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# Influence of leaf position that correspond to whole plant severity and diagrammatic scale for white spot of corn

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ABSTRACT

The main objective of this research is to determine the influence of leaf position on corn plants with white spot caused by *Pantoea ananatis*, which better represents the infection on the whole plant. A diagrammatic scale to quantify the severity of the disease was elaborated and validated. For scale elaboration, the minimal and maximal limits of the disease severity observed in the field were considered, and intermediate levels followed logarithmic increments according to the Weber–Fechner stimulation law. The scale has nine classes: 0.1, 1, 2, 4, 8, 16, 24, 32 and 64%. For scale evaluation, a severity evaluation for white spot was performed by 10 raters with no experience in disease evaluation. Initially, severity estimation was performed without a scale for 41 leaves with different levels of severity. Afterward, the same raters used the proposed diagrammatic scale. Through linear regression to compare the actual and estimate severity values, the raters' accuracy and precision were analyzed. Satisfactory accuracy and precision were achieved when estimation was performed with a diagrammatic scale. To determine the best leaf disease severity evaluation, correlation and regression analyses were performed with 25 plants of five genotypes, for a total of 284 leaves analyzed. Results analysis leads us to conclude that the severity of white spot on corn plants significantly correlates with the disease mean severity of leaves 0 and -1, i.e., a leaf of the corn ear and the one immediately below it. This scale provided good levels of accuracy and precision (a mean $R^2$ of 94%), with errors concentrating around 10%. Raters presented increased reproducibility ($R^2 > 90\%$ in 82% of cases) of severity estimates. The proposed diagrammatic scale is considered adequate to estimate the severity of white spot in corn for germplasm evaluations, for epidemiological studies and for evaluation of control strategies for this disease.



## 1. Introduction

Among worldwide producers of corn, Brazil is currently the third largest producer, with 58.5 million of tons produced in the last harvest (Conab, 2008). However, even after exporting 20% of its yearly production, Brazil has one of the lowest productivities among global exporters (Conab, 2008). Among the factors that contribute to this low productivity, diseases deserve important attention due to the losses they cause. However, among diseases that affect corn (*Zea mays* L.), white spot caused by bacteria *Pantoea ananatis* (Paccola-Meirelles et al., 2001; Bomfeti et al., 2008), primarily described as Phaeosphaeria spot, is considered one of the most important. This disease is endemic in Brazil, and its incidence and severity has been increasing significantly since the 1990s; it can likely be found in all regions where corn is cultured (Pereira et al., 2005). In susceptible cultures, the disease can reduce grain production by up to 63.1% (Pinto, 1999). Leaves with 10–20% severity have a 40% decrease in liquid photosynthetic rate, which also results in decreases in grain production by about 60% (Godoy et al., 2001). This negative correlation between corn productivity and disease severity has been reported by several authors under Brazilian conditions (Sawzaki et al., 1997; Brasil and Carvalho, 1998; Pegoraro et al., 2001).

Disease symptoms are small lesions, up to 2 cm, rounded to oblong in shape, with white spotting and dark borders, and coalescence lesions can occur (Pereira et al., 2005). This disease assessment is of great importance in its handling, but it is difficult to execute because it is a hard and relatively onerous procedure. Disease assessment data is important to evaluate different control measures, varietal resistance and to test phytosanitary products efficiency (Vale et al., 2004). The most adequate form to assess

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diseases, such as white spot in corn, is through severity, which represents the percentage of impaired foliar tissue in relation to the foliar area (Bergamin Filho and Amorim, 1996). To precisely assess disease severity, several strategies have been suggested, and among them are diagrammatic scales, which are illustrated representations of a series of plants, leaves, or parts of plants with symptoms at different severity levels (Bergamin Filho and Amorim, 1996). By using diagrammatic scales, the subjectivity of severity estimates can be reduced among raters, improving the accuracy and precision of evaluations (Martins et al., 2004).

