

Evaluation of Postharvest Stem-End Rot on Coconut Fruits

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Abstract

The stem-end rot on coconut fruits is the main postharvest crop disease, causing heavy losses especially when the product is sold fresh. The efficient and practical control of this plant disease has been difficult to date, such as the damage evaluation, which has been done exclusively by the incidence of the disease. However, the incidence parameter alone does not provide a satisfactory understanding of the lesion area and the intensity it can achieve during the storage period, and about the environmental conditions in which the fruits are subjected to the aggressiveness of *Lasiodiplodiatheobromae*, when it is confirmed as the causal agent of the disease, and the control measures applied. Thus, in this study we propose to evaluate the basal rot development in coconut fruits during postharvest and to prepare and validate a diagrammatic scale to enable the estimation of disease severity. Fruits were inoculated and the external and internal symptoms of disease developments were photographed to measure the damaged area, which were grouped into eight disease levels. Sixty images were assessed by 30 evaluators for three consecutive times at intervals of 7 days and data were subjected to linear regression analysis. Hence, it was possible to create and validate a diagrammatic scale to evaluate the disease severity on coconut fruits with an accurate, precise and reproducible manner. Furthermore, the proposed scale allowed us to distinguish the aggressiveness of different isolates of the fungus through the symptoms of internal fruit rot.

Keywords: diagrammatic scale, symptomatology, *Lasiodiplodiatheobromae*, epidemiology

1. Introduction

Coconut crop is extremely important for the supply of raw materials for food industry and crafts (Fontenele, 2005; Jeronimo and Silva, 2013) and, recently, feasibility studies of the use of coconut fruits for biodiesel production have been done (Araújo *et al.*, 2009; Farias *et al.*, 2011). Coconut water and copra are consumed *in natura* and the consumption of the first one has increased because of the health benefits brought about by its chemical composition, nutritional value and functional properties (Carvalho *et al.*, 2006), especially in tropical countries.

Brazil is the one of the four world's largest coconut producing countries with an estimated production of 1,909,705,000 fruits (IBGE, 2015). As long as the production increases also increases the phytosanitary problems and postharvest stem-end rot on fruits caused by the fungus *Lasiodiplodiatheobromae* (Pat.) Griffon and Maubl, which has been the main cause of losses in the coconut marketing (Viana *et al.*, 2008).

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Although plants infected with the fungus develop dry leaves, the greatest damage is recorded in fruits production (Halfeld-Vieira and Nechet, 2005). Losses increase during the period of transport, storage and commercialization, due to the fast fruit decay and loss of water quality.

One of the aggravating factors of the disease is the latency of fungus in fruits. *L. theobromae* internally colonizes the plant moving into the bunch and reaching the fruit stalk. Once the fungus is located below coconut bracts, it causes an externally dry rot (Viana *et al.*, 2002) while internally deteriorates mesocarp tissues causing cracks and exposing the liquid albumen (coconut water) to contamination and deterioration. There are no natural, synthetic or chemical products that provide an efficient control of this postharvest disease, although there are some indications of mechanical control for cooling fruits directed for exportation (Viana *et al.*, 2008).

One disease assessment difficulty is the lack of the effects visualization of the protective products applied to the fruit, since the pathogen action is not externally visible in the early stages of infection. The external signs of the pathogen can only be seen when the region below bracts is already affected (Viana *et al.*, 2002).

The intensity of postharvest stem-end rot on coconut fruits has not been carried out yet. Both the incidence and severity need to be explored as a tool for quantization of this disease. The development of a diagrammatic scale may provide a representation of the percentage of the area occupied by lesions, allowing the raters to position their estimations within levels represented by diagrams considering the limitations of the human visual acuity (Horsfall and Barrat, 1945), simplicity and facility for application.

Diagrammatic scales are essential to leverage the epidemiological understanding of path systems (Vivas *et al.*, 2010; Lima *et al.*, 2011; Menge *et al.*, 2013), especially those little explored, as in the case of *L. theobromae*-coconut, and to contribute, following plant disease control principles, for assessing the disease severity "inter and intra" variety of grown coconuts. Diagrammatic scales also contribute for assessing the aggressiveness of the pathogen (Djougoue *et al.*, 2010) among isolates from different geographic regions and their hosts (Dugan *et al.*, 2016); contribute for assessing the efficiency of products to be used in disease control (Locatelli *et al.*, 2015), as well as enable comparison between this disease and other types of injuries caused by other pathogens (*Ceratocystis paradoxa* (Dade) C. Moreau) and pests (*Aceria guerrerensis* Keifer) (Galvão *et al.*, 2008) that occur on the basal portion of coconut fruits. This study was conducted to evaluate the development of *L. theobromae* infections symptomatology on postharvest coconut fruits and to create and validate a diagrammatic scale to quantify the stem-end rot on coconut fruits.

1. Materials And Methods

1.1. Culture of the fungus

We isolated *L. Theobromae* (LAQUIBIO 10) from coconut fruits with postharvest stem-end rot symptoms. Monosporic culture was kept in vials with sterile distilled water (Castellani, 1964) at room temperature at the Instituto Superiores de Ensino do CENSA – ISECENSA mycological collection. Four millimeters mycelial discs of the fungus obtained from cultures with 7 days of growth were placed on potato dextrose agar (PDA) (Himedia®) in Petri dishes (90 mm in diameter).

