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CROP PRODUCTION

Proposed diagrammatic scale to assess heat injury in coffee seedling canopy

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ABSTRACT. A diagrammatic scale with five levels (0, 0.1 – 2.0, 2.0 – 6.0, 6.0 – 10, and 10 – 14) was developed and evaluated to measure the symptoms of heat injury in a coffee seedling canopy. The scale was constructed to increase assessment efficiency and align the estimations more closely with the actual values. Two assessments with the diagrammatic scale and one without were conducted with an interval of seven days. The evaluators using the proposed scale presented estimates with better levels of precision, accuracy, reproducibility, and repeatability than those using a conventional method. The proposed diagrammatic scale was shown to provide a reliable estimate for assessing the symptoms of heat injury on the canopy of in *Coffea arabica* L. seedlings. Therefore, it is possible to standardize heat injury evaluation methods using this diagrammatic scale, allowing for data comparisons with different cultivars.

Keywords: *Coffea arabica* L.; Lin´s method; temperature and water stress.

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Introduction

Brazil is the world's largest coffee producer and exporter (USDA, 2019), with an estimated production of 62.02 million 60 kg-bags for 2019/2020 (CONAB, 2020). Coffee production can be negatively affected by adverse weather conditions such as irregular rainfall distribution and increased average temperature (IPCC, 2020), which is increasingly relevant as coffee cultivation has expanded to marginal regions where drought and high temperatures are the main limitations to production (Da Matta & Ramalho, 2006).

Coffea arabica L. cultivars do not tolerate large variations in temperature. The ideal annual average for temperature is 19-22°C, and for precipitation is 1,500-1,800 mm per year (Camargo & Pereira, 1994). Changes in water supply can reduce growth, even when the typical responses of plants under these conditions, such as leaf wilt, are not present (Silva, Silva, Coelho, Rezende, & Sato, 2008). In addition, the combined effects of low water availability in the soil and high air temperatures induce alterations in metabolic processes, and in extreme cases, leaf necrosis and plant death (Taiz, Zeiger, Møller, & Murphy, 2017).

Despite the impacts of water-thermal stress on coffee plants, there are no means to quantify damage in the affected area; therefore, it is challenging for producers to estimate the effects of this type of stress in the field and for researchers to propose alternative, effective methods. The use of diagrammatic scales presents a practical and rapid method of performing these estimations (Madden, Hughes, & Vand Den Bosch, 2007).

The scales consist of illustrations of injured plant parts within a range of severity values that are used for symptom assessments and as comparative guides when estimating the degree of damage (Campbell & Madden, 1990). This methodology has been frequently used to estimate plant disease severity (Azevedo de Paula et al., 2016; Perina et al., 2019; Silva, Rafael, Pereira, Peche, & Pozza, 2019), although it can also be employed for the measurement of other plant injuries.

Thus, considering the lack of standardized and evaluated methods to quantify the effects of stress on coffee plants, the objective of this study was to develop a diagrammatic scale to analyze temperature and water stress impacts on coffee plants.

Material and methods

Simulation of temperature and water stress to obtain symptoms

An experiment to simulate temperature and water stress was conducted in an environmentally controlled greenhouse with average temperature and relative humidity of 22 ± 2°C and 80 ± 2%, respectively, to obtain a symptom scale for coffee seedlings.

Coffee seedlings (*C. arabica* L.) of the cultivar Catuaí Vermelho IAC 144 were planted with four pairs of leaves in 11-L pots arranged on a stand 1 m from the ground. The substrate moisture was maintained at 100% of field capacity for 30 days to ensure the full establishment of seedlings (Castanheira et al., 2019). Irrigation was then performed according to each treatment.

The experiment was conducted with five levels of irrigation (40, 50, 60, 70, and 80% of field capacity), in a randomized block design with four replications and three vessels per replications. A total of 60 plots were used, with each plot consisting of one pot containing one coffee seedling.

Treatment management

The irrigation water was replaced based on the estimated gravimetric weight difference between the pots under field capacity conditions and those of each treatment using electronic scales (Lanna et al., 2016).

Coffee seedlings were investigated three times a week for irrigation requirements using five reference pots located in the greenhouse, each corresponding to one irrigation level. When the evapotranspiration of water from seedling leaves induced weight loss, the seedlings were irrigated to reestablish the appropriate weight for each treatment (Lanna et al., 2016).

