



## Development and validation of diagrammatic scales to assess septoriose in tomato

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### Abstract

Septoriose or septoria leaf spot (*Septoria lycopersici* Speg.) is an important disease in tomatoes (*Solanum lycopersicum* L.) because of the damage that it can cause. To improve the evaluation of studies aiming to determine the most efficient treatment is necessary accuracy and precision during the assessment. Therefore, this study aimed to develop two diagrammatic scales based on grades and severity to evaluate septoriose severity on tomato leaves. The diagrammatic scale based on grades was developed and validated with eight grades, with severities ranging from 0.67 to 100%. The diagrammatic scale based on severity values varied from 1.79 to 100%. More than 80% of the leaves collected from the field showed severity ranging from 0.1 to 20%, but there was a representative leaf for every interval chosen. Five inexperienced evaluators performed the validation of the scales, and the data were analysed with Lin's statistics. Without the scales, most evaluators overestimated disease severity, whereas the use of the scales resulted in increased precision, accuracy, and reliability of the estimates. In conclusion, the proposed diagrammatic scales proved to be useful for assessments of septoriose severity in tomato leaves. The scales may be of interest to researchers performing studies on epidemiology, fungicides efficacy or breeding for resistance.

**Keywords** – disease assessment – disease severity – phytopathometry – Septoria leaf spot

### Introduction

The *Septoria lycopersici* causes septoriose, one of the most devastating diseases in tomatoes worldwide. It is a severe disease everywhere tomatoes grow (Stevenson 1991). This pathogen is a constant concern, especially when in favourable environmental conditions and the same area are continuously cultivated with tomatoes (Lopes & Ávila 2005). The most favourable weather conditions occur when the relative humidity is above 85% and the temperature is between 20 to

25°C (Kurozawa & Pavan 2005). Under these favourable conditions, the disease causes severe defoliation that can reach 100%, and consequently, losses are significant due to the scalding of tomato fruits by the sun (Sohi & Sokhi 1974).

In each cultivation cycle, the disease begins in the leaves of the plant shallow due to raindrops falling on plant fragments with *Septoria* spores and causing spores to spread (Douglas 2008). *S. lycopersici* infect tomato leaves by both stomata and direct penetration (Martin-Hernandez et al. 2000). Symptoms appear one week after inoculation and after six weeks defoliation is close to 100% when in wet conditions and control measures are not employed (Parker et al. 1997). Symptoms on leaves are circular-shaped spots with darkened edges and a brown/grey coloured centre. After a few days, small black dots appear in the centre of the lesions, which correspond to the reproductive structures of the fungus (pycnidia). Under favourable conditions, lesions may coalesce, turn yellow, then brown, wither, dry and detach from the plant (Douglas 2008). Similar lesions may occur on the stem. Fruits are rarely affected. It may be confused with bacterial spot or with early blight caused by *Alternaria* spp. when lesions are early (Lopes & Ávila 2005).

Methods for disease quantification should be easy, accurate, reproducible, and quickly applied under different conditions (Berger 1980, Campbell & Madden 1990). Diagrammatic scales or standard area diagrams (SADs) are handy tools to minimize the subjectivity of severity estimates (Barbosa et al. 2006). A diagrammatic scale must be elaborated considering the following requirements: the number of leaves collected to develop the scale has to be representative; the upper limit illustrated in the scale must correspond to the maximum disease severity found in the field; real severities needs to be represented in the scale with accuracy (Horsfall & Barratt 1945).

There are no validated diagrammatic scales available to assess septoriose severity in tomato leaves. There are, however, reports of the use of the descriptive scales or adaptations of scale made for other pathosystems to evaluate the disease (Maluf et al. 1985, Boff et al. 1991, Mello et al. 1997, Gondal et al. 2012). The availability of validated diagrammatic scales would help to develop studies on disease epidemiology, fungicides efficacy and breeding for resistance because validated scales provide accurate, precise and reproducible estimates of disease severity (Salgado et al. 2009, Santos et al. 2010).

The objective of this study was to develop and validate two diagrammatic scales to assess the severity of septoriose on tomato leaves.

