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Measuring and Analysis of Plant Diseases

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Abstract: *Knowledge of the quantity of disease is particularly important to decision-makers in Plant breeding program. Quantifying disease on plants by measuring symptoms falls under "remote sensing". Remote sensing is obtaining information about an object without having direct physical contact with it. Digital image quantifying techniques methods are used to quantify the severity of a given disease. In assessment of plant disease severity, the use of various error terms and how these should conform to current approaches used in measurement science include: Reliability, Accuracy of estimates and Agreement Visual rating methods are, continue to be the single most important way of assessing plant disease for the foreseeable future. The various kinds of scales used in visual plant disease assessment include: Nominal or Descriptive Scales, Ordinal Scales, Ratio Scales, and Interval or Category Scales. Digital cameras are an inexpensive and widely used resource, and used for various applications in plant pathology. A Portable Application for Quantifying Plant Disease Severity. Leaf Doctor is a new tool for the semiautomatic assessment of disease severity for individual leaves or other sampling units. Advantages of digital photography and image analysis in the visible spectrum Image analysis can be quick, accurate and reliable when automated. For disease severity, various statistical methods have been used to explore the quality of image analysis measurements compared with true and estimated values, as well as the repeatability and reproducibility of various methods.*

Keywords: *Digital Camera, Leaf Doctor, Plant Diseases, Remote Sensing, Visual Rating*

1. Introduction

There are diverse reasons why we need to estimate or measure disease on plants. Knowledge of the quantity of disease is particularly important to decision-makers in crop situations where disease must be related to yield loss, in plant breeding where various germplasm, varieties and/or cultivars need to be rated, and for disease management decisions, for example, applying pesticides to control disease epidemics, but also for understanding fundamental processes in biology, including co-evolution and plant disease epidemiology (Rutter et al., 2006), (Bock et al., 2010).

Quantifying disease on plants by measuring symptoms generally falls under the broad definition of "remote sensing" (there are one or two exceptions). Remote sensing can be defined as obtaining information about an object without having direct physical contact with it (Abazov et al., 2006). Thus, both visual estimation of disease and using cameras or other imaging technologies to measures disease can be considered as remote sensing. Thus, the pathogens can be detected and quantified by immunological and molecular methods. The disease caused by the pathogen can be detected and quantified as symptoms by laser induced fluorescence, radar, microwave, thermograph, nuclear magnetic resonance imaging, multi- and hyper-spectral imagery, digital (or film) cameras and image analysis, and visual assessment (Bock et al., 2010) A diagrammatic scale was developed to assess the severity of bacterial blight of coffee based on ranges of frequency distribution of severity values. The diagrammatic scale provides better levels of accuracy, precision and reproducibility of the evaluations (Belan et al., 2014)

Digital image quantifying techniques methods are used to quantify the severity of a given disease. Such a severity may be inferred either by the area of the leaves that are affected by the disease, or by how deeply rooted is the affection, which can be estimated by means of color and texture features. Most quantification algorithms include a segmentation step to isolate the symptoms, from which features can be extracted and properly processed in order to provide an estimate for the severity of the disease (Barbedo, 2013).

It is worth noting that the problem of determining the severity of a disease by analyzing and measuring its symptoms is difficult even if performed manually by one or more specialist which have to pair the diagnosis guidelines with the symptoms as accurately as possible. As a result, the manual measurements will always contain some degree of subjectivity, which in turn means that references used to validate the automatic methods are not exactly "ground truth". It is important to take this into consideration when assessing the performance of those methods (Barbedo, 2013).

In assessment of plant disease severity, the use of various error terms and how these should conform to current approaches used in measurement science (Bock and Nutter Jr, 2012). Terms that are used include:

Reliability: 'the extent to which the same measurement of individuals obtained under different conditions yield similar results' (Bock and Nutter Jr, 2012)

Accuracy of estimates: 'the degree of closeness of estimated or measured values to some recognized standard, true or actual values(Bock and Nutter Jr, 2012)

Agreement: The concept of agreement in measurement science is commonly defined as the product of precision and accuracy when comparing estimates with measures that are accepted to provide the 'true' values (Bock and Nutter Jr, 2012)

Comparison of the regression analyses of the Evaluators, with and without the use of the diagrammatic scale, showed that the scale was effective at increasing the accuracy and precision of evaluating the disease of leaf blight severity of coffee trees(Bock and Nutter Jr, 2012).