Characteristics of a good diagrammatic scale involve facility of use, reproducible results, and applicability under a wide range of conditions, with intervals that represent all stages of disease development and that allow immediate evaluation (Berger, 1980). Thus, when building a scale, some important aspects must be considered: 1st) the upper and lower limits of scale must correspond, respectively, to the maximal and minimal intensity of the disease observed in the field, 2nd) the symptoms represented must be as close as possible to those observed in plants, and 3rd) when determining the intermediate levels of the scale, human eye acuity limitations must be considered, which are defined by the Weber–Fechner stimulation-response law. Visual acuity is proportional to logarithm of the stimulation intensity (Horsfall and Barrat, 1945). Diagrammatic scales must be validated before being proposed as a standard method of disease assessment, and in the event of producing non-satisfactory results, these must be corrected (Martins et al., 2004).

The most commonly used scale to evaluate white spot in corn in Brazil is the one created by Chester (1950) and modified by Agroceres (1996). Evaluation can be performed on the whole plant or on the leaf positioned below the insertion point of the main corn ear. The method of disease severity evaluation using the whole plant proved to be the most practical for the evaluation of white spot in corn (Silva, 2002), but it has been not validated in relation to visual acuity laws. In addition, because the scale has been developed for whole plant evaluations, its use is limited because assessment is more subjective. To increase the objectivity of the evaluation, it would be interesting to perform a study in which it is determined whether a leaf from a corn with the disease better correlates to the severity of the whole plant, optimizing disease severity evaluations.

Thus, the purpose of this research is to determine the influence of leaf position on corn plants with white spot caused by *Pantoea ananatis*, which better represents the mean severity of the whole plant. The second objective is to elaborate and validate a diagrammatic scale for the evaluation of white spot severity in corn.

## 2. Material and methods

For diagrammatic scale elaboration and to determine which leaf (or leaves) better correlate to disease severity in the whole plant, 25 plants of four genotypes (BRS1010, DAS657, HS200, 2B710) were collected, for a total of 284 leaves of corn. The collection of leaves was performed at random in the experimental fields of EMBRAPA Corn and Sorghum, in the city of Sete Lagoas-MG, Brazil, characterized by samples with variation in disease severity. Collected leaves were individually scanned, and images with 300 dpi resolution were transferred to a microcomputer. Then, each leaf was analyzed for the proportion of the impaired area using the QUANT program (Vale et al., 2003), by the discriminant analysis method, which gave the actual disease severity (%). This method was used because these results have more reliability; other methods can return divergent results because, for this pathosystem, the lesion color is similar to the color of leaves aging naturally.

From the minimal and maximal severity of the disease found in the leaves analyzed, using the Weber–Fechner visual acuity law (Horsfall and Barrat, 1945) and following a logarithmic scale, another seven intermediate levels of disease were established to compose the diagrammatic scale. Therefore the diagrammatic scale was establishing with nine severity levels.

Scale validation was performed in two steps. First, 10 raters, all of them inexperienced in assessing white spot in corn, analyzed 41 images of corn leaves. These images, with different levels of disease severity, were inserted in individual slides for view in a Power Point program and were not used in the proposed diagrammatic scale. In the second step of scale validation, raters received another set of 41 images of corn leaves to perform estimates using the proposed scale.

From the data of each rater, the accuracy and precision were determined through simple linear regression between the actual severity (independent variable) and the estimated severity (dependent variable) with and without the scale. The precision was evaluated through the determination of the regression ($R^2$) coefficient and error variance (the estimated value minus the actual severity). The accuracy of the estimates was determined by the $t$ test applied to the intercept of the linear regression ($a$) to verify whether it was significantly different than 0, and to the line angular coefficient ($b$) to test whether it was significantly different than 1, at the level 5% probability. Intercept values significantly different than 0 indicate the presence of constant deviations, while values of line angular coefficient that significantly deviate from 1 indicate the presence of systematic deviations (Nutter et al., 1993).

The reproducibility of the evaluations was determined based on the $R^2$ values of the linear regression between the estimated severities by different raters combined into pairs, as proposed by Nutter and Schultz (1995). Regressions between the actual and estimated severity for each rater, as well as among raters, were performed with the MINITAB program, version 14.