## **Materials & Methods**

### **Leaf sampling, image analyses and scales elaboration**

To elaborate the scale, 1313 tomato leaves with different severities of septoriose were obtained from the field at Caçador, Santa Catarina, Brazil. The aetiology of the disease was confirmed by spore morphology and via Kock's postulates. Leaves with septoriose were photographed and loaded into the software Measure picture v. 1.0 (Kassler 2016) to determine their health and lesioned leaf areas, which resulted in the real severity for each leaf. Lesions included the chlorotic area as part of the symptoms. We proposed two different scales based on severity percentages and grades. When evaluators use the latter, the estimates were given in grades, which comprise a range of severity. The distribution of frequencies of lesioned leaves determined the diagrammatic scales grades and severities intervals. A diagrammatic scale based on grade was made with eight grades (level 0 – 0.0%, level 1 – 0.1-3.99%, level 2 – 4.0-7.99%, level 3 – 8.0-18.99%, level 4 – 19-39.99%, level 5 – 40-59.99%, level 6 – 60-89.99% and 7 –  $\geq 90$ ) and diagrammatic scale based on severity only was made with nine illustrations with the severity of 0, 1.79, 3.93, 10.91, 20.63, 43.63, 63.14, 84.05 and 100%.

### **Evaluation of the diagrammatic scales**

#### **Estimates performed by five inexperienced evaluators**

Each of the five inexperienced evaluators gave estimates of 100 images of leaves, without and with scales based on severity and grades. These images were randomly chosen and contained different disease severity. The first set of assessments was performed without the aid of a diagrammatic scale. After seven days, the same evaluators received the same images rearranged to perform estimates with the aid of the proposed scale based on severity. Then, seven days later, they did a second assessment with the scale based on grades. Those estimates were analysed with Lin's statistics to validate the scales, comparing the estimates with and without using the scales.

### Analysis with Lin's statistics

Analyses were performed comparing the evaluators' performance without and with the use of the scales to measure agreement, precision, accuracy, bias, and interevaluator reliability of the estimates. Lin's concordance correlation (LCC) analysis (Lin 1989) calculated the agreement between the estimate and true severity for each evaluator. LCC coefficient assesses the fitness of pairs of observations to the line of concordance ( $45^\circ$  = perfect concordance) by combining the measurements of accuracy and precision. It is a method to judge the agreement between estimates and true values and has been previously employed in plant disease severity assessments (Spolti et al. 2011, Yadav et al. 2013). Pearson's correlation coefficient, an indicator of precision, and the bias correction that measures accuracy were used to calculate Lin's concordance correlation coefficient. The bias correction factor (Cb) is calculated from location bias (where 0 = perfect match between x and y) and scale bias (where 1 = perfect match between x and y) and are derived from the means and standard deviations of x and y, respectively. The absolute error was also calculated (the estimated value minus the real severity). The interevaluator reliability indicates the reproducibility of the visual estimates between evaluators matched in pairs with and without the use of the scales (Shrout & Fleiss 1979). The scale's influence on the interevaluator reliability was measured with the LCC coefficient. All statistical analyses were performed with the R software (R Core Team 2013), and the `epi.ccc` function of the `epiR` package (Stevenson et al. 2012) was used to obtain Lin's CC statistics.

### Results

Septoriose severity for the 1313 tomato leaves ranged from 0.01 to 100%. Approximately 63% of the leaves showed severities between 0.00 and 10% (Table 1). The lowest and the highest severities of symptomatic leaves represented in the diagrammatic scale based on severity percentages were 1.79 and 100%, respectively (Fig. 1a). For the diagrammatic scale based on grades, those values varied from 0.67 to 100% (Fig. 1b). The scales were developed to represent values that commonly occur in the field. Since septoriose can occur any time during tomato cultivation, it can severely defoliate the plant reaching 100% of severity.

**Table 1** Distribution of septoriose frequencies in 1313 tomato leaves used to develop the scales

Disease severity (%)	N of leaves	%
0.00 – 10 %	828	63
10.01 – 20 %	160	12
20.01 – 30 %	78	6
30.01 – 40 %	40	3
40.01 – 50 %	27	2
50.01 – 60 %	32	2
60.01 – 70 %	14	1
70.01 – 80 %	15	1
80.01 – 90 %	11	1
90.01 – 100 %	108	8
<b>Total of leaves assessed</b>	<b>1313</b>	<b>100%*</b>

