Ciência Rural, Santa Maria, v.51:3, e20200033, 2021 http://doi.org/10.1590/0103-8478cr20200033
ISSNe 1678-4596
CROP PROTECTION

Diagrammatic scale for assessment of anthracnose severity in feijoa fruit

Ana Carolina Ferreira[1] **Idemir Citadin**[1*] **Silvia Scariotto**[1] **Rafael Henrique Pertille**[1] **Marcos Robson Sachet**[2] **Marieli Teresinha Guerrezi**[3] **Joel Donazzolo**[4]

[1]Programa de Pós-graduação em Agronomia, Universidade Tecnológica Federal do Paraná (UTFPR), 85503-390, Pato Branco, PR, Brasil. E-mail: idemir@utfpr.edu.br. *Corresponding author.
[2]Escola Agrotécnica, Universidade Federal de Roraima (UFRR), Boa Vista, RR, Brasil.
[3]Curso de agronomia, Universidade Federal de Roraima (UFRR), Boa Vista, RR, Brasil.
[4]Curso de Agronomia, Universidade Tecnológica Federal do Paraná (UTFPR), Campus-Dois Vizinhos, Dois Vizinhos, PR, Brasil.

ABSTRACT: *A diagrammatic scale of anthracnose in feijoa fruit was elaborated and validated in order to standardize disease severity assessments. The proposed scale showed six disease severity levels: 2, 10, 20, 40, 70 and 100% of the injured fruit surface. The scale took into account the minimum and maximum limits of disease severity observed in the field and the intermediate values followed logarithmic increments according to the Weber-Fechner stimulus-response law. Eight inexperienced raters validated the scale by quantifying the disease severity (using/not using the scale) of 50 feijoas with anthracnose symptoms. In conclusion, the scale improved the assessment of anthracnose in feijoa. Eight genotypes from different crosses were tolerant to anthracnose.*
Key words: *Acca sellowiana (O. Berg) Burret, Colletotrichum spp., pineapple guava.*

Escala diagramática para avaliação da severidade de Antracnose em frutos de feijoa

RESUMO: *Com o objetivo de padronizar avaliações da severidade da antracnose em frutos de feijoa elaborou-se e validou-se uma escala diagramática com os seguintes níveis de severidade: 2, 10, 20, 40, 70 e 100% de área superficial do fruto com lesão. A escala considerou os limites de severidade mínima e máxima da doença observados em campo e os níveis intermediários seguiram incrementos logarítmicos, obedecendo-se a "Lei do estímulo de Weber-Fechner". A severidade foi estimada, por oito avaliadores sem experiência, em 50 frutos sem e com o auxílio da escala proposta. Em conclusão, a escala melhorou a avaliação da antracnose na feijoa. Oito genótipos de diferentes cruzamentos foram tolerantes à antracnose.*
Palavras-chave: *Acca sellowiana (O. Berg) Burret, Colletotrichum spp., goiaba serrana.*

Feijoa was classified as *Acca sellowiana* (O. Berg) Burret. The species belongs to the myrtle family (Myrtaceae), native to the southern Brazilian highlands and northern Uruguay. It is cultivated commercially in New Zealand, Colombia and the United States (MORETTO et al., 2014). Moreover, due to its profit-making potential, its production has sparked the interest of other countries such as Brazil, Chile and Uruguay, which are carrying out diverse researches to develop more productive genotypes and improve cultivation techniques (DUCROQUET, 2008).

Anthracnose is the main disease that affects feijoa in Brazil and is caused by the fungus *Colletotrichum* spp. The characteristic symptoms are well-defined irregularly-shaped dark spots on the fruit surface, followed by necrosis, with more advanced stages resulting in fruit rot, which in severe cases may lead to a production loss of 75% in the orchards, or even higher in the post-harvest period (FANTINEL et al., 2017).