2. Measuring And Analysis Of Plant Diseases

2.1. Visual Assessment of Plant Diseases

The eye acts as a remote sensing device, and combined with the brain acts as an image analysis system. The way the eye functions and creates an image compares with the way a camera operates. The sharp vision, as when concentrating on an individual leaf, or reading a book, as now, takes place in the center 10% of the retina, which is called the macula, the rest of the retina being responsible for peripheral vision. The eye and the relevant parts of the brain work together extremely rapidly to acquire the image, analyze and interpret it. This process allows individuals to estimate the type and quantity of disease tissue on a particular leaf or plant. The response of visual raters is not the same every time, and is dependent on several factors, some of which are known. There are individual differences in how light and color is perceived among individuals, and thus in estimating disease – quite apart from the cognitive ability to estimate disease that might vary with individual and with assessment occasion. Conditions such as color blindness have also been demonstrated to influence ability to estimate disease (Bock et al., 2010).

2.2. Methods Used to Visually Estimate Disease Severity

(Madden et al., 2007b) provide information on various aspects of visual disease assessment. The various kinds of scales used in visual plant disease assessment include:

2.2.1. Nominal or Descriptive Scales

These are the simplest and probably most subjective of the scales for grading disease severity. Disease is graded into a number of classes with descriptive terms such as "slight," "moderate," or "severe." Due to the subjectivity involved and lack of quantitative definition, these scales have very limited value beyond the individual performing the rating in a particular season and location (Bock et al., 2010).

2.2.2. Ordinal Scales

These are descriptive disease scales, but they grade disease severity into arbitrary classes that represent increasing severity of symptoms. Very simple ordinal scales of this type are subjective and not particularly transferrable between raters, locations or seasons. Ordinal scales can be based on, or accompanied by, diagrams or descriptions indicating the intensity of symptoms. Another example is the nine-point ordinal scale developed to measure severity of fungal pathogens. The scale relied on descriptions of the symptoms and was used to assess genotypes for resistance in the breeding program. Ordinal scales are still quite widely used for specific diseases, particularly for rating some virus diseases where symptoms are not easy to measure quantitatively (Madden *et al.*, 2007). Allocating a number to a symptom description allows the stage of development of the disease to be numerically assessed or denoted (Bock et al., 2010)

2.2.3. Interval or Category Scales

A disease interval (or category) scale comprises a number of categories where the numeric values are known – in the case of plant disease this is generally the percent area with symptoms. The scale included a standard area diagram (SAD) set that had five levels of rust 1–5 (representing 1, 5, 10, 20 and 50% disease), and the rater placed the sample leaf in the most appropriate category. This diagrammatic scale was modified over the years to improve accuracy (Bock et al., 2010)

2.2.4. Ratio Scales

The percent scale is a ratio scale and has been widely used to estimate disease severity in numerous patho systems in plant disease assessment. Some of the advantages of the continuous percent scale in assessing disease severity to the best of the rater's ability and States that: the upper and lower limits of a percent scale are consistently defined (0 and 100%), the scale is universally familiar, it is easily divided and subdivided, and it is widely accepted as a way to measure area coverage. Raters vary substantially in the accuracy and reliability of assessment (Bock et al., 2010)

2.3. The Future of Visual Rating Methods

Visual rating methods are, continue to be the single most important way of assessing plant disease for the foreseeable future. Other technologies being developed will no doubt continue to make a greater contribution. Thus, the need to elucidate the best ways to assess disease severity, identify and gauge the magnitude of error in estimates, and reduce that error is highly desirable. The reliability and agreement that is required for the disease assessment task at hand should be considered when choosing an assessment system (surveys, rating germplasm, predicting disease progress, etc.). Wherever possible studies should adopt methods that maximize both rater reliability and agreement with the actual value (Bock et al., 2010).

2.4. Advantages of Visually Assessed Disease

The process can be quick.

With some training it is relatively easy to recognize and differentiate multiple diseases.

The use of assessment aids and training markedly improves results.

There are several techniques that can be used to suit a particular need (ordinal scales, interval scales, category scales and ratio scales).

No equipment required.

