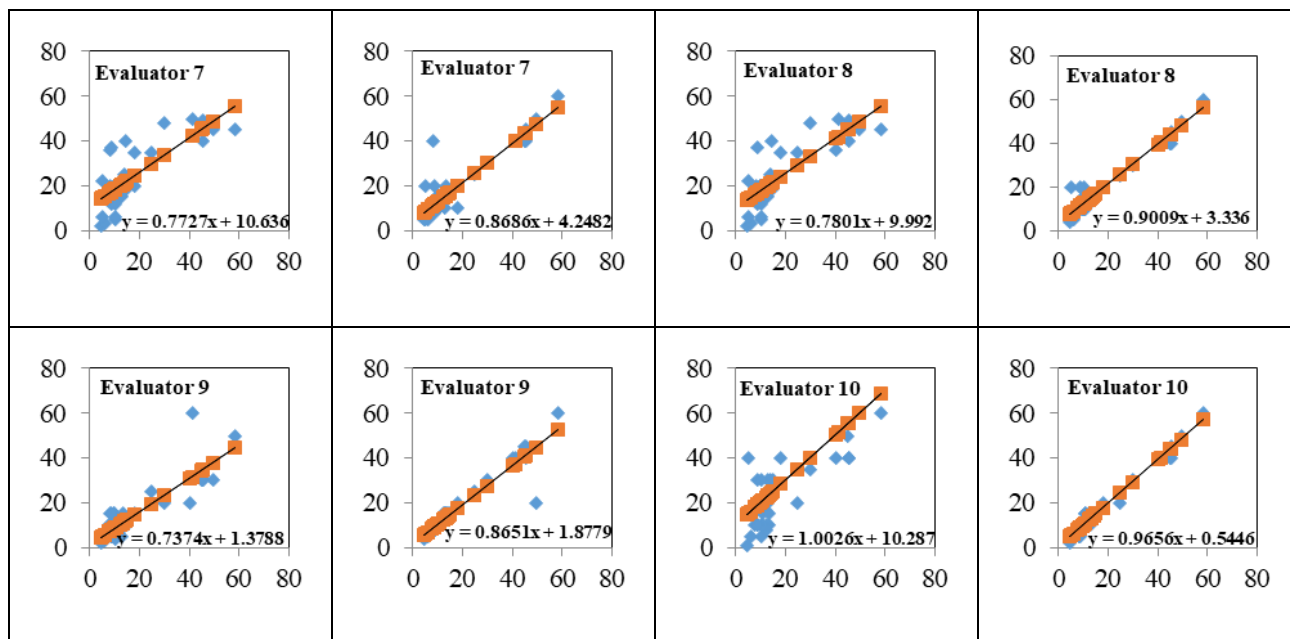
Table 1. Intercept (a), angular coefficient (b) and coefficient of determination (R^2) of the linear regressions between the severity obtained between the actual and the proposed estimated severity of the different evaluators without the aid of scale and the aid of scale

Evaluator	Without			With			
	a	b	R^2	Evaluator	a	b	R^2
1	10,21*	0,89	0,65	1	0,79	0,97*	0,99
2	5,24*	0,91	0,89	2	1,34	0,96*	0,97
3	5,58	0,93	0,72	3	1,79	0,95	0,96
4	5,78	1,15	0,59	4	0,97	1,01	0,96
5	5,18*	0,88	0,79	5	0,73	0,99	0,99
6	9,37*	0,77	0,76	6	0,54	0,96	0,99
7	10,63*	0,77	0,63	7	4,24	0,86	0,80
8	9,99*	0,78	0,68	8	3,33	0,90	0,93
9	1,37	0,73*	0,73	9	1,87	0,86	0,88
10	10,28	1,00	0,39	10	0,54	0,96*	0,97
Average	7,36	0,88	0,68		1,61	0,94	0,94

* situations in which the null hypothesis ($a = 0$ or $b = 1$) was rejected by the t test ($P < 0.01$).

The accuracy of a proposed disease quantification scale is defined as the accuracy of an operation where the measurement is accurate or refined, and can be assessed by regression determination coefficient (R^2), acceptable values close to 1, and by variation of absolute errors, obtained by the difference between estimated and real severities (NUTTER JUNIOR; SCHULTZ, 1995; BERGAMIN FILHO; AMORIM, 1996). In the present study it was found that without the use of the scale the determination coefficients obtained by the evaluators ranged from 0.39 to 0.89 with an average of 0.68, and with the use of the scale the determination coefficients ranged from 0.80 to 0.99 with an average of 0.94 (Table 1). Through the coefficient of determination values, it is possible to verify that the use of the scale gave greater accuracy to all evaluators (Figure 4). Some authors recommend training with evaluators to reduce disease overestimation, as human visual acuity detects more diseased tissue than healthy tissue (HOSFALL; BARRATT, 1945).





REAL SEVERITY (%)

Figure 4. Estimated severity of leaf spots caused by *Pestalotiopsis* sp. in coconut palm seedlings by 10 inexperienced evaluators without and as a help of the scale and regression equations obtained between the real severity (red full points) and the estimated severity (blue full points)

Absolute errors, the difference between estimated and actual severity absolute values, show that there was a reduction with the proposed scale. The absolute error without scale ranged from -14.59 to 29.66%, and using the scale ranged from -6.59 to 9.13% (Figure 5). Scale error values are considered to be within acceptable standards -10% and + 10% for disease assessment (TOMERLIN; HOWELL, 1988; NUTTER JUNIOR; WORAWITLIKIT, 1989).

