# IMAGISTIC ANALYSIS FOR ESTIMATING THE DEGREE OF BACTERIOSIS ATTACK (*Xanthomonas juglandis*) IN WALNUT

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***Abstract.*** *Walnut (Juglans regia* L.*) is a very important and appreciated species from the group of nuts for fruits rich in nutritional principles (minerals, vitamins, fats and so on). Bacteriosis (Xanthomonas juglandis) is one of the main diseases of the walnut. The present study used image analysis methods to evaluate the degree of bacterial attack on walnut leaves. The total leaf area (TLA), healthy leaf area (HLA) and affected leaf area (ALA) were determined. For image analysis, the Scan Sick & Healthy Leaf application, made in Processing with Java code lines Java, was used. There were analyzed 15 case studies, represented by walnut leaflets with different degree of bacteriosis attack. The single factor ANOVA test was used to evaluate the variance in the experimental data set. PAST software was used for statistical analysis. The total leaf area (TLA) studied ranged from 35.89 $cm^2$ in sample 4 to 92.35 $cm^2$ in sample 7. The healthy leaf area (HLA) ranged from 29.85 $cm^2$ for sample 14 to 90.13 $cm^2$ for sample 7. The area affected by bacterial attack (ALA) varied between 0.26 $cm^2$ in case of sample 1 and 9.24 $cm^2$ in case of sample 15. The healthy leaf area (HLA) as a percentage, compared to the TLA, recorded values between 81.24% (sample 14) and 99.58% (sample 1). The leaf area affected by bacteriosis (ALA) recorded percentage values between 0.42% (sample 1) and 18.76% (sample 14). The relation that accurately described the distribution of ALA values against HLA values (%) is given by a linear equation, under conditions of $R^2 = 1$, $p << 0.001$, which indicates a very high accuracy of determining the two types of surface ( HLA, ALA) in relation to the total surface of the studied leaves. The Scan Sick & Healthy Leaf application can be extended to evaluate other attacks by plant pathogens, at different plant species, cultivated or in spontaneous flora.*

**Keywords**: bacteriosis, Java, leaf area, Processing, walnut, *Xanthomonas*

## INTRODUCTION

The leaves represent the receptive organs of plants in relation to sunlight (FIORUCCI and FANKHAUSER, 2017; LI et al., 2018), and the architecture, structure and functionality of the leaves have been adjusted over time in relation to genetic, environmental, and technological factors, in the case of cultivated plants (SCHUMANN et al., 2017). The leaves are actively involved in the process of respiration, photosynthesis, synthesis and translocation of assimilated to growth and storage organs (MESSINGER et al., 2006; TANAKA and MAKINO, 2009; DUAN et al., 2014). The general functioning of plants and the productivity of ecosystems or agricultural systems depends on the health of the leaves (WOOD and LAVERY, 2001; CAVENDER-BARES and BAZZAZ, 2004; BARDGETT and GIBSON, 2017; SHELEF et al., 2017).

The state of the plants and implicitly of the leaves is ensured by nutrition (RAWASHDEH and SALA, 2013, 2014, 2015; JIVAN and SALA, 2014), by water supply and water regime in plants (SCOFFONI et al., 2014; SALA et al., 2019; GENTZEL et al., 2019) and, respectively plant health (MACLEOD et al., 2010; PAUTASSO et al., 2010; MILLS et al., 2011).

The leaf blade is very expressive and has been studied for the characterization and recognition of some plant species (BACKES et al., 2009; DU et al., 2013), for determining the leaf surface (EASLON and BLOOM, 2014; SALA et al., 2015; KUMAR et al., 2017; CÂNDEA-CRĂCIUN et al., 2018), for the characterization and classification of genotypes in apple trees (SALA et al., 2017).

The health of the plants is very important and intensively studied in relation to different pathogens (diseases and pests), under pathological, economic and environmental protection aspects (MACLEOD et al., 2010; MILLS et al., 2011; ZIV et al., 2018). It is important to be identified early the attack of the pathogen, to be establish the degree of attack in relation to the economic threshold of harm and to establish prophylactic measures.

The methods based on the imaging analysis are very useful and appreciated in evaluating the health of the plants, the degree of attack at the foliar level because they are fast, precise, in real time and with minimal costs (FANG and RAMASAMY, 2015; MAHLEIN, 2016; VEYS et al., 2019).