2.5. Disadvantages of Visually Assessed Disease

Raters may tire and lose concentration, thus decreasing their accuracy.

There can be substantial inter- and intra-rater variability (subjectivity).

There is a need to develop standard area diagrams to aide assessment.

Training may need to be repeated to maintain quality. Raters are expensive.

Visual rating can be destructive if samples are collected in the field for assessment later in the laboratory.

Raters are prone to various illusions (for example, lesion number/size and area infected).

3. Digital Imagery and Image Analysis in Visible Spectrum for Plant Disease Measurement

Digital cameras are an inexpensive and widely used resource, and used for various applications in plant pathology. Photography (digital, and previously film) has been used to detect, quantify and study diseases and pathogens for many decades. Aerial film-photography of diseases fields started in the 1920s and has continued to be used and developed in plant disease detection and quantification At the microscopic scale digital image analysis has also been used in plant pathology to measure and observe pathogen and host physiology and development It is in the mid range of plant disease severity estimation that the pertains specifically to measurement of symptoms on individual plant organs, plants and quadrates which is in concert with the resolution of most digital cameras at the sub-plot level (Bock et al., 2010).

3.1. Digital Cameras used in Image Acquiring for plan Diseases Measurements

Digital cameras have fast become the primary device for imaging samples. There is a tremendous range of capability, and it would be unrealistic to attempt a breakdown of their pros and cons. Digital images can be obtained from a flat bed scanner used to digitize old photographic prints and negatives, or even plant leaves directly, and slides can be scanned and digitized by film scanners. Once obtained, there is a flow of information from the choice of the sample unit to record to the measurement of diseased area in the imaging process (Bock et al., 2010)

Digital cameras consists of a lens, a viewfinder (LCD display), and a light sensitive screen on which the light from the image falls. First considering monochromatic digital cameras (sensing only one color), the screen, comprised of an array of photo sensors, measures the intensity of the incoming light. The photo sensors are memory cells and convert the incoming light into electrons – an electric charge - the accumulated charge is released and is proportional to the intensity of the light. The contents of the photo sensors on the screen are then converted from an analogue to a binary digital signal (1, 0) by a frame grabber (an analogue-to-digital converter), and transmitted to the computer where they are drawn on the screen based on the 1 and 0 readings Thus, each photo sensor is represented on the computer monitor by a pixel, with brightness and location identified through image processing. Color digital camera images are acquired by incorporating sensors for each of the primary colors red, green and blue in the screen. The photo sensors can be co-arranged in different ways, from a regular mosaic to a layered arrangement. Each photo sensors records only a single color and the actual color for the image for an individual pixel is obtained by an interpolation algorithm that compares it with the surrounding pixel color measurements thereby generating an estimate of color. The algorithm uses the RGB color model to accurately portray the original color (Bock et al., 2010)

4. Leaf Doctor: A Portable Application For Quantifying Plant Disease Severity

Leaf Doctor is a new tool for the semiautomatic assessment of disease severity for individual leaves or other sampling units. Evaluation of the Leaf Doctor algorithm for a range of lesion types, lea shapes, and disease intensities demonstrated that this application provided estimates that were highly accurate when compared with estimates from a commonly used discipline standard, precise, and robust. Linear relationships were significant among estimates for all diseases studied with approximately 1:1 relationships and high coefficients of determination (R2 $ 0.79) and standard errors of the y estimate, indicating a low degree of error associated with the predicted y-value. The accuracy of estimates by Leaf Doctor was relatively higher in diseases featuring necrotic lesions (R2 $ 0.94) with almost perfect concordance correlation coefficients (Cb $ 0.99). Coefficients of determination were slightly lower with correspondingly higher standard errors for estimates of disease severity in mallow rust and powdery mildew of lilac. This was likely due to the higher contrast in color between healthy and diseased sections of leaves in diseases with necrotic symptoms. Powdery mildew of lilac was the only dataset where a systematic and constant bias was identified in disease severity assessments made by Leaf Doctor in comparison with Assess with a slope and intercepts deviating significantly from one and zero, respectively. A reduction in accuracy and robustness of algorithms used for disease severity estimation has also been reported in powdery mildew and other diseases where contrast is compromised (Barbedo, 2014)**.** Although coefficients of variation in most datasets were minimal among the diseases included in this study, significant negative linear or nonlinear relationships between the coefficient of variation and mean disease severities were observed. Similar phenomena have also been reported in the accuracy of manual disease severity estimations conducted by individual raters (Barbedo, 2014).