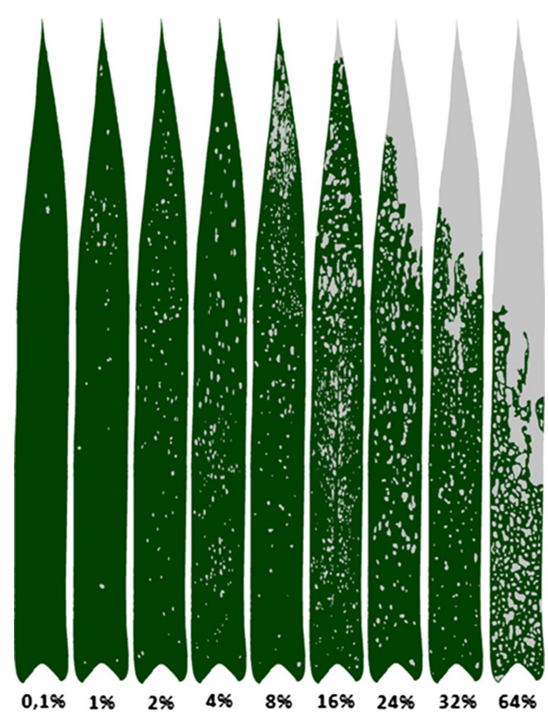


**Fig. 1.** Diagrammatic scale for the evaluation of the severity of white spot in corn (*Zea mays* L.) caused by *Pantoea ananatis*. Values are the percentage (%) of foliar area with disease symptoms.

To determine which corn leaf better represents the severity of the infection, the same leaves used for the construction of the diagrammatic scale were used. The main corn ear leaf was termed leaf 0; the leaf above leaf 0 was leaf +1, while the leaf below leaf 0 was leaf -1, and then each leaf was numbered consecutively.

The analyses consisted of the determination of the correlation between the severity of each leaf and the respective severity of the whole plant (e.g., the correlation of leaf +1 from a plant to the severity of this same plant). Therefore, this procedure was performed for the leaves of each plant for all 25 plants analyzed. The leaves analyzed ranged from leaf +7 to leaf −5, including combinations of more than one leaf. Such combinations of leaves had the purpose of obtaining higher values of correlation.

In addition, simple linear regression was performed to determine the standards *a* and *b* of regression. This analysis helps to choose the leaf or combination of leaves that better represent the actual severity value of the whole plant, which increases the reliability of this determination.

## 3. Results and discussion

The diagrammatic scale proposed in this paper, with nine classes of severity (0.1, 1, 2, 4, 8, 16, 24, 32 and 64%) follow the Weber–Fechner stimulation law, as described in previous papers on scale elaboration (Michereff et al., 2000; Diaz et al., 2001; Leite and Amorim, 2002; Rodrigues et al., 2002; Martins et al., 2004; Mazaro et al., 2006; Halfeld-Vieria and Nechet, 2006) and can be observed in Fig. 1. At severity levels above 8% lesions, coalescence is observed, which is typical of white spot in corn (Pereira et al., 2005).