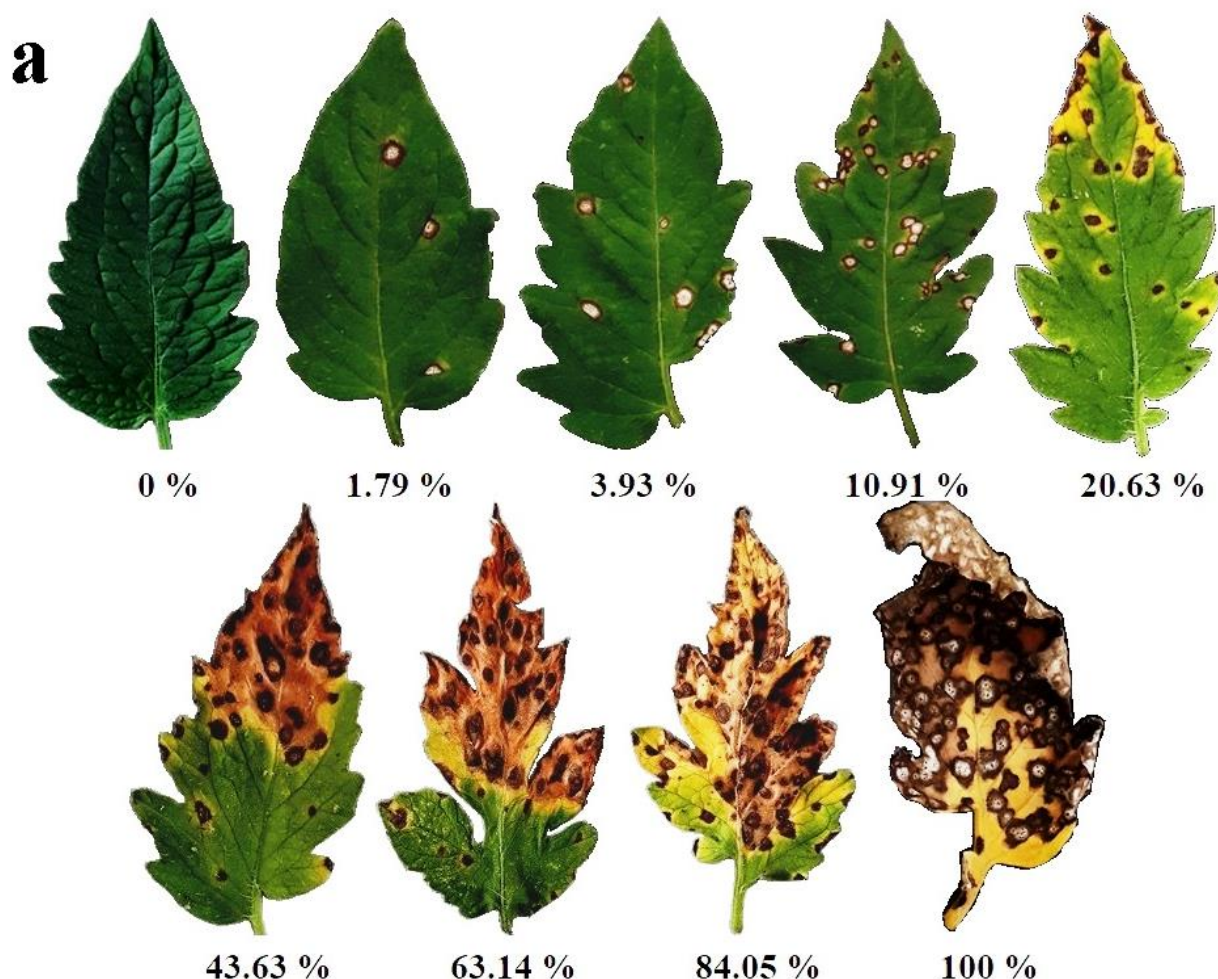
\*Approximately value

The estimates of 500 tomato leaves were nearest to the actual value and the absolute errors (the estimated value minus the real severity) were lower when evaluators used both diagrammatic scales (Figs 2, 3a, b, c). Without scale, the evaluators tend to overestimate the severity.

The most expressive gain in agreement, accuracy and precision when they used the scales was obtained for the evaluators with the poorest estimates when without the use of the scale (Fig. 3d, e, f).