1.2. Inoculation of coconut fruits

Coconut fruits were harvested from crop fields not sprayed with fungicides or insecticides, in São João da Barra, Rio de Janeiro, Brazil. Fruits were surface-disinfested with sodium hypochlorite (0.3%) for 5 minutes and rinsed with water. After air-drying, blocks of tissue with 10 mm diameter x 10 mm depth were removed from the basal portion of each fruit (the bracts were removed) using a cork borer in order to create injuries. We set up an assay with six groups of ten fruits that were inoculated with plugs of mycelium and medium (0.6 mm diameter), taken from the marginal growth zone of the colony, reversely placed on the fruit inoculation site. Control fruits were treated only with PDA disks without the fungus. All the fruits were maintained for 12 days at 28 °C.

Furthermore, a group of intact fruits unharmed and without inoculum was kept under the same conditions as a control to assess any probable natural inoculation in the harvest fields.

1.3. Disease progress assessment

After a two-day interval, one fruit from treatment group and one from the control group were opened up. In this procedure, the coconut water was extracted with a manual punch and then the fruits were sectioned longitudinally in order to expose the central axis of the coconut. The choice of the type of diagrammatic scale to be developed was based on the evolution of the disease images in three angles. Images were made from the side and basal regions of the whole fruit and from the two halves after a longitudinal cut, during several days after the inoculation in order to monitor and record symptom logical aspects. The severity of the disease presented in three different types of images were compared using the Quant program (Vale *et al.*, 2001) and analyzed by the mean square error, the residues dispersion and the Pearson's correlation coefficient were used for choosing a unique image angle to develop the scale to be used for validation.

Before the oxidation of the internal tissues and to facilitate the symptom visualization caused by the fungus, we painted only the damaged area with black ink. Thereafter, each half of the coconut fruits was photographed with a Canon® Ti5 camera and the lesion area was evaluated in accordance with the limits of human acuity, defined by Weber-Fechner stimulus-response law (Campbell and Madden, 1990).

1.4. Evaluation and scale validation

All selected images were grouped together creating a disease gradient, so that it was possible to distinguish the selected percentage levels among evaluators. In order to validate the scale we elaborated a PowerPoint presentation (MS Office 2013®) containing images of 60 fruits with different levels of severity. During this presentation 18 people (inexperienced raters) estimated the severity levels.

Initially, the raters assessed the severity without the aid of diagrammatic scale and only after seven days they were allowed to use the scale. Again, after an interval of seven days, the same pictures were displayed for a third visual estimate (with the aid of the diagrammatic scale), performed by the same raters in order to assess repeatability. We compiled the data of each rater and determined the accuracy and precision using simple linear regression (Microsoft Excel 2013®) between the actual severity, estimated at Quant® program (independent variable) and the visually estimated severity (dependent variable) with and without the use of scale. The estimated accuracy was determined by the determination coefficient (R^2) and the variance of the absolute errors (differences between actual and estimated value).

Accuracy was assessed by regression parameters "a" and "b", comparing them respectively to the values 0 and 1, at "t" test ($p < 0.05$), with and without the use of scale. After the diagrammatic scale elaboration and positive validation, we carried out an assay to compare four different fungal isolates; one from coconut, cocoa and mango fruits, respectively, and one entophytic isolate from *Capparis flexuosa* (L.) L. obtained from leaves collected at Açú Restinga (Freire *et al.*, 2015). Coconuts were arranged entirely at random, with three replicates and a total of 12 fruits. The test was carried out during 12 days under the same conditions described for inoculation process. After fruit longitudinal opening, they were directly evaluated using the proposed diagrammatic scale. The mean percentage of damaged areas was analyzed through confidence interval at 5% probability.

2. Results

The severity of stem-end rot on coconut fruits varied when the symptoms were evaluated under three different angles of the same result (Figure 1). Consequently, it was not possible to define the best fitting among these three angles using the Pearson correlation coefficient (data not shown) in order to develop a rating scale for the disease.

