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CROP SCIENCE

Diagrammatic scale for quantification of severity of white thread blight disease in yerba mate (*Ilex paraguariensis* Saint Hilaire)

MANUELA L. VERESCHUK, FACUNDO G. DOMINGUEZ, ADRIANA E. ALVARENGA & PEDRO D. ZAPATA

Abstract: The yerba-mate industry is one of the most important economic activities in Misiones, a province in the northeast of Argentina that is the world's leading producer and exporter of this crop. White thread blight disease caused by *Ceratobasidium niltonzousanum* affects the cultivation reducing its quality and productivity. Due to the lack of a standardized visual method to quantify the severity of this disease in yerba mate, a diagrammatic scale was developed and validated. Yerba-mate branches were collected in a field in the north of Misiones province, and the actual severity was determined digitally. A six-level scale was developed using the DOSLOG software, based on the Weber-Fechner law. The validation was carried out by twenty raters. One evaluation without the diagrammatic scale and two evaluations with the scale were carried out in 14-day intervals. Accuracy, precision, and reproducibility of the scale were evaluated through linear regressions and correlation analysis, obtaining R^2 values ranged between 0.70 and 0.94. Using the diagrammatic scale developed in this work, raters enhanced the accuracy and precision of the estimates, and the repeatability of the scale improved by 94.74%. The scale was appropriate to assess the damage of white thread blight in yerba mate.

Key words: *Ceratobasidium niltonzousanum*, phytopathology, phytopatometry, Weber-Fechner law.

INTRODUCTION

The yerba-mate (*Ilex paraguariensis* St. Hil.) industry is one of the most important economies in Misiones, a province in the northeast of Argentina that is the world's leading producer and exporter of this crop (López et al. 2020). Native specimens of this evergreen tree can measure up to 30 meters and are part of understory. Under monoculture conditions, it is maintained as a shrub by regular pruning of its branches and leaves, which are consumed as an infusion (Heck & Mejía 2007, Pérez 2016).

White thread blight disease, caused by the fungus *Ceratobasidium niltonzousanum*, affects the cultivation of yerba mate in the north of the province, reducing its quality and the productivity (Lima et al. 2020). The first symptoms are observed in the middle part of the plant. White mycelial threads extend longitudinally along the surface of the stem and branches, and later become leaf blight, growing up on the underside of leaves against sun exposure. Infected leaves gradually turn dark brown; then, blighted leaves remain hanging from strong threads of fungal hyphae. Around affected branches, the fungus produces a compact agglomeration of brown hyphae called sclerotia, which are structures of resistance over long periods of time (Agostini et al. 2014).

Measuring the intensity of a disease is an indispensable requirement in basic epidemiological studies (Kranz 1988, Campbell & Madden 1990). Defined criteria including damage assessment methods that can be easily applied and interpreted are needed to estimate a correlation between disease intensity and reduced crop yield (French & Hebert 1980). The use of scales is the most widely standardized method to assess disease damage. Logarithmic diagrammatic scales are sets of illustrations of plants or plant organs with symptoms that show the percentages of area affected by the disease based on the Weber-Fechner principle, which allows establishing classes based on a logarithmic system that eliminates the arbitrary designation of classes or levels of diseases (French & Hebert 1980, Bergamin & Amorim 1996, Tovar-Soto et al. 2002). These scales should be simple and quick to use under different conditions, as well as accurate, precise and reproducible (Berger 1980).

Three important aspects must be considered when developing a diagrammatic scale: 1) the upper limit of the scale must correspond to the maximum intensity of the disease observed in the field; 2) the representation must be accurate; and 3) scale subdivisions must comply the human visual acuity limitations defined by the Weber-Fechner stimulus-response law (Horsfall & Barratt 1945). Furthermore, the levels of accuracy, precision and reproducibility must be evaluated and validated to verify the quality of the estimates provided by the diagrammatic scale (Kranz 1988, Campbell & Madden 1990, Nutter Jr et al. 1993, Nutter Jr & Schultz 1995).

To date, no scale is known to indicate the severity of white thread blight in *Ilex paraguariensis*. In the present study, we develop and validate a logarithmic diagrammatic scale to quantify the severity of white thread blight caused by *Ceratobasidium niltonsouzanum* in yerba mate.

MATERIALS AND METHODS

Diagrammatic scale development

Branches of yerba mate with signs and symptoms of white thread blight disease were collected in a field located in Caraguatay, Montecarlo (-26.6623; -54.7330), in Misiones province and photographed on a white background with a Nikon D3300 digital camera. The leaves of each branch were isolated and digitized with an EPSON L375 scanner, and the infected leaf area of each branch was determined with the ImageJ 1.47v program (Rasband 1997). The percentage of actual severity of each branch was obtained by calculating the quotient of total leaf area and infected leaf area.