Walnut (*Juglans regia* L.) is a very important and appreciated species from the group of nuts for fruits rich in minerals, vitamins, fats and so on (TAHA and AL-WADAAN, 2011; POLLEGIONI et al., 2017). Among walnut diseases, bacteriosis (*Xanthomonas juglandis*) is one of the most important (SHARMA et al., 2012).

The degree of bacterial attack on walnut leaves was evaluated in the present study by imaging analysis, based on an application made in Processing, with Java code lines.

## MATERIAL AND METHODS

The study aimed to assess the degree of bacterial attack (*Xanthomonas juglandis*) in walnut leaves.

For analyzing the foliar surface and the degree of bacteriosis attack, the Scan LeafArea, and Scan Sick & Healthy Leaf were used (DRIENOVSKY et al., 2017a,b), figure 1.

The folioles with different degree of attack were analyzed (15 samples), figure 2. The folioles were scanned in a 1:1 ratio, in the RGB color system. The total leaf area (TLA), healthy leaf area (HLA) and affected leaf area (ALA) were determined. The values obtained for the leaf surface (TLA, HLA and ALA) were expressed in $cm^2$ and in percentages.

The applications for the determination of the leaf area, for the differentiation between the normal and the affected surface, as well as the degree of attack, were made in the Processing program. Java programming language was used for writing code lines.

The experimental data obtained were analyzed by ANOVA (single factor) test for variance evaluation. For statistical analysis, the statistical module from EXCEL, Office 2007, and PAST software were used (HAMMER et al., 2001).

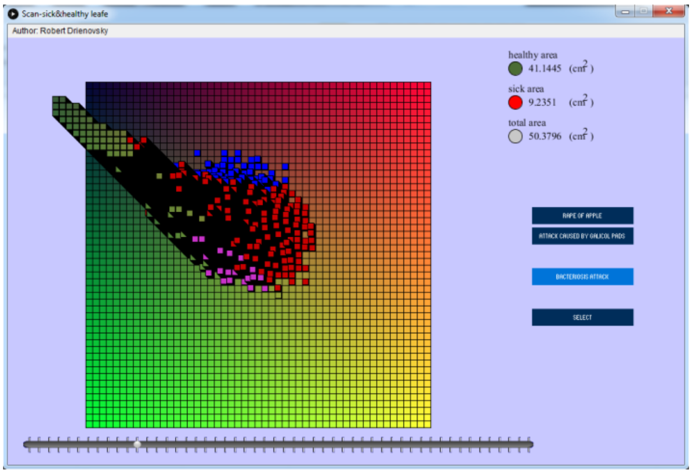


Fig. 1. 3D rendering in the Scan Sick & Healthy Leaf application for recognizing and determining the healthy and affected leaf surface of the leaves