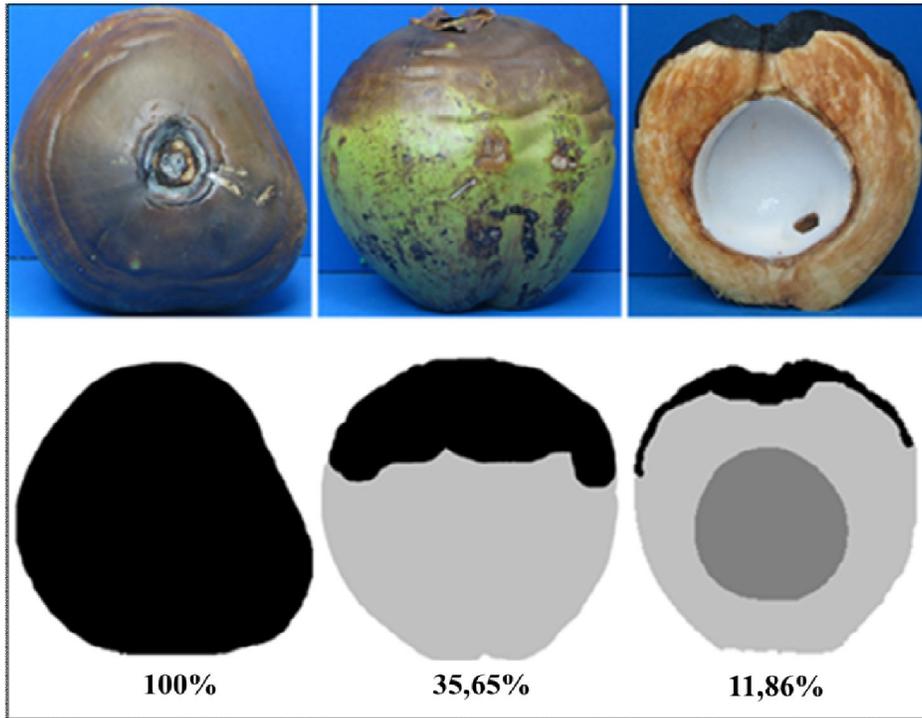


Figure 1: Comparison of the severity of postharvest stem-end rot on coconut fruits caused by *Lasiodiplodiatheobromae*. At the top (from left to right), images of the fruit disease viewed from top, side and internally after a longitudinal cut. Bottom, illustration of the injured areas (black) compared to healthy ones (gray). Values represent percentages of disease severity.

According to the symptoms of the fruits the best settings of severities of coconuts were chosen after 10-day of evaluation. Then, they were cut longitudinally, defining this group of images for the preparation and validation of the diagrammatic scale for disease evaluation. We proposed a scale containing eight levels of percentages starting from the lowest to the highest severity found: 0.4; 1.2; 3.8; 6.5; 10.5; 16.5; 25.0 and 36.5% (Figure 2).

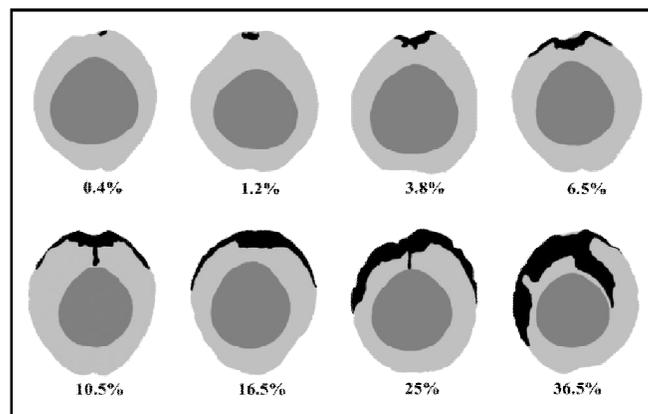


Figure 2: Diagrammatic scale for the assessment of postharvest stem-end rots severity on coconut fruits caused by *Lasiodiplodiatheobromae*. Fruits were cut longitudinally to show disease internal symptoms. Values represent percentages of disease severity.

The scale presented here provided a good repeatability and a high reproducibility rate among evaluations from different raters (Table 1). We need to highlight that these evaluators had not been subjected to any training (evaluation with simultaneous exposure of actual and estimated results), which could further improve the accuracy.

Initially in assessments conducted without the use of the scale, intercept values (a) for 78% of the raters were significantly different from zero ($p=0.05$), with a strong trend to overestimate the severity, indicating the presence of constant positive errors for all severity disease levels (Table 1). With the use of scale, the intercept was reduced to 17% in both evaluations. Still using the scale, 50% of the raters showed negative deviations, in other words, tended to a discrete underestimation of severity and the other 50% slightly tended to overestimate it (Table 1 and Figure3).

Table 1: Performance of the disease evaluators with and without the use of the postharvest stem-end rots on coconut fruits diagrammatic scale. Intercept (a), angular coefficient (b) and coefficient of determination (R^2) of linear regressions of estimated and real severity values