As its cultivation is relatively new in Brazil, many anthracnose management and control techniques still need improvement. Methods that quantify the disease are fundamental, mainly when the aim is to conduct epidemiological studies to evaluate severity, select more tolerant genotypes, describe the progression of the epidemic, validate control forecast models, or apply integrated management (DUARTE

Received 01.16.20 Approved 09.11.20 Returned by the author 11.26.20
CR-2020-0033.R2

et al., 2013). Given the absence of standardized methods to quantify the disease, this research aimed to develop the first diagrammatic scale to evaluate anthracnose severity in feijoa fruit. The orchard was established in 2011 in the Experimental Area of the Universidade Tecnológica Federal do Paraná-UTFPR, Pato Branco, Brazil (26º10'S; 52º41'W; alt.: 760 m). The climate of the site is humid subtropical (*Cfa*), according to the Köppen classification (ALVARES et al., 2013). Plants used in the study are crosses between the cultivars Nonante, Helena and Alcântara from the feijoa germplasm bank of the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina-EPAGRI, São Joaquim, Santa Catarina State. The combinations of crosses between the three parents were: Nonante x Nonante, Nonante x Alcântara, Nonante x Helena, Alcântara x Nonante and Alcântara x Helena. A total of 60 plants were sown (12 individuals from each progenie).

A total of 50 feijoa fruits with natural occurrence of anthracnose symptoms were randomly collected from different plants. Each was sectioned longitudinally (one side keeping the greater part of the injury) and photographed in order to create an image bank. The injured areas were estimated using the replica method (CITADIN et al., 2008).

The diagrammatic scale was elaborated with six levels: 2, 10, 20, 40, 70 and 100% from images generated with the aid of Image J software (RASBAND, 1997), with the first and last levels equivalent to the minimum and maximum observed severities, respectively. Intermediate values were calculated following the law of visual acuity proposed by Weber-Fechner, with successive logarithmic increments (HORSFALL & BARRAT, 1945).

As regards validation, eight inexperienced raters, using the diagrammatic scale and anthracnose in feijoa during the evaluations, made severity estimates based on the analyses of 50 random images of feijoas having different disease severities. They made their evaluations without (1st) and with (2nd) use of the scale. To this end, images with known severity were inserted into a Disease Plan spreadsheet (randomizes image presentation, records estimates and statistics of accuracy and makes concordance) (SACHET et al., 2017). Precision, accuracy and the correlation coefficient were obtained using Lin's concordance correlation analysis (P_c), which combines measurements of accuracy and precision in order to evaluate the degree to which pairs of observations fall on the concordance line of 45º (intercept = 0; slope = 1), of the set of individuals with and without the proposed scale.

The following formula was used to calculate Lin's concordance correlation (P_c): $P_c = C_b.r$, where C_b is a deviation correction factor that measures how far the best-fit line deviates from the concordance line (measure of accuracy) and r is the correlation coefficient between estimated severity (Y) and real severity (X) (measure of precision) (YADAV et al., 2013). The scale effect on the concordance indices was compared through a two-tailed paired t test. The graphs and analyses referring to the scale were made with the aid of R software (R DEVELOPMENT CORE TEAM, 2018).

During the harvest that occurred from December 2015 to March 2016, 30 feijoas were collected from each individual that produced. They were assessed with the aid of the proposed diagrammatic scale as regards anthracnose severity. Incidence (%) was calculated by counting the number of feijoas that had disease symptoms. The results are presented in the form of graphs created using R language. Standard deviations for varying severity were also calculated and presented.

The proposed diagrammatic scale in this work contains six classes of anthracnose intensity scores for feijoas: 2, 10, 20, 40, 70 and 100% (Figure 1). Based on Lin's concordance correlation analysis, accuracy and precision, in addition to the regression line obtained between the real and estimated severity, the assessments were closer to the real value when the proposed scale was used. The measures of concordance correlation, accuracy and precision increased significantly ($p<0.05$) as a result of using the scale (Figure 2). Thus, the proposed diagrammatic scale to assess the anthracnose severity on the fruits has improved the accuracy, precision, and reproducibility of the evaluations, proving to be an essential tool for the evaluation of this disease in feijoa.