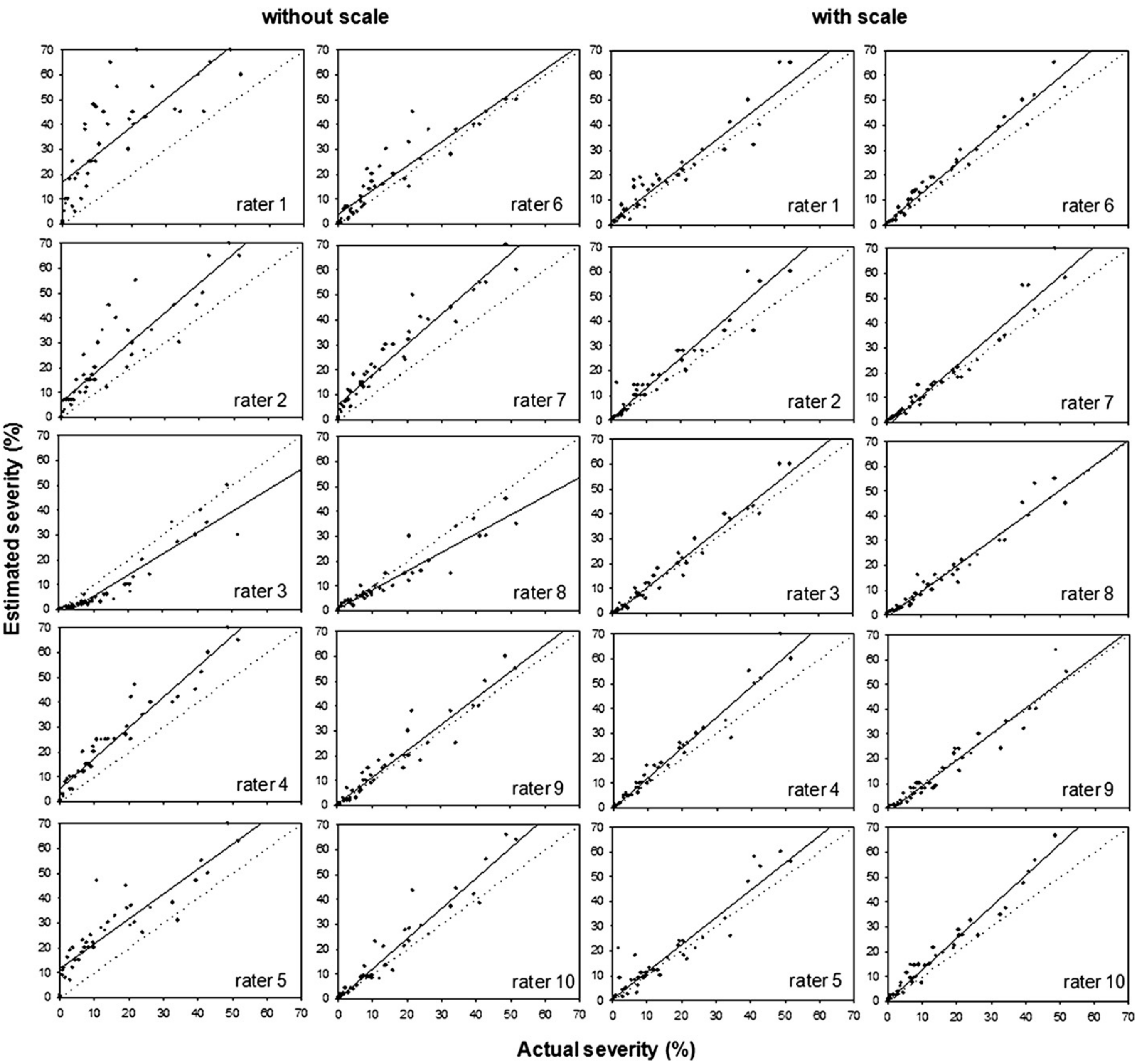


**Fig. 2.** Estimated severity without and with the diagrammatic scale elaborated (full points) and the regression line obtained between the actual and estimated severity (full line) of white spot (*Pantoea ananatis*) in corn (*Zea mays*) for all ten raters. Traced line represents the ideal situation with estimates equal to the actual ones.

For most raters, when using a diagrammatic scale, the estimated severity values were closer to the actual severity values (Fig. 2). The raters' accuracy, determined by the proximity of the estimated and actual values, is defined as the exactness of a measure with no systematic errors, which is measured by the intercept ($a$) and angular coefficient ($b$) of the linear regression between the estimated and actual severity (Bergamin Filho and Amorim, 1996). Thus, accurate raters have average severity estimates close to the overall mean (Martins et al., 2004).

For diagrammatic scale validation, the intercept values ($a$) differed from zero ($P < 0.05$) for 70% of the raters (raters 1, 2, 3, 4, 5, 6 and 7) when they did not use the scale. When the raters used the diagrammatic scale, the intercept values ($a$) did not differ from zero ($P < 0.05$) for 100% of the raters, indicating that constant deviations did not occur with scale use (Table 1). The linear angular coefficient ($b$) differed from one ($P < 0.05$) for 60% of the raters (raters 2, 3, 4, 6, 7 and 10) when they did not use the scale. When they used the diagrammatic scale, the linear angular coefficient ($b$) differed from one ($P < 0.05$) for 60% of the raters (2, 3, 4, 7, 8 and 10), indicating that when using the diagrammatic scale some systematic deviations remain (Table 1). When the raters used the diagrammatic scale, for each 1% increment in actual severity of white spot in corn, the severity estimated by the raters was on average 1.145. This show a small trend to overestimate the disease severity (Table 1). The majority of the raters (70%) had positive errors (Fig. 3).