| Raters | Without Scale | | | With Scale (1st Evaluation) | | | With Scale (2nd Evaluation) | | | Repeatability ¹ | | |
|-----------------|---------------|-------|------|-----------------------------|-------|------|-----------------------------|-------|------|----------------------------|-------|------|
| | a | b | R2 | a | b | R2 | A | B | R2 | a | b | R2 |
| 1 | 5.86 | 0.77* | 0.22 | 0.50* | 0.78* | 0.89 | 0.16* | 0.91* | 0.93 | 0.14* | 1.11 | 0.93 |
| 2 | 5.79 | 0.74* | 0.35 | 0.23* | 0.87* | 0.85 | - 0.51* | 0.96* | 0.95 | 0.67* | 0.95* | 0.84 |
| 3 | 8.02 | 1.31 | 0.59 | 1.08* | 0.97* | 0.85 | 0.94* | 0.94* | 0.92 | 1.15* | 0.85* | 0.85 |
| 4 | 3.88 | 0.75* | 0.49 | 2.44 | 0.80* | 0.77 | 1.28* | 0.82* | 0.82 | 0.71* | 0.84* | 0.73 |
| 5 | 2.93 | 0.58* | 0.49 | - 0.28* | 0.66* | 0.84 | 1.24 | 0.77* | 0.87 | 2.47 | 1.04* | 0.81 |
| 6 | 14.28 | 1.82 | 0.59 | 1.24* | 0.95* | 0.80 | - 0.10* | 1.06* | 0.90 | 0.99* | 0.90* | 0.73 |
| 7 | - 0.12* | 0.84* | 0.57 | 0.96* | 0.82* | 0.73 | - 0.02* | 0.89* | 0.81 | 0.76* | 0.90* | 0.77 |
| 8 | 4.34 | 1.62 | 0.69 | - 1.95* | 0.89* | 0.81 | - 1.30* | 1.01* | 0.94 | 2.48 | 0.93* | 0.78 |
| 9 | - 1.07* | 0.83* | 0.26 | 4.45 | 1.03* | 0.65 | 2.76 | 1.25 | 0.80 | 1.77* | 0.92* | 0.73 |
| 10 | - 0.55* | 0.83* | 0.49 | - 1.36* | 0.94* | 0.87 | - 0.13* | 0.92* | 0.90 | 1.70 | 0.92* | 0.91 |
| 11 | 3.35 | 0.96* | 0.53 | 0.92* | 1.01* | 0.74 | 1.33 | 0.90* | 0.89 | 2.88 | 0.69* | 0.73 |
| 12 | 6.74 | 1.05* | 0.41 | - 0.15* | 0.80* | 0.84 | 0.17* | 0.95* | 0.95 | 1.48 | 1.05* | 0.88 |
| 13 | 15.15 | 1.88 | 0.50 | - 0.24* | 0.93* | 0.84 | - 0.88* | 0.99* | 0.90 | 0.19* | 0.97* | 0.90 |
| 14 | 1.70 | 0.37* | 0.60 | 0.79* | 0.70* | 0.82 | 0.26* | 0.87* | 0.92 | 0.55* | 1.09* | 0.85 |
| 15 | 3.57* | 1.28* | 0.56 | 1.21 | 0.78* | 0.85 | - 0.07* | 1.01* | 0.92 | 0.28* | 1.08* | 0.78 |
| 16 | 9.93 | 1.62 | 0.47 | 0.39* | 0.90* | 0.94 | - 0.01* | 1.00* | 0.94 | 0.18* | 1.06* | 0.88 |
| 17 | 11.74 | 0.66* | 0.21 | - 0.50* | 1.02* | 0.96 | - 0.05* | 0.98* | 0.98 | 0.64 | 0.95* | 0.97 |
| 18 | 11.98 | 0.96* | 0.49 | 0.39* | 0.93* | 0.95 | - 0.10* | 0.98* | 0.99 | - 0.06* | 1.01* | 0.95 |
| Average | 5.97 | 1.05 | 0.47 | 0.56 | 0.88 | 0.83 | 0.28 | 0.96 | 0.91 | 1.05 | 0.96 | 0.83 |
| Amplitude | 16.22 | 1.51 | 0.48 | 6.4 | 0.37 | 0.31 | 4.06 | 0.48 | 0.18 | 2.74 | 0.42 | 0.24 |
| Overall Average | 5.97 | 1.05* | 0.80 | 0.56* | 0.88* | 0.96 | 0.28* | 0.96* | 0.98 | -0.13* | 1.07* | 0.98 |

*= Indicates that the null hypothesis ($a = 0$ and $b = 1$) has been accepted by T test ($t= 0.05$); ¹Simple linear regression equation estimates the relation of the second to the first severity evaluation using the scale.