Without using the proposed scale, evaluators with no experience underestimated the severity of the disease (Figure 2). The opposite was observed by (GODOY et al. 2006), in which inexperienced evaluators overestimated the severity of Asian soybean rust. The evaluators also overestimated the white spot severity on maize (CAPUCHO et al. 2010) and brown spot on coffee fruits (AZEVEDO de PAULA et al. 2016). Errors in assessments may be related to the characteristics of the disease assessed. BOCK et al. (2009) observed that the error in the estimates varied according to the real severity. In this research, evaluators underestimated the disease severity, when it was above 70%, even with the use of the scale (Figure 2). In assessing the leaf symptoms of citrus canker, the evaluators underestimated the

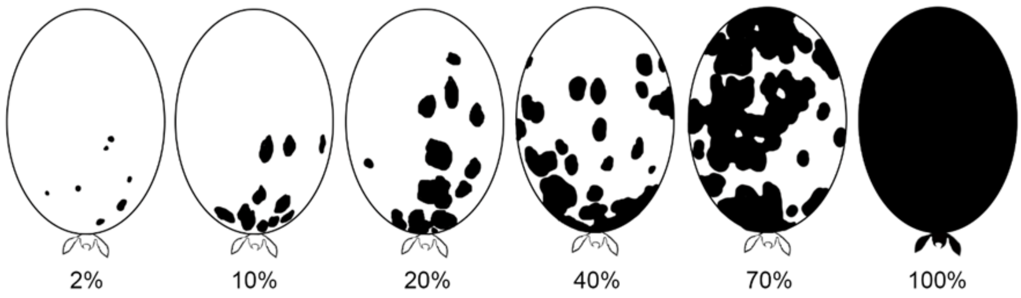

Figure 1 - Diagrammatic scale proposed to assesses anthracnose (*Colletotrichum* spp.) in feijoa (*A. sellowiana*) fruit, indicating severity levels of 2, 10, 20, 40, 70 and 100%.

actual severity (which was between 5 and 10%) (BOCK et al., 2009). SANTOS et al. (2010) observed that the evaluators overestimated the *Cercospora* leaf spot severity on castor bean when it was below 20%. When there are more small injuries, the error of evaluators is more pronounced compared to the case where there are fewer injuries (BRAGA et al., 2020). This fact was observed in this research, where errors