For most studies involving diagrammatic scale validation, raters tend to overestimate disease severity levels (Newton and Hachett, 1994; Parker et al., 1995; Diaz et al., 2001). In some cases, underestimation of the disease severity levels occurs (Michereff et al., 2000; Gomes et al., 2004). The solutions to correct overestimation of disease severity levels vary according to the error magnitude and can be corrected by training the raters (Nutter and Schultz, 1995).

Precision is also a factor to be considered in diagrammatic scale validation, and it is defined as the exactness of an operation where there is hardness or refinement in measure (Bergamin Filho and Amorim, 1996). It can be evaluated using the regression determination coefficient, which must be close to 100%, as well as by the variation in the error. The precision levels of visual estimates of white spot with the diagrammatic scale were close to those found in other studies of scale validation (Michereff et al., 2000; Gomes et al., 2004; Martins et al., 2004) because the $R^2$ value was higher than 90%, which is considered optimal for this kind of evaluation (Bergamin Filho and Amorim, 1996). It was observed that with use of the scale, the determination coefficient was between 90 and 97%, with a mean of 94%, while, without use of the scale, this value was between 61 and 90%, with a mean of 86% (Table 1), indicating that, when using scale, the raters give estimates that are systematically related to the actual value. The increased precision was also confirmed by the decrease in errors (Fig. 3). Without using the diagrammatic scale, the raters had more deviations in errors, and 50% of raters (raters 1, 2, 4, 5 and 7) had errors higher than 30% (Fig. 3). However, with the diagrammatic scale, few errors were above 15%, and most were concentrated in the range of 10% (Fig. 3). The values of these residues were found to be good according to the results obtained with computer programs for rater training, such as Disease.Pro (Nutter and Worawitlikit, 1989), which classifies a rater as excellent if the errors do not exceed 5% and good if the errors are lower than 10%. The presence of some error level in measurements can be balanced by speed and standardization resulting from the use of diagrammatic scales (Stonehouse, 1994).

With use of the scale, constant errors were eliminated, but it was observed that systematic errors continued, even with raters with high precision, indicating a mild divergence between accurate and precise estimates, as observed by Nutter Jr. et al. (1993), in measuring foliar spot of *Agrostis palustris* caused by *Sclerotinia homoeocarpa* and by Gomes et al. (2004) measuring lettuce cercosporiosis caused by *Cercospora longissima*. Evaluations performed with less accuracy than precision do not represent serious problems, given that deviations normally follow the same pattern (Gomes et al., 2004).

In addition to accuracy and precision, another indicator to develop the diagrammatic scale efficiency is the reproducibility of estimates between raters (Berger, 1980). Different raters, when using the same scale to evaluate the same disease on a plant, should give the same severity values (Nutter and Schultz, 1995). According to these authors, the reproducibility can be estimated by the correlation of severities estimated by raters in pairs. So, when the determination coefficient of the correlation reaches 100%, estimates of the raters are repeated (Leite and Amorim, 2002; Belasque et al., 2005). Scales with good reproducibility means that assessments of the disease could be performed by different people. In the regression of severities estimated by raters in pairs, it was observed that without the scale, the determination coefficients were lower than 80% in 48% of comparisons to other raters (Table 2). With the proposed scale, the determination coefficient was higher than 80%, and in 82% of the cases the determination coefficients were higher than 90%, indicating that estimates performed with the scale are reproducible (Table 2).

For determination of the best leaf, or combination of leaves, to represent the severity of white spot in the whole plant, it was found that all leaves (from leaf +7 to −5) were statistically correlated to the severity of the whole plant (Table 3). The severity on the plant ranged from 0.13% to 26.38%, while severities of the leaves ranged from 0.01% to 64%. In total, 284 leaves were analyzed, and it was observed that more than 60% (174 leaves) had a severity between 0 and 5%, showing that the scale needs more points in this severity range.