In addition to the statistical comparisons to quantify the accuracy, precision, and robustness of Leaf Doctor, faster processing times than Assess were also a substantial advantage.

Another use of Leaf Doctor is the rapid construction of standard area diagrams from actual patho systems based on estimates of disease made by trained raters. The benefits of standard area diagrams include improved accuracy and precision of intra-rater estimates and better inter-rater reproducibility, (Braido et al., 2014)**,** (Pethybridge and Nelson, 2015)

5. Image Resolution and Subject Orientation

It is useful to understand the factors that influence digital image quality, and how these influence disease severity measurement during image analysis. These include focus, reflection (glare) of light on the object, uniformity of lighting prior to image acquisition (Bock et al., 2010)

5.1. Image Processing

Once the image has been obtained, it can be edited in various ways in many different image analysis and image processing software packages. Color and contrast can be corrected, images rotated sharpened inverted or further manipulated. Programs such as Adobe Photoshop (Adobe Systems Inc., San Jose, CA) are powerful software package that offer many options for enhancing images. Most image analysis programs also offer image editing and modification including enhancing edges and geometric corrections (Bock et al., 2010).

5.2. Image Analysis Software and Image Measurement

Many different proprietary and custom image analysis software programs have been used in plant disease severity assessment. Before exploring the application of these it is worth going through the image analysis protocol, which has many common processes regardless of the software program being used. Firstly, with color images the image is composed of three colors and each pixel in the image has a particular value for each of the primary colors red, green and blue based on the RGB color model, which is a three-dimensional color space used to generate the correct color (Bock et al., 2010)

5.3. Advantages of Digital Photography and Image Analysis in the Visible Spectrum

Image analysis can be quick, accurate and reliable when automated.

If a good automated system can be developed (there are a few reported instances of agreement verification) then it can be extremely powerful.

Technology exists to make the assessment both reliable and accurate.

Image analysis equipment is relatively inexpensive.

There is specific software adapted for the applications and specific needs and issues in plant disease measurement.

5.4. Disadvantages of Digital Photography and Image Analysis in the Visible Spectrum

Coping with plant-to-plant variation in color and various image artifacts or flaws is not straight forward.

Not established how to deal with multiple diseases, damage or physiologic conditions on sample leaves.

It requires some training in the program to become proficient.

Truthing is often required to ensure the quality of the measurement.

6. Statistical Methods To Gauge The Ability Of Image Analysis In Plant Disease Measurement

Using image analysis techniques, correct detection of disease has been analyzed by comparing symptoms identified using the image analysis system with those identified by visual confirmation, thereby judging the incidence of correct disease identification, false positives and false negatives (Zhao et al., 2009). For disease severity, various statistical methods have been used to explore the quality of image analysis measurements compared with true and estimated values, as well as the repeatability and reproducibility of various methods. (Bock and Nutter Jr, 2012)

Regression analysis is the most widely used tool for testing the quality of disease severity measured using image analysis and other techniques. It has been used to judge reproducibility, reliability, precision and accuracy. It is a useful method, but it should be applied cautiously as incorrect conclusions might be drawn from the analysis under certain, circumstances (Madden et al., 2007a). A number of regression parameters and statistics are used to assess the quality of estimates. These include the slope, intercept and associated standard errors (if slope=1 and intercept=0, the assessment is completely accurate). The coefficient of determination (r^2) provides a measure of reliability or precision (the higher the r^2, the higher the precision). Statistically, the coefficient of determination describes the proportion of variability accounted for by the regression model (a measure of the proportion of overlapping variance). Accuracy and precision of measurements of disease using image analysis have been analyzed using regression analysis for several diseases for both color and monochrome images (Bock and Nutter Jr, 2012)

7. Regression Analysis

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7.1. Lin's Concordance Correlation Coefficient