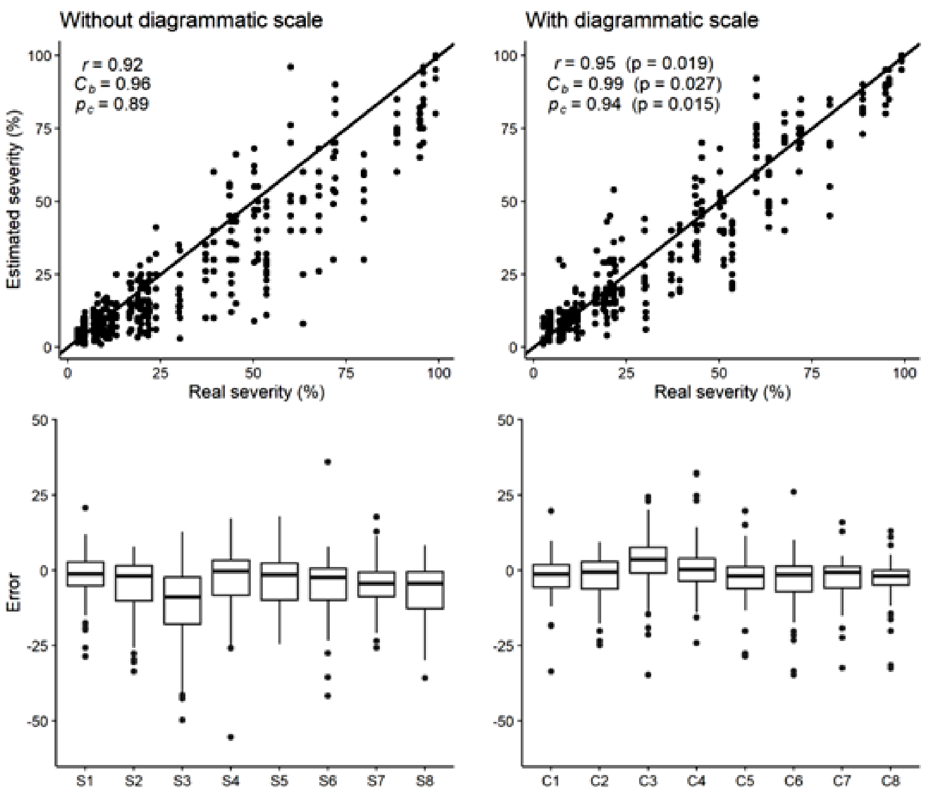

Figure 2 - Distribution of severity estimates of anthracnose and boxplot with the distribution of errors in the assessment of *Colletotrichum* spp. in 50 feijoas (*A. sellowiana*) assessed by 8 raters without and with the aid of the diagrammatic scale proposed in this research. r = precision, C_b = accuracy, p_c = concordance correlation and P = t test error probability.

in the evaluation were more pronounced when the severity was between 25% and 70%.

Estimate precision increased for every rater who used the proposed diagrammatic scale. The correlation coefficient (95%) was considered high for this type of assessment, and significantly higher compared to the no-scale correlation coefficients. Scale use decreases errors in the estimates. Without its use, 27.5% of the severity estimates presented errors above 10 and up to 55.4. Using the scale, only 15% of the severity estimates presented errors above 10 (maximum = 34.8) (Figure 2). Evaluator training can reduce errors in severity estimation (BOCK et al., 2016; CHIANG et al., 2016). BARDSLEY & NGUGI (2013) tested evaluators with different levels of experience. They reported that inexperienced and experienced evaluators could evaluate if they have enough instructions. The reliability between evaluators was higher when the evaluator used the proposed scale. The same results were reported by YADAV et al. (2013) using the scale to evaluate the pecan scab symptoms on fruit, AZEVEDO de PAULA et al. (2016) working on with brown eye spots on red and yellow coffee cherries, and DOLINSKI et al. (2017) for the assessment of peach rust.

Every evaluated individual presented feijoas with the presence of anthracnose, which indicated that immunity to the disease does not occur between these accessions (Figure 3). As regards severity, the individuals presented contrasting values,