The higher correlation values were observed in combinations of leaves, except leaf −2, which had a correlation of 0.90 (Table 3). It is important to mention the need to analyze $a$ and $b$ from the linear regression because a leaf can have a high correlation to the mean of the whole plant, but this value can be different than the actual one. In the analysis of 25 leaves at leaf position −2 with severity of their respective plants, a severity correlation of 0.90 was observed between these leaves and the whole plant, but this correlation value did not represent the actual severity of the whole plants. Therefore, when determining the best leaf, or combination of leaves, there is a need for additional analysis of the $a$ and $b$ values from the linear regression and comparing them to 0 and 1,

**Table 1**
Estimates of intersection standards ($a$), of angular coefficients ($b$) and determination coefficients ($R^2$) of linear regression equations calculated between the actual and estimated severity of white spot in corn, performed by non-experienced raters, without and with the diagrammatic scale.

| Raters | Without scale | | | With scale | | |
|---|---|---|---|---|---|---|
| | $a$ | $b$ | $R^2$ | $a$ | $b$ | $R^2$ |
| 1 | 16.821*[a] | 1.118$^{ns}$ | 0.61 | 1.235$^{ns}$ | 1.086$^{ns}$ | 0.91 |
| 2 | 6.206* | 1.195* | 0.81 | 0.651$^{ns}$ | 1.223* | 0.92 |
| 3 | −3.102* | 0.852* | 0.89 | −0.917$^{ns}$ | 1.118* | 0.97 |
| 4 | 5.272* | 1.230* | 0.93 | −0.105$^{ns}$ | 1.223* | 0.96 |
| 5 | 11.733* | 1.001$^{ns}$ | 0.81 | 0.498$^{ns}$ | 1.102$^{ns}$ | 0.90 |
| 6 | 3.968* | 0.972$^{ns}$ | 0.85 | 0.706$^{ns}$ | 1.167* | 0.97 |
| 7 | 6.002* | 1.214* | 0.93 | −1.463$^{ns}$ | 1.203* | 0.95 |
| 8 | 0.937$^{ns}$ | 0.754* | 0.89 | −0.856$^{ns}$ | 1.021$^{ns}$ | 0.95 |
| 9 | 0.428$^{ns}$ | 1.069$^{ns}$ | 0.92 | −0.343$^{ns}$ | 1.049$^{ns}$ | 0.94 |
| 10 | −0.073$^{ns}$ | 1.217* | 0.93 | 0.238$^{ns}$ | 1.262* | 0.97 |
| Mean | 4.819* | 1.062$^{ns}$ | 0.86 | −0.036$^{ns}$ | 1.145$^{ns}$ | 0.94 |

[a] ns non-significant and * situations where the line intersection value (a) or angular coefficient (b) was different than '0' and '1', respectively, by the $t$ test ($P \leq 0.05$).