From the analyzes it was verified good repeatability of the estimates, where the average amount of variation of the non-scaled evaluators is explained by the scaled evaluation in 94%. In three raters, the angular coefficient was significantly different from 1 ($P < 0.01$) (Table 1). Similar results were found on the scale for *Cercospora* patches in watermelon, with an estimated severity of 96% on average (HALFELD-VIEIRA; NECHET, 2006).

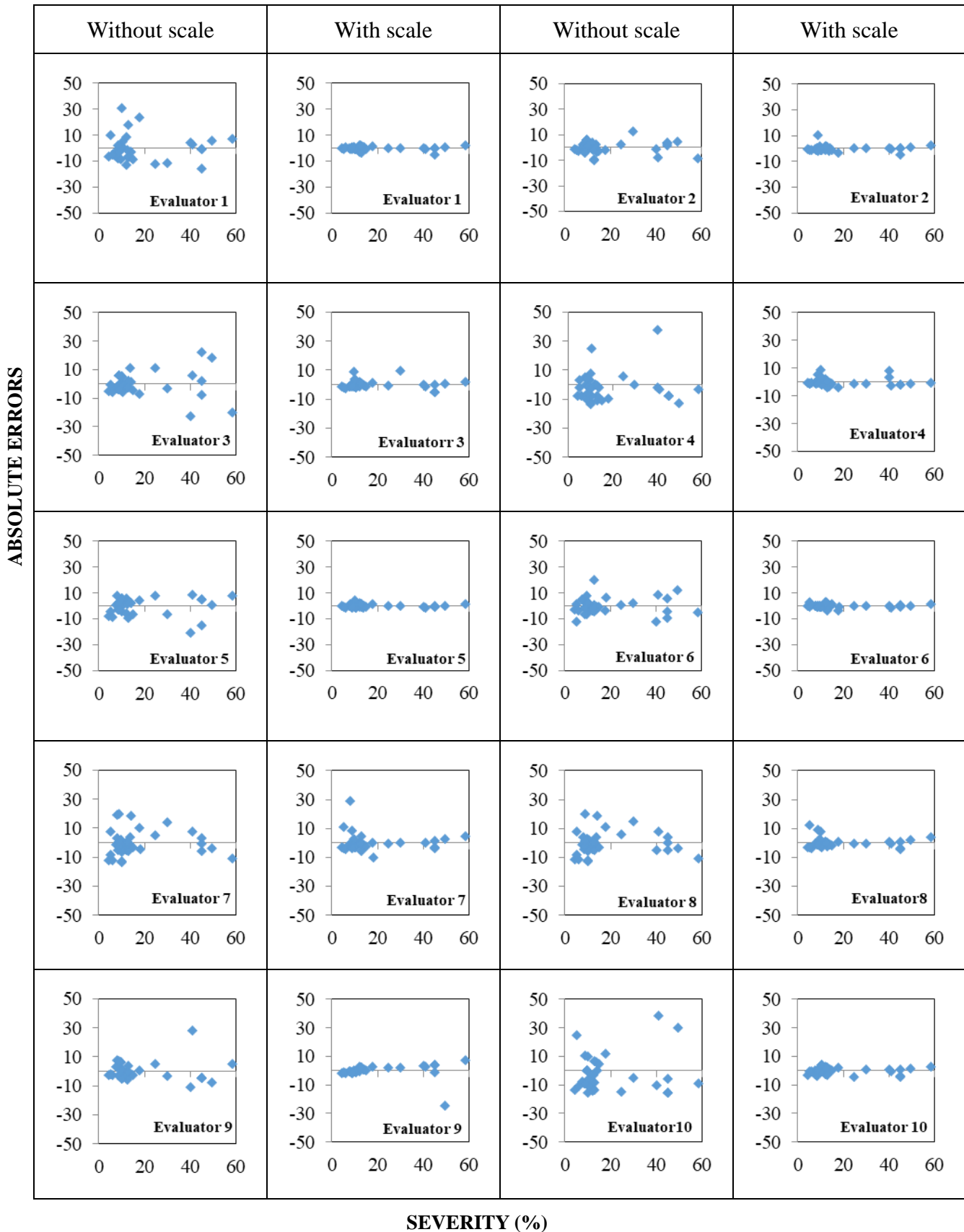


Figure 5. Absolute errors (estimated severity minus actual severity) for 10 raters without and with the aid of leaf spot scale caused by *Pestalotiopsis* sp. in coconut palm seedlings

4. Conclusion

The accuracy, precision and reproducibility levels of *Pestalotiopsis* sp. spot severity measurements in Brazilian dwarf coconut palms improve with the use of the proposed diagrammatic scale, which proves to be a useful tool for severity assessments. The diagrammatic scale allows decision making and optimization of coconut tree nursery management program.

Acknowledgment

We acknowledge the Federal Rural University of Amazonia and the Plant Protection Laboratory for the structure provided, and for the partnership and financial support from FAPESPA, CAPES and Sococo S/A.

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