Simulation of thermal stress in a greenhouse

The experiment was conducted over 130 days. Thermal stress was then simulated in the greenhouse by increasing the temperature and reducing the relative humidity for 4h each morning to 45°C and 38%, respectively. After 10 days under thermal stress, the 60 coffee seedlings were photographed to develop a diagrammatic scale.

Diagrammatic scale development

All plant materials were photographed on a white background, using a Sony Cybershot DSC/-H7/H9 (Sony, Brazil) digital camera, in automatic mode, with an 18-55 mm lens focal length. A photograph from the top displayed the plant canopy. Subsequently, total and injured canopy leaf areas were determined using Assess® software (American Phytopathological Society), and all necrotic tissues formed through heat injury were considered dead. According to the minimum and maximum levels found, a frequency plot was constructed by plotting the percentage of damaged leaf area (x-axis) in severity intervals of 1-14% (y-axis).

These values were then adjusted to simple linear, non-linear exponential, and logarithmic models (Campbell & Madden, 1990). The model that best fit the frequency plot was chosen, as indicated by the largest R^2 (coefficient of determination) and the significance of the parameters of the equations from the *t*-test. The heat injury scale was created based on the interval when the greatest concentration of plants had the same percentage of damaged area. The severity intervals for each score were established according to Weber-Fechner's visual acuity law (Horsfall & Barrat, 1945; Nutter Jr. & Schultz, 1995) and the shape and distribution of the lesions. Photographs of the coffee seedling canopy with heat injury were then used to develop the scale.

Diagrammatic scale validation

To validate the diagrammatic scale, 60 coffee seedlings showing symptoms of heat injury were used, representing various levels of damage severity. In three evaluations, eight evaluators inexperienced in the quantification of plant heat injury observed seedling images using Microsoft PowerPoint 2010. The first evaluation was performed without using the scale, and after an interval of 7 days, a second evaluation was performed, aided by the diagrammatic scale. To assess the repeatability of the observed values, a third evaluation using the proposed scale was performed after 7 days. Based on the data obtained from each evaluator, Lin's method was used to refine the accuracy and precision of the developed scale. Lin's concordance correlation coefficient (Lin, 1989), which assesses agreement between pairs of observations, was used to measure the difference between the actual and estimated heat injury severities. The method included other variables to aid in the validation. The scale shift factor measures the difference between the actual and estimated values, and is calculated as the difference between the slope of the fitted regression lines and the concordant line, where 1 = perfect agreement between x and y. The location shift factor estimates the change in the adjusted regression line relative to the concordant line by measuring the difference in height between the two lines, and 0 = perfect agreement between x and y. The BIAS correction factor, which measures the deviation of the fitted line from the concordant line, was calculated from the location and scale shift factors based on the means and standard deviations of x and y. In addition Pearson's correlation was used to evaluate the precision of the assessments. The confidence interval (CI) ($p < 0.05$) between the groups of evaluators, with and without the use of the scale, was calculated to determine if there were significant differences between the evaluations. The repeatability of the estimates from each evaluator was determined by the R^2 values of the linear regression between the two assessments using the scale (Nutter Jr. et al., 1993). The reproducibility of the estimates was evaluated by the R^2 values from linear regressions between the estimated severities of the same sample unit with different evaluators in pairs (Campbell & Madden, 1990; Kranz, 1988; Nutter Jr. & Schultz, 1995). The data were tabulated, and statistical analyses were performed using the RStudio software (R Core Team, 2016), and the epi.ccc function of the epiR package (Stevenson et al., 2012) to determine Lin's concordance correlation coefficient.

Results

The minimum and maximum severity of heat injury in the coffee seedling canopy were 0% and 13.6%, respectively, with a high proportion of seedlings at the level of 0–2% (Table 1). The scale had a maximum level of 13.6% with some necrotic areas, based on the heat injury severity observed. The exponential model adjusted for the frequency values in the severity intervals was optimal according to Weber Fechner's law, as it returned the greatest R^2 (0.95%) and slope parameter significance of the equations in the *t*-test (Table 2). The severity scale was developed using five scores or percentage intervals (Figure 1), three of which were distributed into intervals reaching 6.0% of the diseased leaf area. The interval up to 2% included 65% of the total plants and constituted the highest frequency. The five percentage severity intervals of the scale were 0, 0.1 – 2.0, 2.0 – 6.0, 6.0 – 10.0, and 10.0 – 14.0%.

Table 1. Frequency distribution of severity values of heat injury in coffee seedlings at unit intervals (%).