The development of a six-level scale was considered. The maximum severity value obtained among the collected branches was used as a parameter to determine the intervals of each level using the DOSLOG 2.0v software for Windows® (Mora-Aguilera & Acevedo-Sánchez 2018), which is based on the Weber-Fechner visual acuity law and employs an adjustment of the method proposed by Horsfall & Barratt (1945).

In general, fungal diseases can affect different organs (branches, leaves, roots, fruits) of the same plant with different degrees of severity, so it is necessary to quantify the total severity (TS) of the organism. In yerba mate, the TS of white thread blight can be quantified using the method proposed for banana black leaf streak caused by *Mycosphaerella fijiensis* (Vicente et al. 2006) with modifications:

$$TS = \left(\frac{\Sigma an}{NT} \right).100$$

where *a* is the degree of severity according to the scale for white thread blight disease (0 - 5), *n* is the number of central branches corresponding to each scale degree in the plant, *N* is the maximum number of the scale (5 in this case), and *T* is the total number of branches evaluated in each plant at the time of severity estimation (da Rocha Júnior et al. 2010, Oddino et al. 2016).

Validation of the diagrammatic scale

Twenty-one images of yerba-mate branches with symptoms of white thread blight disease were used to represent all the scale levels for validation. These images were randomly inserted into a Google Form® and presented to eight raters with expertise in pathogens and plant diseases and twelve raters without expertise in pathogens and plant diseases, in three evaluations.

In the first evaluation, the raters scored the branches without using the scale. After 14 days, the same raters performed the second evaluation using the scale. To evaluate the repeatability of the estimates with the scale, 14 days after the second evaluation, the same raters made a third evaluation with the scale.

The accuracy and precision of each rater visual estimates were determined by linear regression analysis using the InfoStat software (Di Rienzo et al. 2010), considering the data of actual severity as an independent variable and those of estimated severity as a dependent variable. The precision of estimates was determined by the coefficient of determination (R^2) of the same regression line and by the variance of absolute errors; their accuracy was determined by *t*-test applied to the intercept of linear regression (β_0) to verify the hypothesis Ho: $\beta_0 = 0$ and to the slope of the line (β_1) to test the hypothesis Ho: $\beta_1 = 1$ at 5% probability level (p = 0.05) (Nutter Jr & Schultz 1995, Boito et al. 2013, Belan et al. 2014). Intercept values significantly different from 0 indicate overestimation of actual severity, whereas values of slope of the line significantly different from 1 indicate systematic deviations from true severity (Nutter Jr & Schultz 1995, Boito et al. 2013).

The reproducibility of estimates, which indicates whether the scale can be used efficiently by other raters, was evaluated by analyzing R^2 values of linear regressions between severity values of the same sampling unit, estimated by different raters–matched in pairs (Kranz 1988, Campbell & Madden 1990, Nutter Jr & Schultz 1995, Boito et al. 2013).

RESULTS AND DISCUSSION

Diagrammatic scale of branches

For white thread blight disease in yerba mate in Misiones province, Argentina, the minimum and maximum values of actual severity found in the field were 1.79% and 91.37%, respectively. Table I shows the numerical scale obtained with the DOSLOG 2.0v program, using as a parameter the obtained maximum value of actual severity; its midpoint (pmc) and the lower and upper limits are detailed for each level of the scale. Figure 1 shows the diagram of the scale represented by the pmc values obtained for each level (see Supplementary Material - Figure S1).

Table I. Lower and upper limits and pmc for each level on the logarithmic diagrammatic scale for white thread blight (*C. niltonsouzanum*) in yerba-mate branches (*I. paraguariensis*) obtained with the DOSLOG program.

Level	Lower limit	pmc	Upper limit
0	.0	.0	.0
1	.1	1.1	2.8
2	2.9	4.5	9.1
3	9.2	13.6	25.0
4	25.1	36.5	63.7
5	63.8	91.0	91.0

Figure 1. Diagrammatic scale for white thread blight disease (*C. niltonzousanum*) in yerba-mate branches (*I. paraguariensis*). The scale presents six levels: 0 (0%); 1 (0.1 - 2.8%); 2 (2.9 – 9.1%); 3 (9.2 - 25%); 4 (25.1 – 63.7%) and 5 (63.8 - 91%).