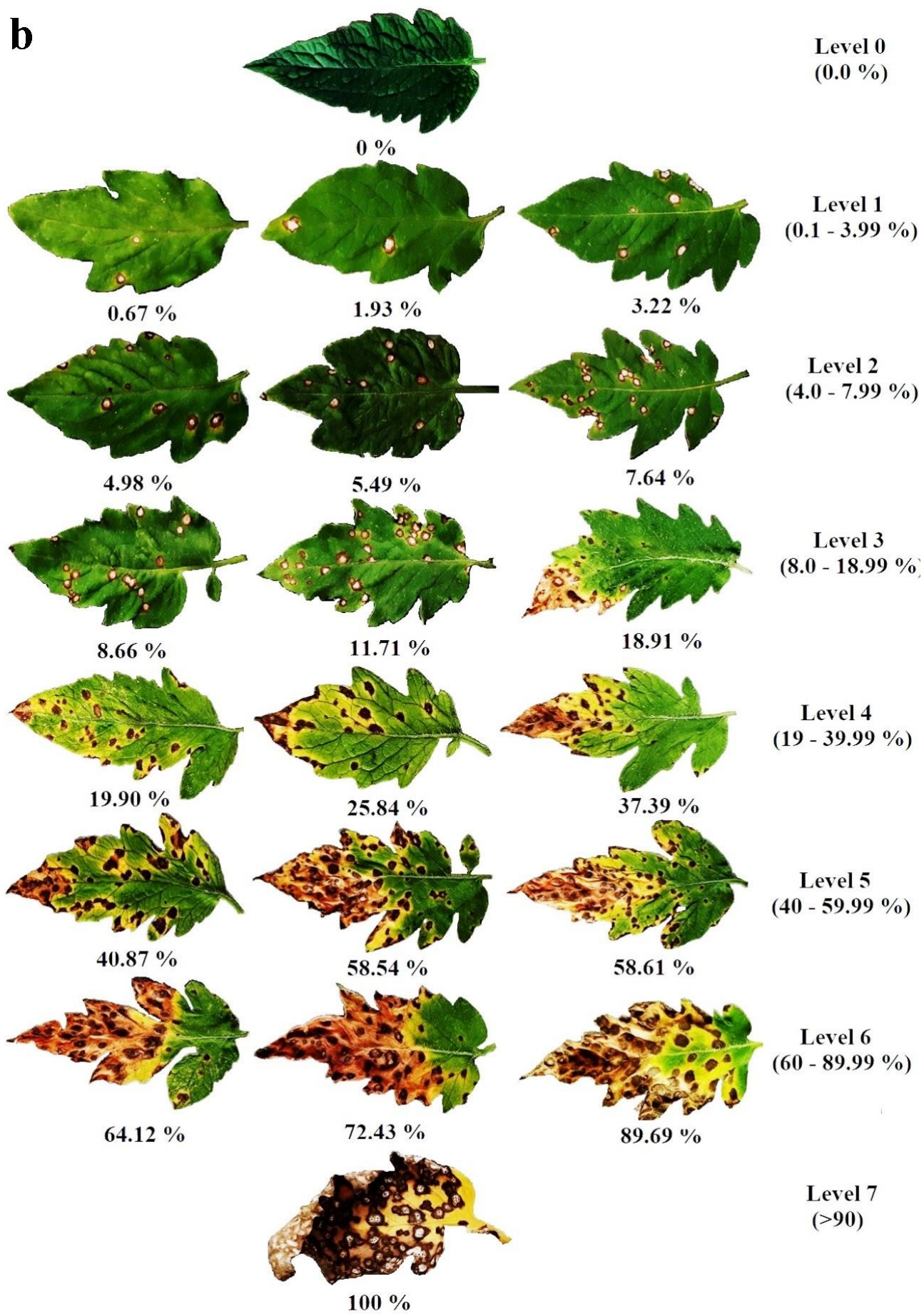
Lin's statistics is a set of calculations used to validate the scales. Except for scale shift, all Lin's statistics (location shift, bias correction, Lin's concordance correlation coefficient) and Pearson's correlation were improved, on average, with the use of the scales (Table 2). The bias correction is a measure of accuracy, and it showed significantly higher values, on average, with the use of the scales ( $C_b = 0.98$  for severity scale and  $C_b = 0.99$  for grade scale) than without the scale ( $C_b = 0.89$ ). Similarly, Lin's  $P_c$  that measures the agreement was higher with the scales ( $P_c = 0.90$ ) than without it ( $P_c = 0.79$ ). Pearson's correlation coefficient, which measures precision was also higher with the scales ( $r = 0.93$  and  $0.91$ ) than without it ( $r = 0.89$ ).