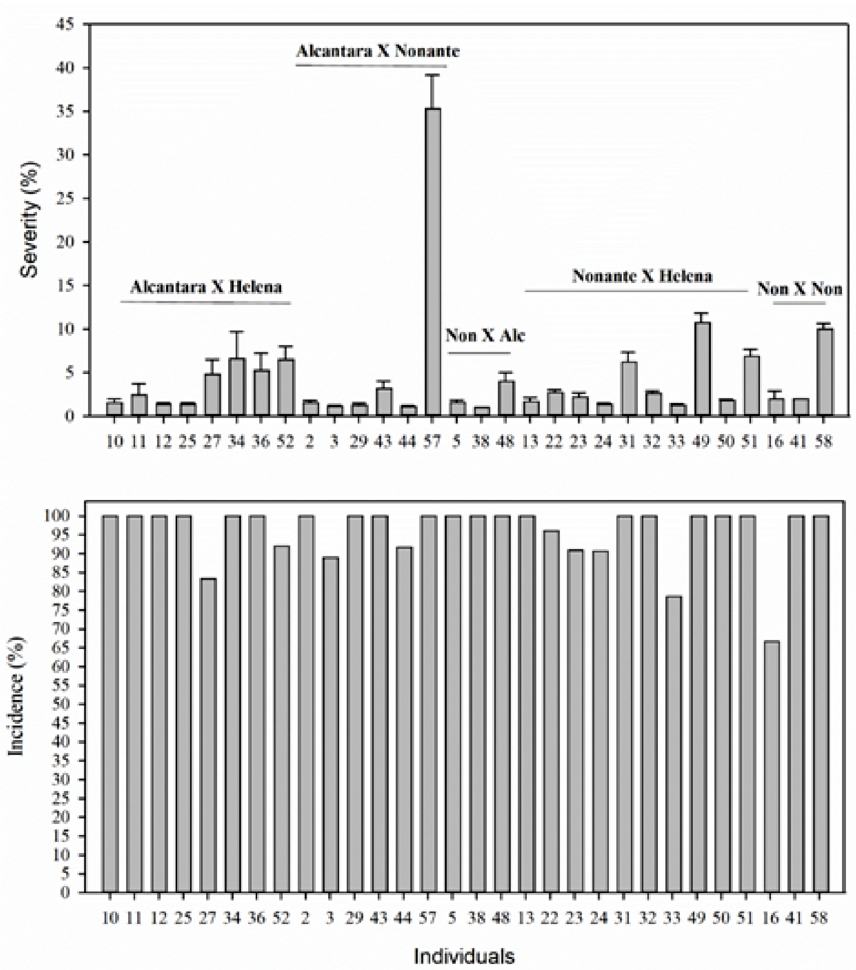

Figure 3 - Severity (%) of injured area (± standard deviation) and incidence (%) of anthracnose in feijoas with different individuals from five different crosses. Nonante (Non); Alcântara (Alc).

varying from 1.0 to 35.3%. Incidence was 100% for 21 of the 30 assessed individuals (lowest value = 66% -individual16). There was no significant relationship between incidence and severity ($r^2 = 0.16^{ns}$); thus, incidence (easily measurable variable) cannot be considered sufficient to select individuals resistant to anthracnose. For example, accession 38 presented 100% incidence, but only 1% severity, which does not compromise its appearance and commercial potential; whereas individual 52 presented lower incidence (90%), but 6.5% severity. Individuals2, 3 and 44 (Alcântara x Nonante) presented the lowest severities and were considered the most tolerant to anthracnose. Conversely, individuals 27, 34, 36 and 52 (Alcântara x Helena) presented the highest severities (Figure 3). The Nonante cultivar, when used in crosses, tends to increase anthracnose severity in the individuals of progenies, whereas the Alcântara cultivar reduces disease severity. DUCROQUET et al. (2008) describe Nonante, as requiring anthracnose control measures in the fruit. On the other hand, Alcântara came from native material and tolerate anthracnose well (DUCROQUET et al., 2007), like the Helena cultivar. In conclusion, the scale improves the assessment of anthracnose in feijoa; the Alcântara genotype is better as a parent as regards resistance to anthracnose; and eight genotypes from different crosses are tolerant to anthracnose.

ACKNOWLEDGEMENTS

The authors would like to thank UTFPR Campus of Pato Branco for providing the physical structure. This research was funded by Conselho Nacional de Desenvolvimento Científico – CNPQ and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES).

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

ALVARES, C. A., et al. Köeppen's climate classification map for Brazil. Meteorologische Zeitschrift, v.22, p.711-728. 2013. Available from: <https://www.schweizerbart.de/papers/metz/detail/22/82078/Koppen_s_climate_classification_map_for_Brazil>. Accessed: Nov. 18, 2019. doi: 10.1127/0941-2948/2013/0507.

AMORIM, L. et al. Diagrammatic scale to evaluate target spot severity in cucumber plant leaves. **Fitopatologia Brasileira**, v.18, p.174-180, 1993.