Scale validation

According to the severity analysis of white thread blight disease in yerba mate, in the evaluation without using the proposed scale, the raters with no expertise were not very accurate (Table II). Both hypotheses $\beta_0 = 0$ and $\beta_1 = 1$ of the linear regression equation between estimated and actual severity were rejected by 8 of the 12 evaluators ($p < 0.05$). In contrast, only one of the raters with expertise presented β_0 values different from 0 ($p < 0.05$).

All the raters presented β_1 values different from 1 ($p < 0.05$), which indicates the presence of systematic deviations in the three evaluations (Table II). With the use of the proposed scale, all the raters improved the accuracy in both evaluations since none presented β_0 values different from 0 ($p < 0.05$).

Precision improved with the use of the proposed scale for both categories of raters. R^2 values in the first evaluation without using the scale ranged between 0.18 - 0.81, and with the use of the scale in the second evaluation ranged between 0.21 - 0.81, whereas in the third evaluation they ranged between 0.70 and 0.94 (Table II). This indicates that it is necessary and advisable to train monitoring personnel to recognize the signs and symptoms of white thread bright disease in the use of the scale as a tool to estimate severity.

According to these values, the proposed scale is precise, since R^2 was equal to or greater than 0.70 in all cases after two evaluations, according to the results obtained by other authors (Spósito et al. 2004, Nascimento et al. 2005, Godoy et al. 2006, Michereff et al. 2006, 2009, Boito et al. 2013).

Furthermore, there was a reduction in absolute errors and lower amplitude values with the scale (Fig. 2). In fact, the minimum and maximum values for the residuals of all the evaluators without the scale were -57.59 and 48.21, respectively, whereas the range was reduced to -37.43 and 37.99 after two evaluations with the scale. This reduction in residual amplitude is greater in the evaluators with no expertise, which shows the importance of prior training to improve the precision and accuracy of evaluations (Tovar-Soto et al. 2002, Téliz-Ortiz et al. 2003, Boito et al. 2013).

To test the reproducibility of the scale, we focus on the coefficient of determination (R^2) values obtained from the linear regressions between the estimates made by both categories of raters combined in pairs (see Figure S2). Without the use of the scale, 36.84% of the cross correlations between the evaluators obtained

Table II. Intercept (β_0), slope of the line (β_1) and coefficient of determination (R^2) of the linear regression equation that relates visual estimates of severity of white thread blight (*C. niltonzousanum*) in yerba mate-branches (*I. paraguariensis*) conducted by evaluators with an without the diagrammatic scale with actual severity determined electronically.

evaluator		coefficients								
		without scale			with scale					
					1st evaluation			2nd evaluation		
		β_1	β_0	R^2	β_1	β_0	R^2	β_1	β_0	R^2
with experience	1	*0.80	1.53	0.67	*0.92	-4.43	0.81	*0.98	-1.53	0.94
	2	*0.73	7.06	0.62	*0.65	2.72	0.56	*0.72	2.94	0.72
	3	*0.50	4.86	0.36	*0.95	-3.08	0.82	*0.98	-2.58	0.93
	4	*0.77	0.15	0.78	*0.94	-1.43	0.86	*0.98	-1.53	0.94
	5	*0.47	*13.91	0.39	*0.63	0.61	0.54	*0.72	1.26	0.71
	6	*0.41	0.47	0.61	*0.64	-4.10	0.55	*0.89	-7.14	0.70
	7	*0.47	-3.25	0.68	*0.97	-7.73	0.79	*1.02	-4.49	0.91
	8	*0.67	2.61	0.81	*0.69	-3.51	0.58	*0.76	-0.02	0.70
without experience	9	*0.32	*53.53	0.19	*0.89	0.91	0.54	*0.98	-0.85	0.90
	10	*0.51	*20.03	0.34	*0.40	4.18	0.21	*0.93	-5.04	0.74
	11	*0.34	*18.36	0.47	*0.55	4.92	0.46	*0.92	-0.11	0.80
	12	*0.40	*21.75	0.45	*0.46	0.50	0.31	*0.93	-7.78	0.73
	13	*0.42	*12.48	0.36	*0.53	3.24	0.38	*0.96	-7.08	0.78
	14	0.38	54.88	0.18	*0.87	14.17	0.37	*1.01	3.48	0.80
	15	*0.64	*30.42	0.25	*0.69	-3.86	0.62	*0.94	-5.54	0.77
	16	*0.60	*14.43	0.45	*0.92	3.84	0.60	*0.97	0.34	0.88
	17	*0.60	4.78	0.58	*0.59	-3.52	0.42	*0.95	-6.80	0.74
	18	*0.78	*22.89	0.55	*0.93	4.44	0.84	*0.93	0.00	0.84
	19	*0.56	-7.04	0.43	*0.90	-6.14	0.67	*1.04	-5.46	0.91
	20	*0.36	4.91	0.39	*0.52	-2.76	0.41	*0.94	-7.86	0.77

*represents situations where the null hypothesis ($\beta_0 = 0$ or $\beta_1 = 1$) was rejected by *t*-test ($p = 0.05$).

values higher than 0.70. However, after two evaluations with the proposed scale, 94.74% of the cross correlations obtained values higher than 0.70, which confirms the reproducibility of the evaluations with the use of the diagrammatic logarithmic scale developed in this work; similar results were obtained by Nascimento et al. (2005), Michereff et al. (2006) and Boito et al. (2013).