The reproducibility of the scales tested with correlation concordance coefficient by comparing pairs of evaluators had values varied from 0.47 to 0.91% (average of 0.65%) for assessments without the scale and from 0.80 to 0.94% (average 0.87%) and from 0.78 to 0.92% (average 0.87%) with the use of the scale based on severity and with the scale based on grades, respectively (Table 3)

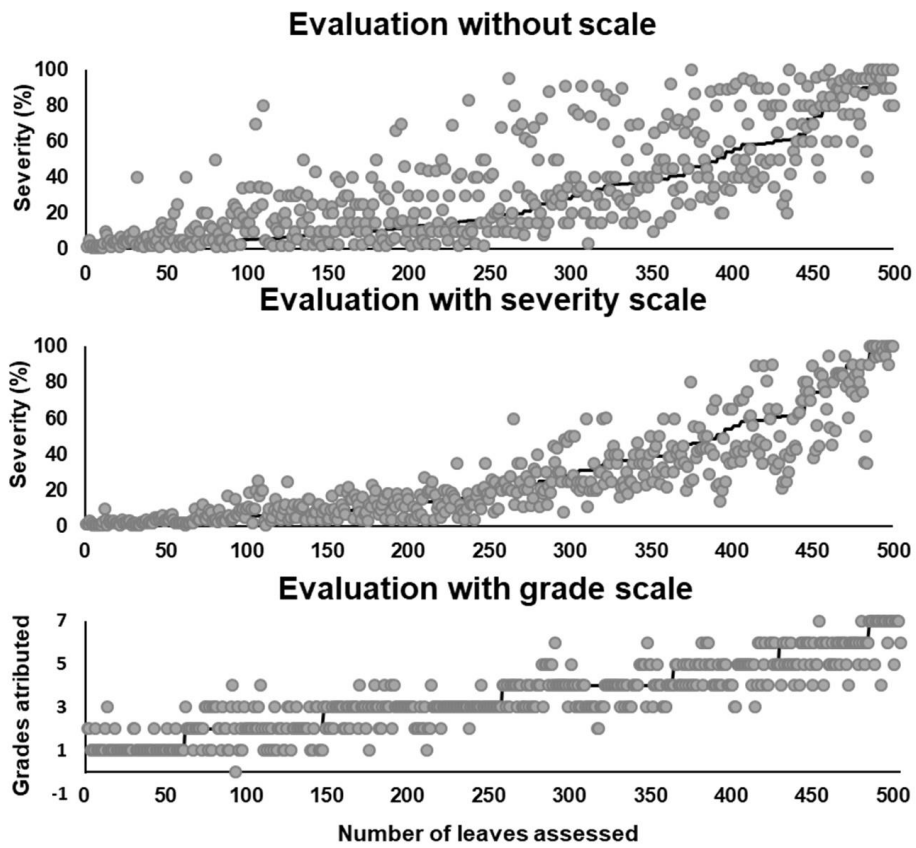


**Fig. 1** – Diagrammatic scales for the assessment of septoriose in tomato leaves. Scale based on severity percentages (a) and scale based on grades representing a range of severity (b). Numbers below each picture represent the real percentage of leaf area affected by the disease. The yellow colour was considered part of the disease.