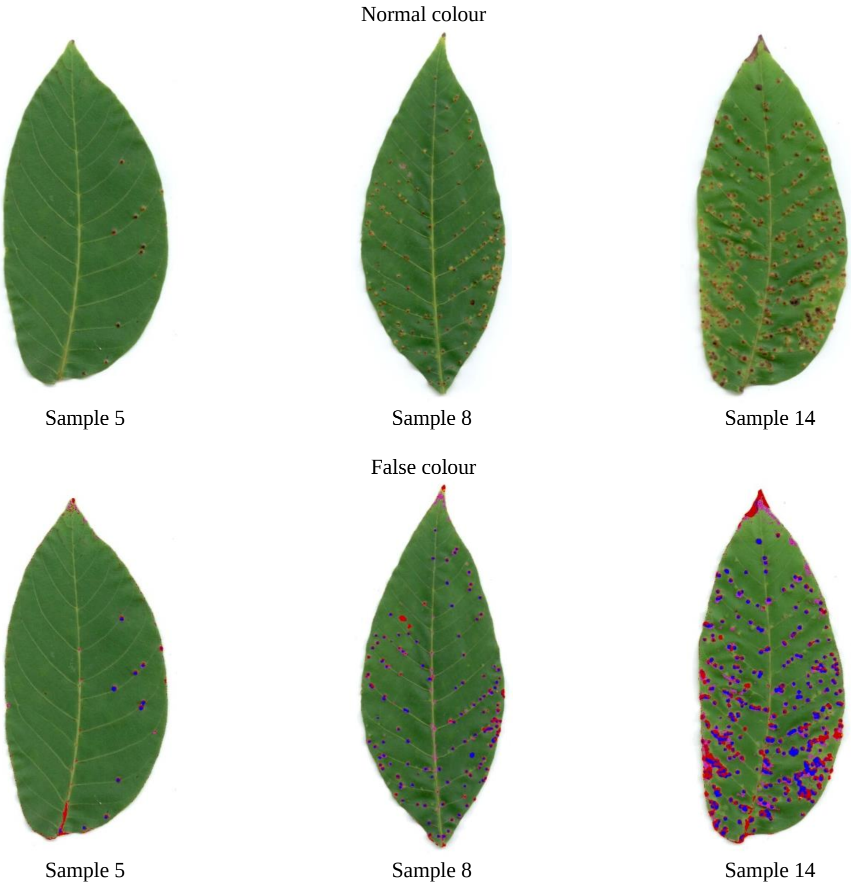


Fig. 2. Walnut folioles attacked by bacteriosis (*Xanthomonas juglandis*); selective presentation from the 15 case studies

## RESULTS AND DISCUSSIONS

Images of walnut leaves, affected by bacteriosis, were analyzed with the Scan LeafArea and Scan Sick & Healthy Leaf (Drienovsky et al., 2017a,b) applications to determine the healthy surface and the degree of attack of the leaves. The experimental data obtained regarding the total leaf area (TLA), the healthy leaf area (HLA) and the surface attacked by bacteriosis (ALA), expressed in $cm^2$ and percentage, are presented in table 1.

15 samples were analyzed, with different levels of intensity of the bacteriosis attack. In the case of total leaf area (TLA), the leaf area obtained varied between 35.89 $cm^2$ in sample 4, and 92.35 $cm^2$ in sample 7. The healthy leaf area (HLA) varied between 29.85 $cm^2$ in sample 14, and 90.13 $cm^2$ in sample 7. The area affected by bacterial attack (ALA) ranged from 0.26 $cm^2$ in sample 1, to 9.24 $cm^2$ in sample 15. The healthy leaf area (HLA), as a percentage compared to TLA, recorded values between 81.24 (sample 14) and 99.58% (sample 1). The leaf area affected by bacteriosis (ALA) recorded percentage values between 0.42% (sample 1) and 18.76% (sample 14). The graphical distribution of the values for TLA, HLA and ALA, respectively, is presented in figure 3.

The ANOVA test (single factor), for Alpha = 0.001, revealed the statistical certainty of the experimental data and the presence of the variance in the experimental data set for the case studies performed on walnuts leaves attacked by bacteriosis (*Xanthomonas juglandis*) $p << 0.001$, F> Fcrit, table 2.

The statistical analysis revealed a normal distribution for the three data sets regarding: total leaf area (TLA), figure 4, healthy leaf area (HLA), figure 5, and respectively, affected leaf area (ALA), figure 6.

*Table 1*

Experimental data on TLA, HLA and ALA in walnut leaves, attacked by bacteriosis (*Xanthomonas juglandis*)

| Case studied | TLA | HLA | ALA | HLA(%) | ALA(%) | HLA+ALA |
|---|---|---|---|---|---|---|
| 1 | 62.24 | 61.97 | 0.26 | 99.58 | 0.42 | 100.00 |
| 2 | 55.19 | 54.90 | 0.28 | 99.49 | 0.51 | 100.00 |
| 3 | 50.39 | 49.94 | 0.45 | 99.12 | 0.88 | 100.00 |
| 4 | 35.89 | 35.40 | 0.49 | 98.62 | 1.38 | 100.00 |
| 5 | 45.20 | 44.67 | 0.52 | 98.84 | 1.16 | 100.00 |
| 6 | 61.26 | 60.10 | 1.16 | 98.10 | 1.90 | 100.00 |
| 7 | 92.35 | 90.13 | 2.22 | 97.59 | 2.41 | 100.00 |
| 8 | 45.15 | 42.58 | 2.57 | 94.31 | 5.69 | 100.00 |
| 9 | 63.56 | 60.68 | 2.88 | 95.47 | 4.53 | 100.00 |
| 10 | 52.71 | 49.76 | 2.94 | 94.41 | 5.59 | 100.00 |
| 11 | 66.39 | 63.20 | 3.19 | 95.19 | 4.81 | 100.00 |
| 12 | 58.73 | 54.68 | 4.05 | 93.11 | 6.89 | 100.00 |
| 13 | 43.46 | 39.14 | 4.32 | 90.06 | 9.94 | 100.00 |
| 14 | 36.74 | 29.85 | 6.89 | 81.24 | 18.76 | 100.00 |
| 15 | 50.38 | 41.14 | 9.24 | 81.67 | 18.33 | 100.00 |
| SE | ±3.63 | ±3.80 | ±0.66 | ±1.53 | ±1.53 | |