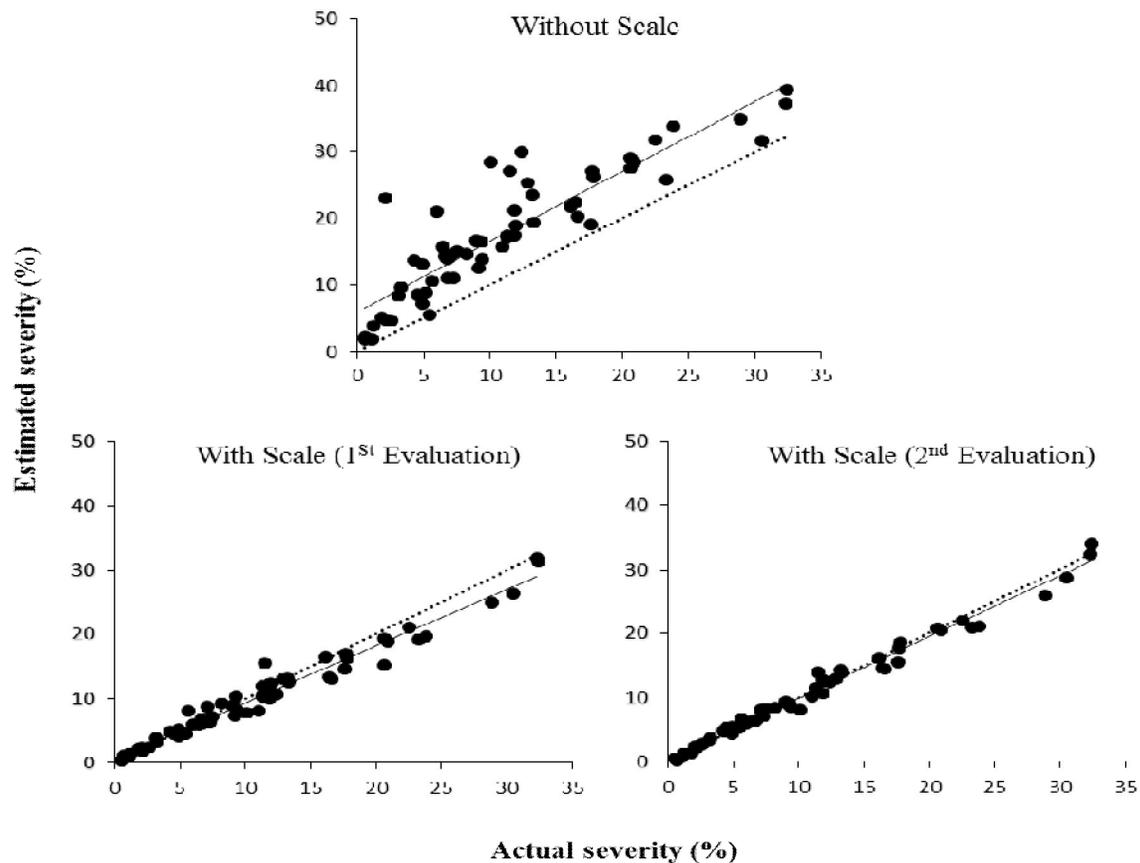


Figure 3. Average of severity estimation with and without the elaboration of diagrammatic scale (full points) and regression line obtained between the actual and estimated severity (full line) of postharvest stem-end rot of coconut (*Lasiodiplodiatheobromae*). Dotted lines represent the ideal response with equal estimation of the actual ones.

The linear angular coefficient (b) shows that 28% of the raters had significantly different values from 1 without using the diagrammatic scale (Table 1). When using the scale, 0% (first evaluation) and 5% (second evaluation) of the raters had significantly different linear angular coefficients 1 ($p=0.05$), indicating that the use of scale improved the accuracy of visual estimates (Table 1).

In the precision analysis, visual estimates of severity without the use of diagrammatic scale, occasioned determination coefficient (R^2) values with an average of 0.47, ranging from 0.21 to 0.69 and amplitude of 0.48. In the first evaluation using the scale, the values of R^2 had an average of 0.83, ranging from 0.65 to 0.96 (range of 0.31). In the second evaluation with scale, R^2 ranged from 0.80 to 0.99 with amplitude of 0.18 (Table 1).

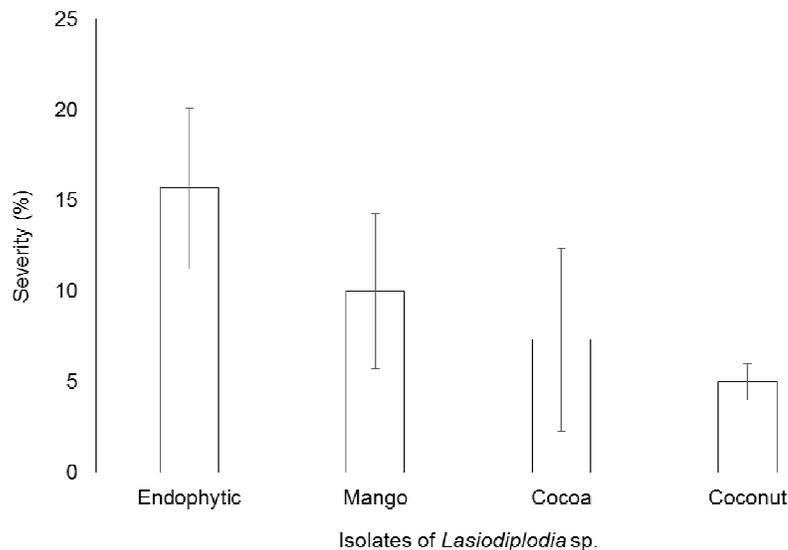


Figure 4: Severity means of *Lasiodiplodia* isolates inoculated in coconut fruits evaluated through diagrammatic scale. Means were compared by confidence interval ($\alpha = 0.05\%$).

After coconut fruits were inoculated with four fungal isolates from different sources (coconut, cocoa, mango and endophytic from leaf) and evaluated using the diagrammatic already validated, it was observed a variation in the severity of basal rot (Figure 4). It was possible to use the scale in a practical and efficient way to compare disease aggressiveness among the isolates tested. So, the most aggressive isolate for coconut fruit was the endophytic one and the less aggressive was the one isolated originally from a coconut fruit.

3. Discussion

The main basal lesions on postharvest coconut fruits can be caused by two pathogen types, *L. theobromae* and *Ceratocystisparadoxa*, the latter has different symptomatic features, although not pronounced externally in the early disease stages. Losses in coconut production by *L. theobromae* infections have been reported in Brazil since the beginning of the export for the European market. At that time the fruits were wrapped in plastic films and kept under refrigeration for 15 days, reaching the destination unsuitable for consumption (Viana *et al.*, 2002). The same occurred with Quissamã production (Rio de Janeiro state), when the fruits were wrapped in paraffin.

The incidence of fruit rots can often be easy to estimate. However, when the intensity is measured by the severity, it becomes difficult because studies focusing on this theme are still limited, making it difficult to estimate production losses (Widiastuti *et al.*, 2015). Consequently, there is a need for tools such as validated diagrammatic scales to help in the understanding of each path system (Pedro so *et al.*, 2011). While it is easy to assess the incidence of basal rot in coconut, external symptoms do not correlate with the internal infection. In our studies the visual evaluation of the fruits did not give us a real estimation of the disease severity observed externally either in side view or in the top view, when compared with injury presented internally (Figure 1). This occurred as a result of differential development of inner lesion which tends to be more discreet than the external necrosis. Symptoms appeared externally on the fruit and advanced on the still green surface under the bracts, towards the apex. Affected tissues became dark-brown necrotic lesions (Halfeld-Vieira and Nechet, 2005). At the same time, internally the fungus colonized the monocarp in the lower middle third and the lesion was formed closer to the base.

Considering the visual change in the estimation of the disease severity associated with the Pearson correlation data we decided to propose the diagrammatic scale from coconuts cut longitudinally (Figure 1; Figure 2) highlighting the internal injury, different from the external lesions that are always larger than the inner ones, making it difficult to know the real internal fungi colonization.