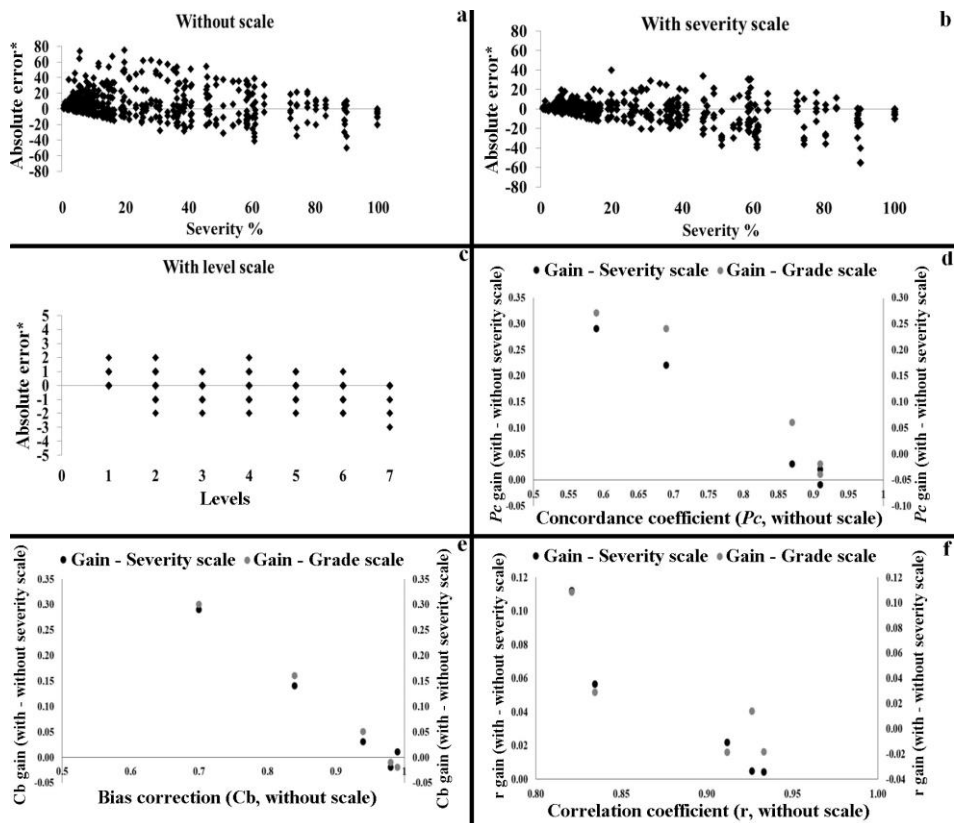
**b**



**Fig. 1** – Continued.



**Fig. 2** – Assignment of 500 estimates given by the five evaluators compared to the real severity without the use of scales and with it. The black line is the real severity assessed digitally.



**Fig. 3** – Distribution of residuals of the estimates obtained from evaluators in 500 estimations done without a scale (a), with the severity scale (b) and with grade scale (c). Average distribution of

residuals (estimated severity – true severity) of septoriose (*Septoria lycopersici*) and gain in Lin’s statistics in estimations done with and without the scales. Gain on estimates obtained with the use of scale in agreement measured with Lin’s concordance correlation coefficient (d); and accuracy measured by bias correction (e); and precision measured by Pearson’s correlation coefficient (f).

**Table 2** Lin’s concordance coefficients of the estimated severity of septoriose in tomato leave evaluated by five evaluators with and without the proposed scales

Evaluator	Without					With scale based on the severity					With scale based on grade				
	$P_c^2$	$C_b^3$	<i>S.shift</i> <sup>4</sup>	<i>L. shift</i> <sup>5</sup>	<i>Pearson</i> <sup>6</sup>	$P_c$	$C_b$	<i>S.shift</i>	<i>L. shift</i>	<i>Pearson</i>	$P_c$	$C_b$	<i>S.shift</i>	<i>L. shift</i>	<i>Pearson</i>
A	0.91	0.98	1.02	-0.20	0.93	0.90	0.96	0.91	-0.27	0.94	0.89	0.97	0.88	-0.22	0.92
B	0.59	0.70	1.14	0.91	0.83	0.88	0.99	0.89	-0.10	0.89	0.86	1.00	1.00	-0.01	0.86
C	0.91	0.99	0.89	0.03	0.91	0.93	1.00	0.92	0.01	0.93	0.87	0.97	0.95	-0.24	0.89
D	0.87	0.94	0.78	-0.25	0.93	0.90	0.97	0.83	-0.16	0.93	0.93	0.99	0.96	-0.12	0.94
E	0.69	0.84	1.06	0.61	0.82	0.91	0.98	1.15	0.16	0.93	0.93	1.00	1.06	0.04	0.93
<b>Means</b>	0.79	0.89	0.98	0.22	0.89	0.90	0.98	0.94	-0.07	0.93	0.90	0.99	0.97	-0.11	0.91
<b>Global evaluation<sup>1</sup></b>	0.76	0.97	1.10	0.23	0.79	0.91	1.00	0.96	-0.06	0.91	0.90	1.00	0.98	-0.11	0.90

<sup>1</sup> Global evaluation was performed analysing the data as one without discriminate evaluators.