Interval (Severity %)	Frequency
0 – 1	28
1 – 2	11
2 – 3	1
3 – 4	3
4 – 5	6
5 – 6	1
6 – 7	0
7 – 8	1
8 – 9	1
9 – 10	1
10 – 11	1
11 – 12	0
12 – 13	1
13 – 14	1

Table 2. Parameters of the linear and non-linear models for the frequency of heat injury in coffee by severity interval.

Models	Y_0^c	R^b	QMR	R^{2a}
Linear	25.43*	-2.18	123.29	0.46
Monomolecular	340.42	1.09*	6.81	0.97
Exponential	253.17	-0.94*	10.11	0.96
Gompertz	2049.04	0.37*	5.38	0.98

a) Coefficient of determination (R^2); b) Progress rate (R); c) Initial heat injury (Y_0); *significant according to the *t*-test ($p = 0.05$).

According to Lin's method, estimates of disease severity improved with the use of the proposed scale (Table 3), which was verified by the concordance coefficient and correlations between the actual and estimated values, which showed greater estimation efficiency using the scale (a = 0.82) than conventional evaluations (a = 0.24). Without the scale, the evaluators overestimated disease severity (c =1.18) while underestimations occurred when using the scale (c = -0.26). The Pearson's correlation coefficient indicated increased precision with the scale (e = 0.86), than without (e = 0.76), and the BIAS correction factor without the use of the scale (d = 0.31) was lower than that of the estimates obtained using the scale (d = 0.94). This indicates an increase in the accuracy of the evaluators. Considering the confidence intervals, the assessments for heat injury in seedlings with and without the diagrammatic scale differed significantly at the 95% confidence interval.

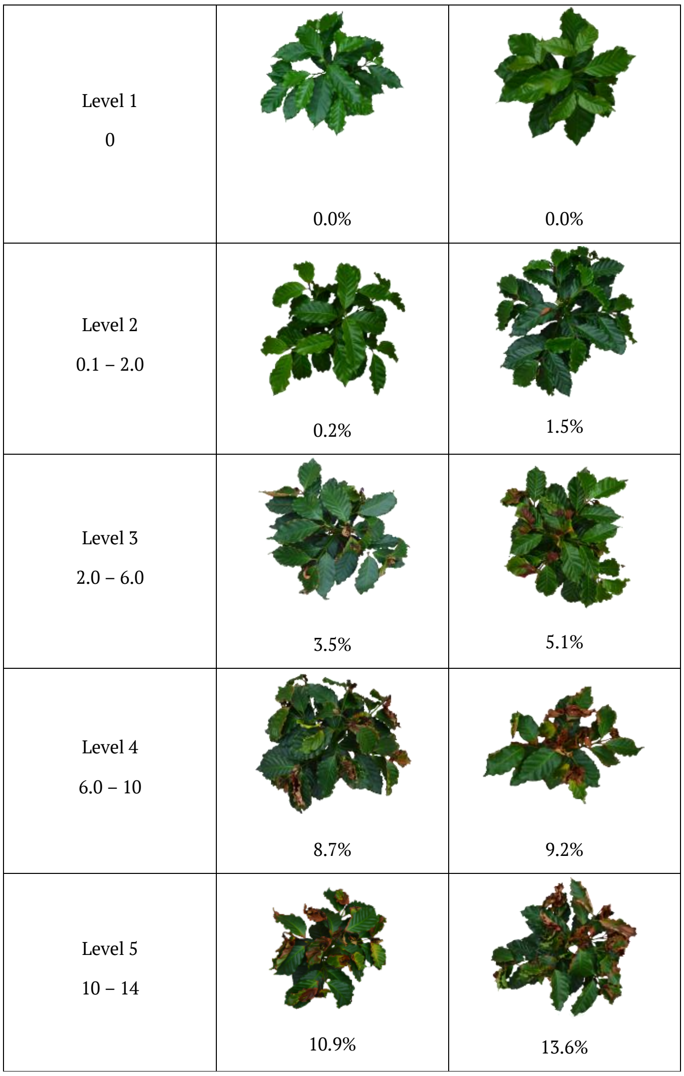

Figure 1. Diagrammatic scale to quantify the percentage severity of heat injury in coffee seedlings (*Coffea arabica* L.). Numbers below each picture represent the actual percentage of leaf area affected by thermal and water stress.

Table 3. Lin's concordance correlation coefficients for 8 evaluators without or with the use of the diagrammatic severity scale for estimating heat injury in coffee seedlings.