CONCLUSIONS

A six-level diagrammatic scale was developed to quantify in the field the severity of white thread blight disease in yerba-mate branches. The scale was statistically validated and provided better levels of accuracy, precision and reproducibility in the 94.74% of the evaluations. It was also demonstrated the need for training prior to the use of the scale to correctly recognize the signs and symptoms of the disease in the field and improve the estimation of severity.

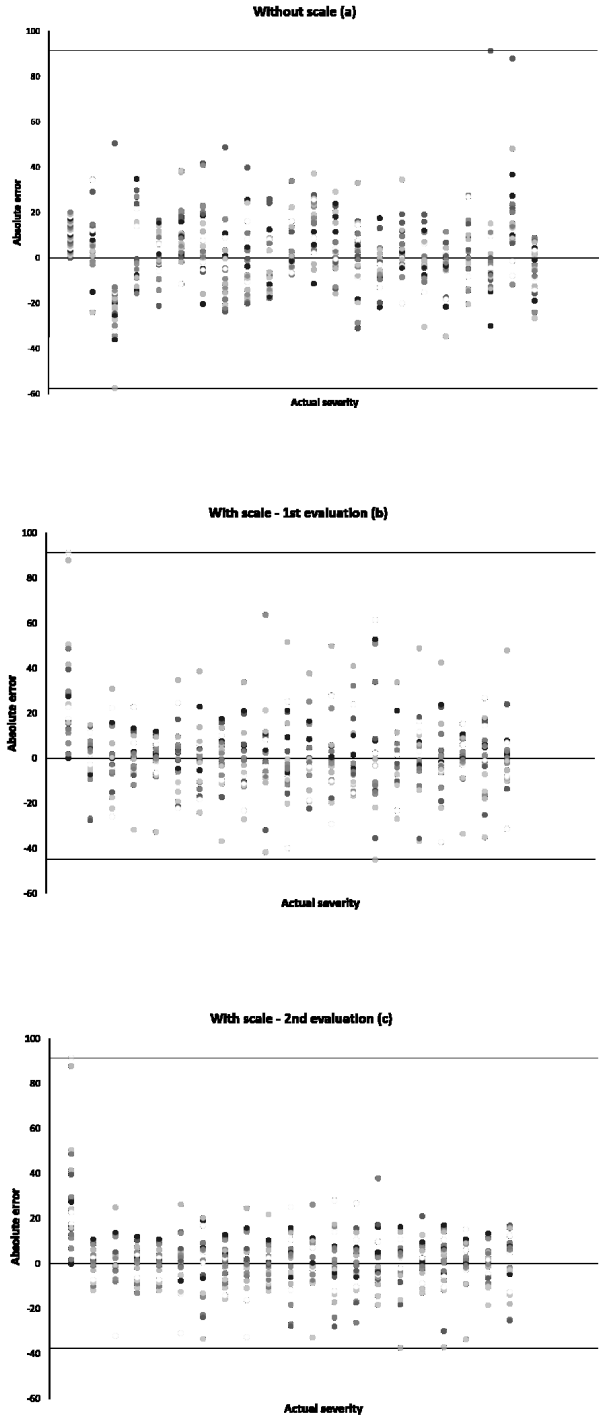

Figure 2. Distribution of residuals (estimated severity – actual severity) of estimates of white thread blight (*C. niltonzousanum*) in yerba-mate branches (*I. paraguariensis*) without (a) and with (b, c) the diagrammatic scale in two evaluations.

This scale is an important tool for the quantification of severity, estimation of productivity losses and decision-making for the management of this disease in yerba-mate cultivation.

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SUPPLEMENTARY MATERIAL

Figures S1, S2.

How to cite

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Author contributions

M.L.V. performed the scale and the validation. F.G.D. recollected the samples and digitalized the images. A.E.A. and P.D.Z. conceived the study and supervised the project. M.L.V. and F.G.D. wrote the manuscript with support from A.E.A. and P.D.Z. All authors discussed the results and contributed to the final manuscript.