The disease evaluation requires training, preparation and knowledge of the symptoms associated with pathogen-plant interaction. The selection of cashew plants infected with *L. the obromae* was done through gummosis identification. However, according to Muniz *et al.*, (2014) a major problem with visual assessment of infected plants is the difficulty to detect this delayed observation and to interpret the initial symptoms, which requires an expert eye. In cocoa plant, branches disease severity has been estimated by necrosis between 2 and 4 mm from the point of inoculation (Adu-Cheampong *et al.*, 2012) that is, the visible lesion length and the distance from the inoculation point.

The estimated severity values with the help of scale were more accurate and precise to all raters. The tendency of evaluators to underestimate levels of disease severity can occur in some cases, as occurred in this study, and in other path systems (Vivas *et al.*, 2010; Santos *et al.*, 2011). Disease severity over estimation can be corrected by the initial training of raters (Nutter and Schultz, 1995). Therefore, the use of diagrammatic scale increased the precision of visual estimates, similar to what is seen in other studies of diagrammatic scale validation (Capucho *et al.*, 2010; Vieira *et al.*, 2014; Freitaset *et al.*, 2015).

In general, the evaluators who used our diagrammatic scale (Figure 2) showed good repeatability because the linear regression analyzes of the data in which the scale was used presented R^2 values between 0.73 and 0.97, with an average of 0.83 (Table 1). The evaluation of *L. Theo brome* infection in the coconut crop has been difficult, due to the path system own characteristics, as well as it has been for disease estimation when focusing on plants (Montero *et al.*, 2013) and basal rot in fruit (Vienna *et al.*, 2008).

There is no study correlating the association of plant disease incidence with postharvest losses to date. Since this disease always occurs in the field associated with other fungi (*Phyllachorales*), the combination of the symptoms of both ones may increase the leaf area affected. Consequently, this may have turned it difficult to apply pathogen city tests through Koch's Postulates for each disease separately. An alternative to this problem would be the use of fruit inoculation to evaluate the pathogen city and then compare the aggressive isolates. The scale proposed in this work (Figure 2) may be essential for the development of studies aimed to elucidate the interaction *L. the obromae*-coconut, regardless of the type and infection occurrence because no tool to assess fungus damage on fruits has been developed yet. Moreover, there are no studies about the aggressiveness of different isolates on the development of this disease.

In the case of mango fruit, the severity of this fungus and the virulence of the isolates was evaluated by measurement of the lesion length 72 h after inoculation in two perpendicular directions on each fruit (Marques *et al.*, 2013). Although this is a scale showing the severity index of stem-end rot in mango (Alvindia *et al.*, 2000) it may not represent the injury evolution, since the size of the lesion on the one side of the fruit can be quite different from the other as it does not occur homogeneously.

Another use for diagrammatic scale proposed here would be the comparison of isolates from other hosts by cross inoculation of coconut fruits, as the isolates from cocoa and mango fruits and the endophytic one from a Restinga plant (Figure 4). In this case there were variations in the extension of the lesions indicating different degrees of aggressiveness among isolates from different hosts. In *Lasiodiplodia*-mango relationship the fungus infects and settles on the skin of developing fruit generating the stem-end rot post-harvest disease (Alvindia and Acda, 2015). The same was already registered for *Alternaria alternata* (Fr.) Keissl. in studies detecting latent infection on the surface of mature mango fruits and postharvest rot (Prusky *et al.*, 1983). It is known that *Lasiodiplodia* is an entophyte in several plant species and can be present in many plant parts like bark, twig and leaf segments (Sunayana *et al.*, 2014). The evaluation of different isolates from different hosts, through inoculations in coconut fruits could be an efficient tool for selecting them by the degree of pathogenicity, aggressiveness and perhaps endophytism (Figure 4).

Plant disease control studies suggest that the contribution of diagrammatic scale is essential to compare the efficiency of products, such as the ones used as barriers to the fungus. *In vitro* evaluation of *L. theobromae* control has been promising, as well as using synthetic fungicides (Locatelli *et al.*, 2015) or natural products with little toxicity to the environment (Freire *et al.*, 2013). However, studies of basal rot severity on coconut fruits have not been made because of the lack of tools to enable an ease and practical evaluation of the disease evolution and postharvest control.

Initially, products for coconut disease control must be inevitable tested in the laboratory and/or on fruits, thus avoiding the high costs and the difficulties of conducting epidemiological studies in the field (Monteiro *et al.*, 2013).

4. Conclusions

The diagrammatic scale developed in this work proved to be adequate to evaluate the severity of the disease in coconut fruits in an accurate, precise and reproducible manner. Moreover, the scale may be used in field works and in laboratory conditions to evaluate *L. theobromae* isolates aggressiveness or either in experiments of chemical or alternative control of the disease.