<sup>2</sup> Concordance correlation coefficient ( $P_c$ ) combines precision and accuracy to measure agreement with the true values.

<sup>3</sup> Bias correction ( $C_b$ ) measures how much the best-fit line deviates from the 45° line. No deviation from the 45° line occurs when  $C_b = 1$ .  $C_b$  is a measure of accuracy calculated from scale shift and location shift.

<sup>4</sup> Scale shifts relative to the perfect match (1 = perfect match between  $x$  and  $y$ ).

<sup>5</sup> Location shift relative to the perfect match (0 = perfect match between  $x$  and  $y$ ).

<sup>6</sup> Pearson’s correlation coefficient measures precision ( $r$ ).

**Table 3** Concordance correlation coefficient between evaluators matched in pairs of visual estimates of septoriose severity on 100 leaves of tomato by five evaluators

Without scale	A	B	C	D	E
A	1	0.47	0.85	0.85	0.57
B	-	1	0.50	0.39	0.77
C	-	-	1	0.91	0.68
D	-	-	-	1	0.52
E	-	-	-	-	1

**Table 3** Continued.

<b>With scale based on severity</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>
<b>A</b>	1	0.89	0.88	0.92	0.80
<b>B</b>	-	1	0.90	0.94	0.82
<b>C</b>	-	-	1	0.92	0.86
<b>D</b>	-	-	-	1	0.81
<b>E</b>	-	-	-	-	1
<b>With scale based on grade</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>
<b>A</b>	1	0.87	0.88	0.92	0.85
<b>B</b>	-	1	0.78	0.89	0.88
<b>C</b>	-	-	1	0.89	0.82
<b>D</b>	-	-	-	1	0.91
<b>E</b>	-	-	-	-	1

### Discussion

In this study, two diagrammatic scales to evaluate septoriose severity in tomato leaves were elaborated and validated. Those are the first validated diagrammatic scales made available to assess septoriose severity for tomato plants. The proposed diagrammatic scales were elaborated with real images of tomato leaves, which facilitates the assessments and improves precision and accuracy. On the other hand, most scales reported in the scientific literature do not use real images of plant parts to illustrate disease severity.

The scale based on grades was developed with eight grades and three severity values per grade (except for the first and last one), which help the evaluators to interpolate their samples and obtain better severity estimates. The development of scales with grades has increased in the last years (Nuñez et al. 2017). The assessments of septoriose severity with the scales were done with acceptable accuracy and precision by evaluators without any previous experience, showing the usefulness of the proposed diagrammatic scales. Without using a scale, evaluators tend to overestimate disease severity, especially at severity lower than 20%, whereas at mid-to-high severity, estimates tend to be underestimated (Salgado et al. 2009, Santos et al. 2010, Menge et al. 2013). The use of a diagrammatic scale provides speed and allows users to standardize their estimates. In this study, the evaluators were considered to have had a good performance as their estimates were in the 6.10% interval of the real severity values, whereas to be considered as excellent evaluators, their estimates should have been in the 5% interval (Nutter Junior & Worawitlikit 1989). One of the reasons for the good performance of the evaluators may be related to the large size of the lesions, which according to other authors (Sherwood et al. 1983, González-Domíngue et al. 2014) facilitates rating and increases precision. In this context, training the evaluators to use the scales may further reduce the subjectivity of the estimates by improving their performance (Nutter Junior & Schultz 1995). Some evaluator (A) might have a natural ability, giving good estimates even without scales, and in this situation, the scale had little effect to improve the estimate of the individual.

Validation of the scale based on grade and severity values yielded similar conclusions about the performance of the evaluators. Both scales were useful to evaluate septoriose and had similar results. The use of one or another depends on the preference of the evaluator. There were no differences



between the scales, and both showed improvements in precision, accuracy and reproducibility. Some authors do not consider regression analysis an appropriate approach for testing agreement, which combines precision and accuracy, and recommends Lin's statistics for scale validation (Lin 1989, Bock et al. 2010). Researchers working on breeding programs and phytopathology (epidemiology) will possibly benefit from using the proposed scales in experiments designed to assess septoriose severity and allow comparisons across different experiments.

The proposed diagrammatic scales proved to be useful for assessments of septoriose severity in tomato leaves. The scale may interest research performing studies on epidemiology, fungicides efficacy, or breeding for resistance.

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