AZEVEDO de PAULA, P. V. A., et al. Diagrammatic scales for assessing Brown eye spot (*Cercospora coffeicola*) in red and yellow coffee cherries**. Journal of Phytopathology**, v.164, p.791–800, 2016. Available from: <https://doi.org/10.1111/jph.12499>. Accessed: Jul. 20, 2020. doi: 10.1111/jph.12499.

BARDSLEY, S. J.; NGUGI, H. K. Reliability and accuracy of visual methods to quantify severity of foliar bacterial spot symptoms on peach and nectarine. **Plant Pathology**, v.62, p.460–474, 2013. Available from: <https://doi.org/10.1111/j.1365-3059.2012.02651.x>. Accessed: Jul. 20, 2020. doi: 10.1111/j.1365-3059.2012.02651.x.

BOCK, C. H., et al. Comparison of assessment of citrus canker foliar symptoms by experienced and inexperienced raters. **Plant Disease**, v.93, p.412–424, 2009. Available from: <https://doi.org/10.1094/PDIS-93-4-0412>. Accessed: Jul. 20, 2020. doi: 10.1094/PDIS-93-4-0412.

BOCK, C. H., et al. Assessing disease severity: Accuracy and reliability of rater estimates in relation to number of diagrams in a standard area diagram set. **Plant Pathology**, v.65, p.261–272, 2016. Available from: <https://doi.org/10.1111/ppa.12403>. Accessed: Jul. 20, 2020. doi: 10.1111/ppa.12403.

BOCK, C. H., et al. Comparison of assessment of citrus canker foliar symptoms by experienced and inexperienced raters. **Plant Disease**, v.93, p.412–424, 2009. Available from: <https://doi.org/10.1094/PDIS-93-4-0412>. Accessed: Jul. 20, 2020. doi: 10.1094/PDIS-93-4-0412.

BRAGA, K., et al. Development and validation of a diagrammatic scale for the assessment of the severity of bacterial leaf streak of corn. **European Journal of Plant Pathology**, v.157, p.367–375, 2020. Available from: <https://link.springer.com/content/pdf/10.1007/s10658-020-02008-7.pdf>. Accessed: Jul. 20, 2020. doi: 10.1007/s10658-020-02008-7.

CAPUCHO, A. S., et al. Influence of leaf position that correspond to whole plant severity and diagrammatic scale for white spot of corn. **Crop Protection**, v.29, p.1015–1020, 2010. Available from: <https://doi.org/10.1016/j.cropro.2010.04.012>. Accessed: Jul. 18, 2020. doi: 10.1016/j.cropro.2010.04.012.

CHIANG, K. S., et al. Effects of rater bias and assessment method on disease severity estimation with regard to hypothesis testing. **Plant Pathology**, v.65, p.523–535, 2016. Available from: <https://doi.org/10.1111/ppa.12435>. Accessed: Jul. 20, 2020. doi: 10.1111/ppa.12435.

DOLINSKI, M. A., et al., Development and validation of a standard area diagram set for assessment of peach rust. **European Journal of Plant Pathology**, v.148, p.817–824, 2017. Available from: <https://doi.org/10.1007/s10658-016-1138-9>. Accessed: Jul. 20, 2020. doi: 10.1007/s10658-016-1138-9.

DUARTE, H. S. S. et al. Development and validation of a set of standard area diagrams to estimate severity of potato early blight. **European Journal Plant Pathology**, v.137, p.249-257, 2013. Available from: <https://link.springer.com/article/10.1007/s10658-013-0234-3>. Accessed: Nov. 23, 2019. doi: 10.1007/s10658-013-0234-3.