5. References

- Adu-Cheampong, R., Archer, S., & Leather, S. (2012). Resistance to dieback disease in cacao caused by *Fusarium* and *Lasiodiplodia* species in cacao (*Theobroma cacao* L.) genotypes. *Experimental Agriculture*, 48(1), 85–98. <http://doi.org/10.1017/S0014479711000883>
- Alvandia, D. G., & Acda, M. A. (2015). Revisiting the efficacy of hot water treatment in managing anthracnose and stem-end rot diseases of mango cv. "Carabao." *Crop Protection*, 67, 96e101. <http://doi.org/10.1016/j.cropro.2014.09.016>
- Alvandia, D. G., Kobayashi, T., Yaguchi, Y., & Natsuaki, K. T. (2000). Symptoms and the associated fungi of postharvest diseases on non-chemical bananas imported from the Philippines. *Japanese Journal of Tropical Agriculture*, 44(2), 87–93.
- Araújo, G. S., Carvalho, R. H. R., & Sousa, E. M. B. D. (2009). Produção de biodiesel a partir do óleo de coco (*Cocos nucifera* L.) bruto. In *2nd International Workshop - Advances in Cleaner Production. São Paulo - Brazil* (pp. 1–10).
- Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. New York: John Wiley & Sons.
- Capucho, A. S., Zambolim, L., Duarte, H. S. S., Parreira, D. F., Ferreira, P. A., Lanza, F. E., ... Cota, L. V. (2010). Influence of leaf position that correspond to whole plant severity and diagrammatic scale for white spot of corn. *Crop Protection*, 29(9), 1015–1020. <http://doi.org/10.1016/j.cropro.2010.04.012>
- Carvalho, J. M., Maia, G. A., Sousa, P. H. M., & Maia Jr., G. A. (2006). Água-de-coco: propriedades nutricionais, funcionais e processamento waterofcoconut: nutritionalandfunctionalpropertiesandprocessing. *Semina: Ciências Agrárias*, 27(3), 437–452.
- Castellani, A. (1964). The "water cultivation" of pathogenic fungi. *Annales Des Societes Belges de Medecine Tropicale, de Parasitologie, et de Mycologie*, 44(2), 217–220.
- Djocgoue, P. F., Simo, C., Mbouobda, H. D., Boudjeko, T., Nankeu, D. J., & Omokolo, N. D. (2010). Assessment and heritability of productivity and tolerance level to *Phytophthora megakarya* in two hybrid populations of *Theobroma cacao*. *Journal of Plant Pathology*, 92(3), 607–617.
- Dugan, F. M., Lupien, S. L., Osuagwu, A. N., Uyoh, E. A., Okpako, E., & Kisha, T. (2016). New records of *Lasiodiplodia theobromae* in seeds of *Tetrapleuratetraptera* from Nigeria and fruit of *Cocos nucifera* from Mexico. *Journal of Phytopathology*, 164(1), 65–68. <http://doi.org/10.1111/jph.12384>
- Farias, A. C. M., Santana, J. S., Oliveira Filho, M. F., Santana, J. S., Barbosa, C. R. F., & Medeiros, J. T. N. (2011). Os combustíveis verdes do Brasil - avaliação da lubrificidade do biodiesel B5 e óleos de mamona e coco. *HOLOS*, 3, 3–17.
- Fontenele, R. E. S. (2005). Cultura do coco no Brasil: caracterização do mercado atual e perspectivas futuras. In *XII Congresso da SOBER* (pp. 1–20).