Lin´s statistic	Without scale	With scale	95% CI[c]
Lin's concordance correlation coefficient	0.24	0.82	0.47–0.69
Scale shift factor [a]	4.74	0.82	1.61–3.28
Location shift factor [b]	1.18	-0.26	0.05–0.74
BIAS correction factor	0.31	0.94	0.53–0.78
Pearson's correlation	0.76	0.86	0.86–0.90

[a]Scale shift factor relative to perfect agreement. [b]Location shift factor relative to perfect agreement. [c]Upper and lower limits of the 95% confidence intervals. [Bold]represents a significant difference ($p \leqslant 0.05$) between the two evaluations, according to the *t*-test.

For reproducibility without the diagrammatic scale, the value of the determination coefficient (R^2) ranged from 40 to 90%, with a mean of 74.4% (Table 4). Using the scale, R^2 values ranged from 46 to 92% (mean = 73.5%) for the first evaluation, and 46 to 93% (mean = 70%) for the second evaluation.

Table 4. Coefficients of determination (R^2) of the linear regression equation between pairs of different evaluators, with or without the use of the heat injury severity assessment scale in two evaluations estimating the damage severity on coffee seedlings.

Without scale							
Evaluator	B	C	D	E	F	K	L
A	0.88	0.87	0.82	0.79	0.59	0.77	0.86
B		0.88	0.86	0.80	0.65	0.77	0.81
C			0.90	0.85	0.69	0.62	0.78
D				0.82	0.74	0.64	0.78
E					0.72	0.52	0.78
F						0.40	0.63
K							0.64
With scale – 1st assessment							
Evaluator	B	C	D	E	F	K	L
A	0.65	0.79	0.74	0.62	0.84	0.46	0.51
B		0.87	0.86	0.81	0.76	0.68	0.85
C			0.92	0.74	0.85	0.66	0.79
D				0.71	0.83	0.63	0.82
E					0.77	0.74	0.65
F						0.63	0.65
K							0.61
With scale – 2nd assessment							
Evaluator	B	C	D	E	F	K	L
A	0.57	0.70	0.73	0.63	0.72	0.72	0.65
B		0.73	0.65	0.70	0.72	0.76	0.46
C			0.93	0.87	0.78	0.89	0.62
D				0.81	0.75	0.85	0.62
E					0.72	0.76	0.75
F						0.72	0.58
K							0.48

Good repeatability was observed between the estimates by the same evaluators (Table 5). Between the two assessments with the use of the scale, only one evaluator (D) exhibited an intercept significantly different from 1 ($p < 0.1$), while high precision levels were found in 75% of the estimates. All evaluators showed acceptable repeatability in the estimates of heat injury severity.

Absolute errors were reduced with the use of the scale, which decreased the range of values between the first and second evaluations (Table 5). However, in the second evaluation using the scale, the minimum and maximum values observed for the residuals of all evaluators were, respectively, 0.46 and 0.89, thereby increasing the range of the determined values.

Table 5. Intercept (β_0), slope (β_1), and coefficient of determination (R^2) of the linear regression equations relating to the first and second estimates of heat injury severity on seedlings, for estimates performed by 8 evaluators using the heat injury severity scale.

Evaluator	β_0	β_1	R^2
A	0.30 ^{ns}	0.68 ^{ns}	0.58
B	0.01 ^{ns}	0.58 ^{ns}	0.76
C	0.12 ^{ns}	0.96 ^{ns}	0.90
D	0.37 *	0.89 ^{ns}	0.83
E	0.25 ^{ns}	1.23 ^{ns}	0.74
F	0.11 ^{ns}	0.61 ^{ns}	0.82
K	0.23 ^{ns}	0.76 ^{ns}	0.67

*ns represent situations where the null hypothesis ($\beta_0 = 0$ or $\beta_1 = 1$) was rejected or not rejected, respectively, according to the *t*-test ($p = 0.05$).

Discussion

Abiotic stresses such as extreme temperature, drought, salinity, or chemical toxicity represent severe limitations to agriculture production by reducing average yields for major crop species to less than 50% (Bray, Bailey-Serres, & Weretilnyk, 2000). The strongest climatic limitations of coffee are frost and drought (Da Matta & Ramalho, 2006). The successful economic exploitation of coffee crops is limited by temperature, because coffee growth is particularly affected by both high and low temperatures (Barros, Mota, Da Matta, & Maestri, 1997; Silva, Da Matta, Ducatti, Regazzi, & Barros, 2004). In addition, frequent drought episodes affect coffee production, decreasing yields by up to 80% in very dry years (Da Matta & Ramalho, 2006). Many of the injuries caused by abiotic factors can be mistaken for biotic symptoms, and according to Da Matta and Ramalho (2006), studies on the effects of drought on coffee physiology have often been conducted on plants grown in small containers under greenhouse conditions.