DUCROQUET, J. P. H. J. et al. Novas cultivares brasileiras de goiabeira-serrana: SCS 414-Mattos e SCS 415-Nonante. **Revista Agropecuária Catarinense**, v.21, n.2, p.79-82, 2008. Available from: <http://andorinha.epagri.sc.gov.br/consultawebsite/busca?b=ad&id=60631&biblioteca=vazio&busca=autoria:%22GUERRA,%20M.P.:%20NODARI,%20R.O.%22&qFacets=autoria:%22GUERRA,%20M.P.:%20NODARI,%20R.O.%22&sort=&paginacao=t&paginaAtual=1>. Accessed: Nov. 23, 2019.

DUCROQUET, J. P. H. J. et al. As primeiras cultivares brasileiras de goiabeira serrana: SCS411 Alcântara e SCS412 Helena. **Revista Agropecuária Catarinense**, v.20, p.77-80, 2007. Available from: <http://andorinha.epagri.sc.gov.br/consultawebsite/busca?b=pc&id=54398&biblioteca=vazio&busca=autoria:%22SANTOS,%20K.L.%20dos.%22&qFacets=autoria:%22SANTOS,%20K.L.%20dos.%22&sort=&paginacao=t&paginaAtual=1>. Accessed: Nov. 23, 2019.

FANTINEL, V. S. et al. First report of *Colletotrichum siamense* causing Anthracnose on *Acca sellowiana* Fruits in Brazil. **Plant Disease**, v.101, n.6, p.1035, 2017. Available from: <https://doi.org/10.1094/PDIS-01-17-0096-PDN>. Accessed: Nov. 25, 2019. doi: 10.1094/PDIS-01-17-0096-PDN.

GODOY, C. V., et al. Diagrammatic scale for assessment of soybean rust severity. **Fitopatologia Brasileira**, v.31, p.63–68, 2006. Available from: <https://doi.org/10.1590/S0100-41582006000100011>. Accessed: Jul. 18, 2020. doi:10.1590/S0100-41582006000100011.

HORSFALL, J. C.; BARRAT, R. W. An improved grading system for measuring plant diseases. **Phytopathology**, v.35, p.665. 1945. Available from: <http://www.garfield.library.upenn.edu/classics1986/A1986A666500001.pdf>. Accessed: Jul. 18, 2020.

MORETTO, S. P. et al. A Introdução e os Usos da Feijoa ou Goiabeira Serrana (*Acca sellowiana*): A perspectiva da história ambiental. **Journal of Social, Technological and Environmental Science**, v.3, n.2, p.67-79, 2014. Available from: <https://doi.org/10.21664/2238-8869.2014v3i2.p67-79>. Accessed: Nov. 21, 2019. doi: 10.21664/2238-8869.2014v3i2.p67-79.

RASBAND, W.S. **Image J**: Image Processing and Analysis in Java. Available from the U.S. National Institutes of Health, 1997.

SACHET, M. R. et al. Disease Plan - a spreadsheet application for training people to assess disease severity and to assist with standard area diagram development. **Ciência Rural**, v.47, p.2016-2224, 2017. Available from: <http://dx.doi.org/10.1590/0103-8478cr20160924>. Accessed: Nov. 21, 2019. doi: 10.1590/0103-8478cr20160924.

SANTOS, W. F., et al. Elaboration and validation of a diagrammatic scale for epidemiological studies of cercospora leaf spot of castor bean. **Journal of Phytopathology**, v.158, p.403–408, 2010. Available from: <https://doi.org/10.1111/j.1439-0434.2009.01636.x>. Accessed: Jul. 20, 2020. doi: 10.1111/j.1439-0434.2009.01636.x.

YADAV, N. V. S., et al., Development and validation of standard area diagrams to aid assessment of pecan scab symptoms on fruit. **Plant Pathology**, v.62, p.325–335, 2013. Available from: <https://doi.org/10.1111/j.1365-3059.2012.02641.x>. Accessed: Jul. 20, 2020. doi: 10.1111/j.1365-3059.2012.02641.x.