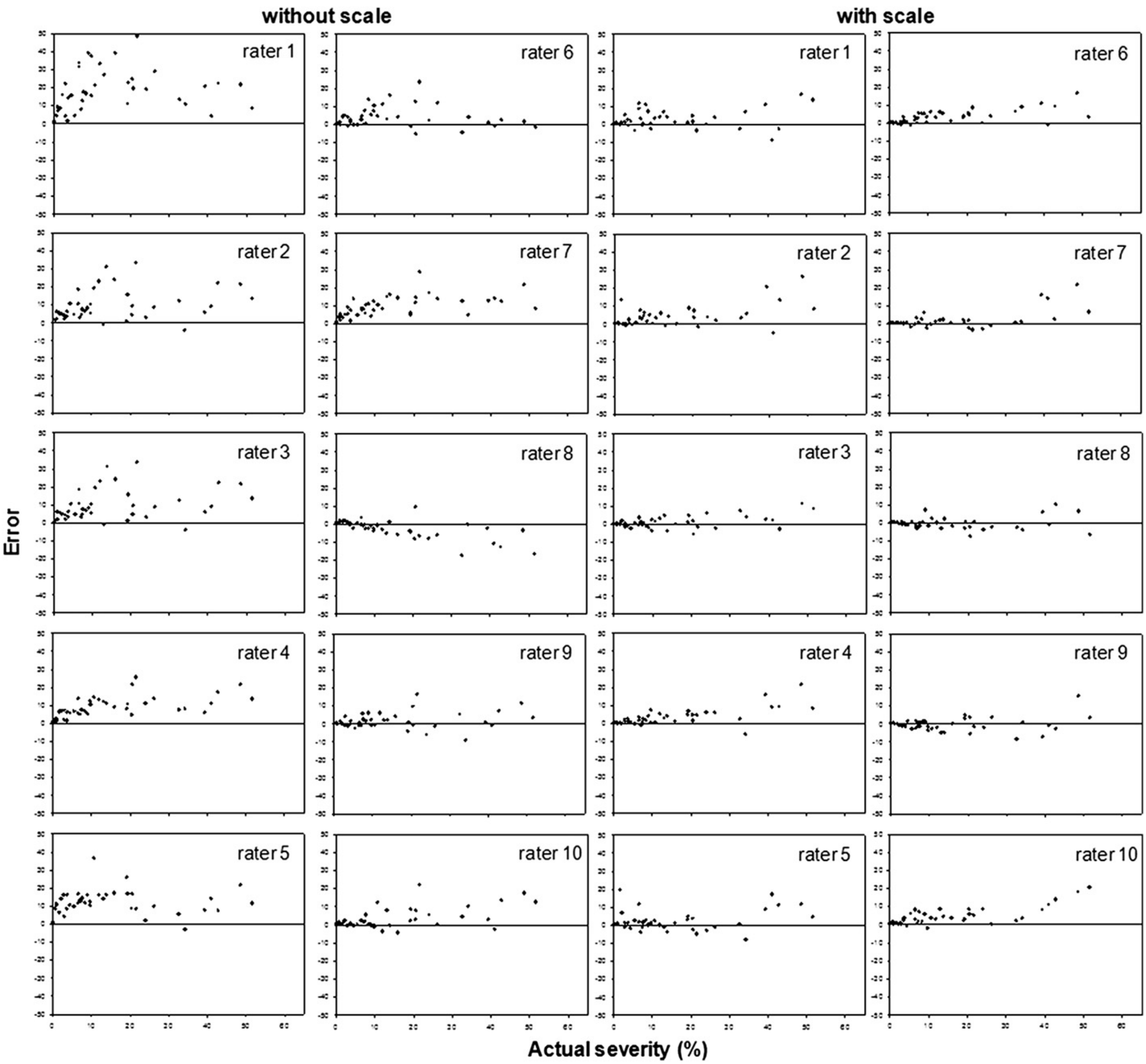


**Fig. 3.** Errors (the estimated values minus the actual severity) for all ten raters without and with the diagrammatic scale to assess the severity of white spot (*Pantoea ananatis*) in corn (*Zea mays*). The use of the diagrammatic scale visibly diminished the error of each rater.

respectively. Thus, this analysis allows us to determine if the intercept and regression line inclination are close to actuality for severity of the analyzed leaf and of the whole plant.

Therefore, leaf −2, the combination of leaves −2 and −1, and the combination of leaves −2, −1 and 0, even with a correlation value higher than 0.90, do not represent the best leaf or combination leaves for the determination of the actual severity of the whole plant because they had *b* values different than 1 (Table 3).

Analyzing this set, the correlations and the values *a* and *b*, the following combinations of leaves were the best for representing the severity of the whole plant: 1st) leaves −1 and 0; 2nd) leaves −1, 0 and +1; 3rd) leaves −2, −1, 0 and +1; 4th) leaves −1, 0, +1 and +2; 5th) leaves −2, −1, 0, +1 and +2 (Table 3). Due to the greater facility of evaluation, we recommend that in evaluations the mean severity of leaves −1 and 0 be adopted because only two leaves would be needed for evaluation, and there would still be no statistically significant losses in the determination of the severity of the whole plant.