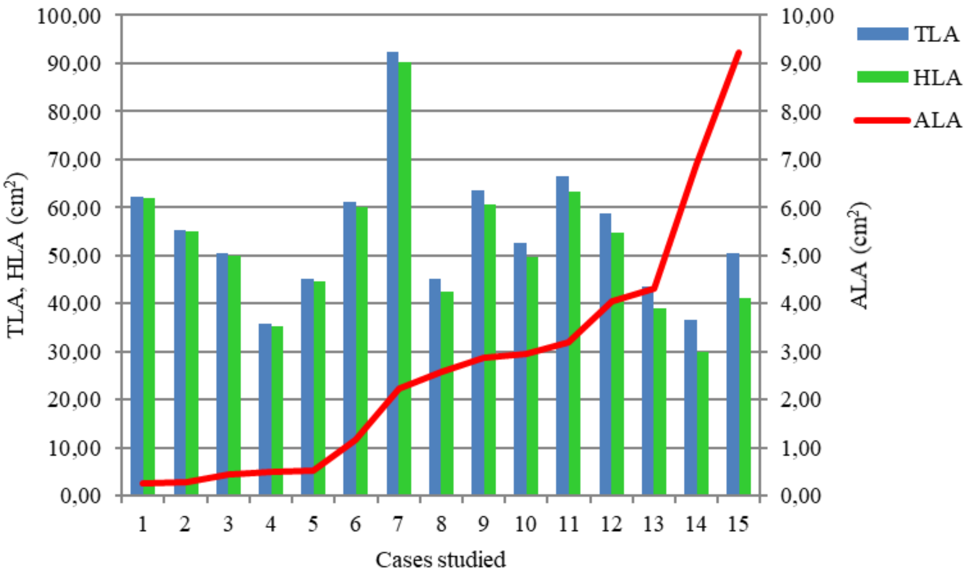


Fig. 3. Graphical distribution of TLA, HLA and ALA values for cases studied in walnut leaves with bacteriosis attack

*Table 2*

ANOVA test, single factor

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 88153.89 | 4 | 22038.47 | 223.8305 | 4.36E-39 | 5.200847 |
| Within Groups | 6892.238 | 70 | 98.46054 | | | |
| Total | 95046.13 | 74 | | | | |

Alpha = 0.001

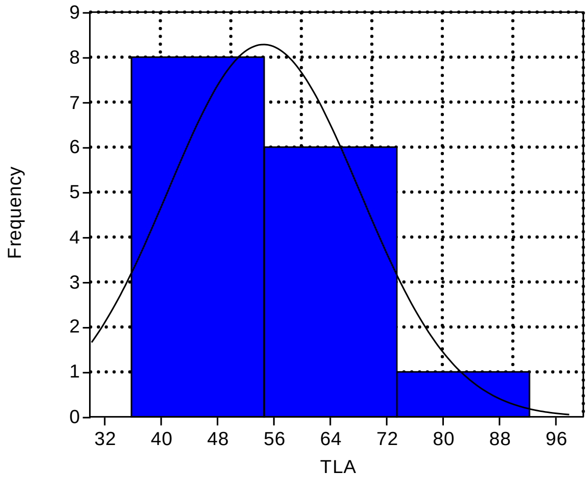


Fig. 4. Distribution histogram of experimental values for TLA (total leaf area)