- Freire, M. G. M., Mussi-Dias, V., Siqueira, G. M. B., & Nascimento, D. F. (2015). *O Tempo e a Restinga/Time and Restinga*. (M. das G. M. Freire, Ed.) (1st ed., Vol. 1). Campos dos Goytacazes: RR Donnelly. Retrieved from <https://issuu.com/otempoearestinga>
- Freire, M. G. M., Souza, C. L. M., Portal, T. P., Alves, R. M. M., Santos, P. H. D., & Mussi-Dias, V. (2013). Effect of castor bean oil on post harvest fungal pathogen of coconut: *Lasiodiplodiatheobromae*. *Journal of Plant Physiology & Pathology*, *1*(3), 1–7. <http://doi.org/10.4172/2329-955X.1000108>
- Freitas, M. L. O., Pozza, E. A., Belan, L. L., Silva, J. L., & Abreu, M. S. (2015). Diagrammatic scale for blister spot in leaves of coffee tree. *African Journal of Agricultural Research*, *10*(19), 2068–2075. <http://doi.org/10.5897/AJAR2014.9465>
- Galvão, A. S., Gondim Jr., M. G. C., & Michereff, S. J. (2008). Escala diagramática de dano de *Aceria guerreronis* Keifer (Acari: Eriophyidae) em coqueiro. *Neotropical Entomology*, *37*(6), 723–728. <http://doi.org/10.1590/S1519-566X2008000600015>
- Halfeld-Vieira, B. A., & Nechet, K. L. (2005). Queda de frutos em coqueiro causada por *Lasiodiplodiatheobromae* em Roraima. *Fitopatologia Brasileira*, *30*(2), 203–203. <http://doi.org/10.1590/S0100-41582005000200022>
- Horsfall, J. G., & Barrat, R. W. (1945). An improved grading system for measuring plant diseases. *Phytopathology*, *35*, 655. <http://doi.org/10.1094/PHYTO-02-16-0074-RVW>
- IBGE (2015). Levantamento sistemático da produção agrícola: pesquisa mensal de previsão e acompanhamento das safras agrícolas no ano civil. *Levant. Sistem. Prod. Agric.*, *29*(1), 1–83.
- Jeronimo, C. E., & Silva, G. O. (2013). Estudo de alternativas para o aproveitamento de resíduos sólidos da industrialização do coco. *Revista Monografias Ambientais*, *10*(10), 2193–2208. <http://doi.org/10.5902/223613086935>
- Lima, G. S. A., Assunção, I. P., Martins, R. B., Santos, H. V., & Michereff, S. J. (2011). Development and validation of a standard area diagram set for assessment of *Alternaria* spot on the cladodes of the prickly pear cactus. *Journal of Plant Pathology*, *93*(3), 691–695.
- Locatelli, T., Freire, M. G. M., Souza, C. L. M., & Mussi-Dias, V. (2015). Toxicidade de fungicidas no crescimento e germinação in vitro do fungo *Lasiodiplodiatheobromae*. *Perspectivas Online: Ciências Biológicas e da Saúde*, *17*(5), 9–22. Retrieved from http://www.seer.perspectivasonline.com.br/index.php/biologicas_e_saude/article/view/678/587
- Marques, M. W., Lima, N. B., Morais Jr., M. A., Barbosa, M. A. G., Souza, B. O., Michereff, S. J., ... Câmara, M. P. S. (2013). Species of *Lasiodiplodia* associated with mango in Brazil. *Fungal Diversity*, *61*(1), 181–193. <http://doi.org/10.1007/s13225-013-0231-z>
- Menge, D., Makobe, M., Shomari, S., & Tiedemann, A. V. (2013). Development and validation of a diagrammatic scale for estimation of cashew blight for epidemiological studies. *International Journal of Advanced Research*, *1*(4), 26–38.
- Monteiro, C. M., Caron, E. S., da Silveira, S. F., Almeida, A. M., Souza-Filho, G. R., & de Souza, A. L. (2013). Control of foliar diseases by the axillary application of systemic fungicides in Brazilian coconut palms. *Crop Protection*, *52*(2013), 78–83. <http://doi.org/10.1016/j.cropro.2013.05.013>
- Muniz, C. R., Freire, F. C. O., Viana, F. M. P., Cardoso, J. E., Sousa, C. A. F., Guedes, M. I. F., ... Jalink, H. (2014). Monitoring cashew seedlings during interactions with the fungus *Lasiodiplodiatheobromae* using chlorophyll fluorescence imaging. *Photosynthetica*, *52*(4), 529–537. <http://doi.org/10.1007/s11099-014-0061-6>
- Nutter, F. W., & Schultz, P. M. (1995). Improving the accuracy and precision of disease assessments: selection of methods and use of computer-aided training programs. *Canadian Journal of Plant Pathology*, *17*(2), 174–184. <http://doi.org/10.1080/07060669509500709>
- Pedroso, C., Lage, D. A. C., Henz, G. P., & Café-Filho, A. C. (2011). Development and validation of a diagrammatic scale for estimation of anthracnose on sweet pepper fruits for epidemiological studies. *Journal of Plant Pathology*, *93*(1), 219–225.
- Prusky, D., Fuchs, Y., & Yanko, U. (1983). Assessment of latent infections as a basis for control of postharvest diseases of mango. *Plant Disease*, *67*(7), 816–818.
- Santos, P. H. D., Vivas, M., Silveira, S. F., Silva, J. M., & Terra, C. E. P. S. (2011). Elaboração e validação de escala diagramática para avaliação da severidade de oídio em folhas de mamoeiro. *Summa Phytopathologica*, *37*(4), 215–217. <http://doi.org/10.1590/S0100-54052011000400011>
- Sunayana, N., Nalini, M. S., Sampath, K. K. K., & Prakash, H. S. (2014). Diversity studies on the endophytic fungi of *Vitexnegundo* L. *Mycosphere*, *5*(August), 578–590. <http://doi.org/10.5943/mycosphere/5/4/10>

- Vale, F. X. R., Fernandes Filho, E., & Liberato, J. R. (2001). Quant - quantificação de doenças: versão 1.0.1. Software. Viçosa, UFV.
- Viana, F. M. P., Freire, F. C. O., Barguil, B. M., Alves, R. E., Santos, A. A., Cardoso, J. E., & Vidal, J. C. (2002). Podridão basal pós-colheita do coco verde no estado do Ceará. *Fitopatologia Brasileira*, 27(5), 545–545. <http://doi.org/10.1590/S0100-41582002000500020>
- Viana, F. M. P., Uchôa, C. N., Vieira, I. G., Freire, F. C. O., Saraiva, H. A. O., & Mendes, F. N. P. (2008). Processamento mínimo, atmosfera modificada, produtos químicos e resfriamento no controle da podridão basal pós-colheita em frutos do coqueiro anão verde. *SummaPhytopathologica*, 34(4), 326–331.
- Vieira, R. A., Mesquini, R. M., Silva, C. N., Hata, F. T., Tessmann, D. J., & Scapim, C. A. (2014). A new diagrammatic scale for the assessment of northern corn leaf blight. *CropProtection*, 56, 55–57. <http://doi.org/10.1016/j.cropro.2011.04.018>
- Vivas, M., Terra, C. E. P. S., Silveira, S. F., Fontes, R. V., & Pereira, M. G. (2010). Escala diagramática para avaliação da severidade da pinta-preta em frutos de mamoeiro. *Summa Phytopathologica*, 36(2), 161–163. <http://doi.org/10.1590/S0100-54052010000200010>
- Widiastuti, A., Ningtyas, O. H., & Priyatmojo, A. (2015). Identification of fungus causing postharvest disease on several fruits in Yogyakarta. *Jurnal Fitopatologi Indonesia*, 11(3), 91–96. <http://doi.org/10.14692/jfi.11.3.91>