The proposed diagrammatic scale to evaluate the severity of white spot in corn allowed the assessment of the disease symptoms

**Table 2**
Reproducibility of estimates of white spot severity in corn, represented by the frequency of determination coefficients ($R^2$) of simple linear regression equations relating estimates between raters, without and with the diagrammatic scale.

| $R^2$ value | Frequency (%)[a] | |
|---|---|---|
| | Without scale | With scale |
| 0.70–0.79 | 48.90 | 0.00 |
| 0.80–0.89 | 37.80 | 17.80 |
| 0.90–1.00 | 13.30 | 82.20 |

[a] Calculated considering the number of occurrences of the interval in relation to the total of 45 possible combinations between ten raters for each evaluation of the disease severity.

**Table 3**
Estimates of correlation e standards of linear regression used to determine the leaf, or leaf combination, which better represents white spot (*Pantoea ananatis*) severity in the whole plant.

| Evaluated leaves[a] | Performed analyses[b] | | |
|---|---|---|---|
| | Correlation | Value of *a* | Value of *b* |
| Leaf +7 | 0.85* [c] | 4.50* | 0.83$^{ns}$ |
| Leaf +6 | 0.78* | 3.91* | 1.47$^{ns}$ |
| Leaf +5 | 0.76* | 4.02* | 1.41$^{ns}$ |
| Leaf +4 | 0.80* | 3.72* | 2.00* |
| Leaf +3 | 0.79* | 4.80* | 0.75$^{ns}$ |
| Leaf +2 | 0.84* | 2.95* | 1.38$^{ns}$ |
| Leaf +1 | 0.83* | 2.88* | 1.04$^{ns}$ |
| Leaf 0 | 0.80* | 3.26* | 0.70$^{ns}$ |
| Leaf −1 | 0.87* | 1.98$^{ns}$ | 0.64* |
| Leaf −2 | 0.90* | 1.75$^{ns}$ | 0.58* |
| Leaf −3 | 0.78* | 2.32$^{ns}$ | 0.48* |
| Leaf −4 | 0.76* | 2.06$^{ns}$ | 0.29* |
| Leaf −5 | 0.88* | 0.22$^{ns}$ | 0.49* |
| Leaf −2 −1 | 0.97* | 0.61$^{ns}$ | 0.73* |
| Leaves −1 and 0 | 0.94* | 1.17$^{ns}$ | 0.84$^{ns}$ |
| Leaves 0 and +1 | 0.84 | 2.81* | 0.89$^{ns}$ |
| Leaves −2, −1 and 0 | 0.96* | 0.81$^{ns}$ | 0.79* |
| Leaves −1, 0 and +1 | 0.94* | 1.13$^{ns}$ | 0.97$^{ns}$ |
| Leaves −2, −1, 0 and +1 | 0.96* | 0.92$^{ns}$ | 0.88$^{ns}$ |
| Leaves −1, 0, +1 and +2 | 0.95* | 1.16$^{ns}$ | 1.10$^{ns}$ |
| Leaves −2, −1, 0, +1 and +2 | 0.96* | 1.00$^{ns}$ | 0.98$^{ns}$ |

[a] Leaf 0 corresponds to the leaf of the corn ear. Leaves +1 and −1 are immediately superior and inferior to leaf 0, respectively.
[b] Analyses of correlation and linear regression were performed, both at 5% of probability, the latter represented by values of *a* and *b* of the regression.
[c] $^{ns}$ non-significant and * situations where the line intersection value (a) or angular coefficient (b) was different than ‘0’ and ‘1’, respectively, by the *t* test ($P \leq 0.05$).

in an accurate, precise and reproducible manner. The assessment of disease in only two leaves (0 and −1) is suggested to estimate the severity in the whole plant, which can optimize studies in corn pathosystem vs. white spot. Therefore, the presented scale is a worthy tool for research, such as in epidemiological studies and in comparison among methods of disease control, providing more adequate information for this pathosystem.

## 4. Conclusion

1. Assessment of disease in only two leaves (leaf 0 and leaf −1) was proven to estimate the severity of the in the whole plant.
2. The proposed diagrammatic scale allowed the assessment of the disease symptoms in an accurate, precise and reproducible manner.

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