Lin's concordance correlation coefficient has been used to quantify agreement, in disease severity assessment (Bock et al., 2009a). It has some advantages over regression analysis and evaluates the degree to which pairs of observations fall on the concordance line of (Bock et al., 2008b) (slope=1, intercept=0). The concordance correlation coefficient rc combines the measures of accuracy (systematic and constant bias) with precision (r, the correlation coefficient, described subsequently) to assess the relational fit to the line of concordance. (Bock et al., 2009a). used Lin's concordance correlation coefficient to investigate accuracy and precision of citrus canker symptom measurement on grapefruit leaves using image analysis. (Bock and Nutter Jr, 2012)

7.2. Analysis of Variance (ANOVA) and General Linear Modeling (GLM)

Analysis of variance (ANOVA) and general linear modeling have long been used to investigate sources of error in estimates of disease severity but not as a tool to judge image analysis per severity. However, a non-parametric equivalent of ANOVA, the Kruskall–Wallis test, was used to judge the quality of image analysis compared with visual assessments of powdery mildew (Podosphaera clandestina) severity on cherry leaves. In such particular study, image analysis was found to be inferior to visual rating. (Bock and Nutter Jr, 2012)

7.3. Correlation Coefficient

Correlation analysis measures the strength and nature (positive or negative) of an association between two variables. In the case of disease severity assessment, it can indicate the degree of precision (reliability) between estimates or measurements obtained using different methods or at different times. Disease severity (measured as % necrotic area) had correlation coefficients as high as 0.80. The correlation coefficient measures precision as a component of Lin's concordance correlation coefficient – used in assessments of disease severity rating methods of Phomopsis on strawberry , and when studying assessment methods used to quantify the severity of citrus canker on grapefruit using visible-wavelength images and image analysis (Bock et al., 2009b)**.** It should be remembered that if measured alone, a high correlation coefficient (_1.00) can occur in the presence of bias, and thus no assumption can be made regarding equality of the two assessments based on correlation alone . (Bock and Nutter Jr, 2012)

The coefficient of variation (CV) provides a good overall index as to the degree of precision with which raters or assessment methods such as image analysis can be evaluated and compared. The CV expresses the experimental error as a percentage of the mean, and thus has the advantage of normalizing units of measure (% severity, % reflectance, etc.) and so each rater or assessment method can be compared with other raters or methods, when the same sampling units are assessed (the CV should not be used to compare rater precision on different samples. The lower the CV (%) of a rater or assessment method, the higher the precision of that rater or method. Other methods that have been used to gauge precision and accuracy from image analysis compared with either visual assessments or true values include Bland–Altman plots (Bock et al., 2008a), chi-square hypothetical variance tests and plots of the residuals or absolute error (estimate minus true disease) and relative error (absolute error/true severity_100) (Bock and Nutter Jr, 2012).

8. Summery And Conclusion

Quantifying disease on plants by measuring symptoms generally falls under the broad definition of "remote sensing" (there are one or two exceptions). Remote sensing can be defined as obtaining information about an object without having direct physical contact with it. Digital image quantifying techniques methods are used to quantify the severity of a given disease Such a severity may be inferred either by the area of the leaves that are affected by the disease, or by how deeply rooted is the affection, which can be estimated by means of color and texture feature. In assessment of plant disease severity, the use of various error terms and how these should conform to current approaches used in measurement science Include: Reliability, Accuracy of estimates and Agreement Visual rating methods are, continue to be the single most important way of assessing plant disease for the foreseeable future. Other technologies being developed will no doubt continue to make a greater contribution. The various kinds of scales used in visual plant disease assessment include: Nominal or Descriptive Scales, Ordinal Scales, Ratio Scales, and Interval or Category Scales. Digital cameras are an inexpensive and widely used resource, and used for various applications in plant pathology. Photography (digital) has been used to detect, quantify and study diseases and pathogens for many decades. A Portable Application for Quantifying Plant Disease Severity. Leaf Doctor is a new tool for the semiautomatic assessment of disease severity for individual leaves or other sampling units. Evaluation of the Leaf Doctor algorithm for a range of lesion types, lea shapes, and disease intensities demonstrated that this application provided estimates that were highly accurate when compared with estimates from a commonly used discipline standard, precise, and robust. Advantages of digital photography and image analysis in the visible spectrum Image analysis can be quick, accurate and reliable when automated. For disease severity, various statistical methods have been used to explore the quality of image analysis measurements compared with true and estimated values, as well as the repeatability and reproducibility of various methods.

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