Quantifying the effects of stress on coffee plants is essential for assessing and standardizing the evaluations. The use of diagrammatic scales is useful for estimating plant disease severity, and as observed in this study, the effects of temperature and water stress on coffee plants. Diagrammatic scales exist for coffee crops (Azevedo de Paula et al., 2016; Belan et al., 2014; Belan et al., 2020; Capucho, Zambolim, Duarte, & Vaz, 2011; Custódio et al., 2011) in relation to injury from various pathosystems. However, consideration must be given to conditions arising from abiotic factors, such as the injury by heat as discussed here. From the information acquired, it was possible to develop a diagrammatic scale representing the symptoms of heat injury in coffee seedlings. As the majority of the coffee seedling canopy samples were concentrated in the first and second levels of the scale, the higher degrees of injury were reduced.

The maximum amount of heat injury observed in the greenhouse was 13.6%. The intermediate levels of the scale were determined based on the highest frequency intervals of heat injury and an exponential increase in heat injury values that was obtained by adjusting the exponential model on the frequency sampled coffee canopy. Based on the characteristics of the scale responsible for the ease of severity estimate interpolation we adapted the estimation for heat injury. These increments follow the principles of the "Horsfall and Barratt Scale" (Horsfall & Barratt, 1945), based on the Weber-Fechner Law (Campbell & Madden, 1990). The symmetry of the 50% severity intervals was not adopted because of the maximum acquired heat injury value (13.6%). The diagrammatic scale should follow the "Weber-Fechner Law" (logarithmic increments), without necessarily using the same intervals chosen by Horsfall and Barratt (1945), depending on the individual characteristics of the disease (Campbell & Madden, 1990), or as in this case, heat injury.

For researchers and plant breeders, plants or cultivars should be selected that have resistance to drought effects. Precision and accuracy are essential in the first intervals for the heat injury scale, and generally the range or maximum level of plant injury is defined considering resistant progeny. In this study, there was a tendency for the evaluators to overestimate when not using the scale (c = 1.18), as in most studies involving the validation of a diagrammatic scale (Andrade et al., 2019; Azevedo de Paula et al., 2016; Belan et al., 2020; Capucho et al., 2011; Custódio et al., 2011; Menge, Makobe, Shomari, & Tiedemann, 2013, Perina et al., 2019) in plant pathology. However, when the scale was employed, the evaluators underestimated the level of heat injury, which has also occurred in certain severity levels evaluated for diseases (Gomes, Michereff, & Mariano, 2004; Michereff, Maffia, & Noronha, 2000). The Pearson's correlation coefficient indicated increased evaluator precision when using the scale (e = 0.86), as opposed to without (e = 0.76). The value of the BIAS correction factor without using the scale (d = 0. 31) was lower than that of the estimates obtained with the scale (d = 0.94), which indicates an increase in the accuracy of the evaluators. Greater accuracy and precision in evaluating disease severity in different pathological systems based on diagrammatic scales has previously been shown (Belan et al., 2014; Belan et al., 2020; Capucho et al., 2011; Custódio et al., 2011; Menge et al., 2013). Therefore, to reduce subjectivity the scale should be used for training, with the aim of improving the accuracy at the time of evaluation (Azevedo de Paula et al., 2016).

By analyzing the coefficients of the linear regression equation, absolute errors were reduced when the scale was used which related to a decreased range of values between the first and second evaluations. The mean of the determination coefficient for repeatability was approximately 67% for heat injury on the seedling diagrammatic scale. In addition, for disease diagrammatic scales, Belan et al. (2014) found a mean determination coefficient for repeatability of 83.2% for the scale of bacterial blight. Belan et al. (2020) also observed an increase in the determination coefficient of the regression analysis for 92% of the evaluators using standard area diagrams constructed from color photographs for the assessment of coffee leaf rust in *Coffea canephora* L. Azevedo de Paula et al. (2016) obtained values of 96% and 97%, for brown eye spot in red and yellow cherries respectively.

Conclusion

The diagrammatic scale proposed for the assessment of heat injury of *C. arabica* coffee seedlings improved the precision, accuracy, and reproducibility levels of the evaluators. Therefore, the diagrammatic scale can standardize heat injury evaluation methods, allowing data comparison with different cultivars.

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