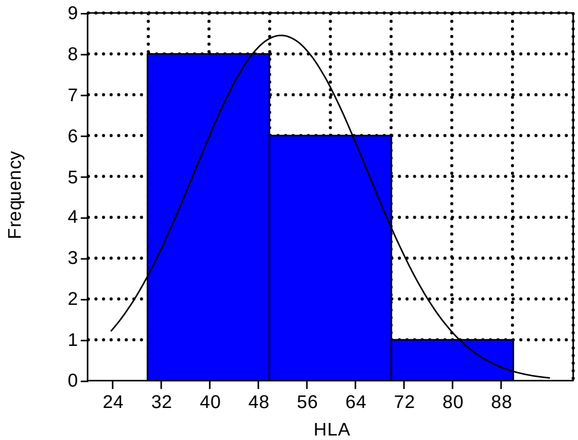


Fig. 5. Distribution histogram of experimental values for HLA (healthy leaf area)

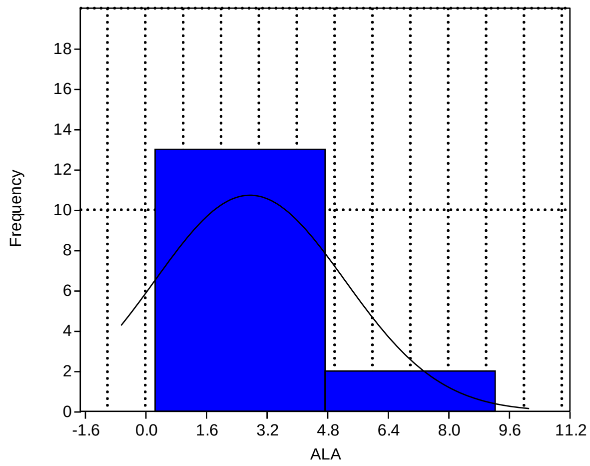


Fig. 6. Distribution histogram of experimental values for ALA (affected leaf area)

The 3D distribution of the values for the leaf surface, healthy leaf area (HLA) and affected by bacteriosis in walnut (ALA), in relation to the total leaf area (TLA), is graphically represented in figure 7.

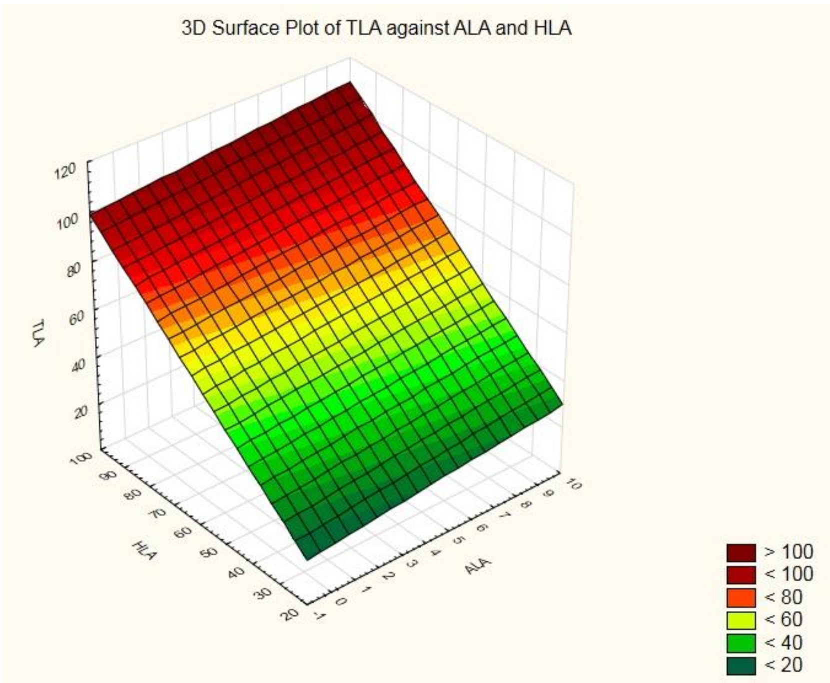


Fig. 7. 3D graphic distribution of values for HLA and ALA in relation to TLA in walnuts leaves studied with bacteriosis attack (*Xanthomonas juglandis*)

The relation that accurately described the distribution of ALA values (%) with respect to HLA values (%), is given by a linear function, relation (1), under conditions of $R^2 = 1$, $p << 0.001$, which indicates a very high accuracy of the determination of the two types of surface (HLA, ALA), in relation to the total surface (TLA) of the studied leaves. Graphical distribution is presented in figure 8.

$$ALA = -1x + 100 \quad (1)$$

where: x – healthy leaf area (HLA).

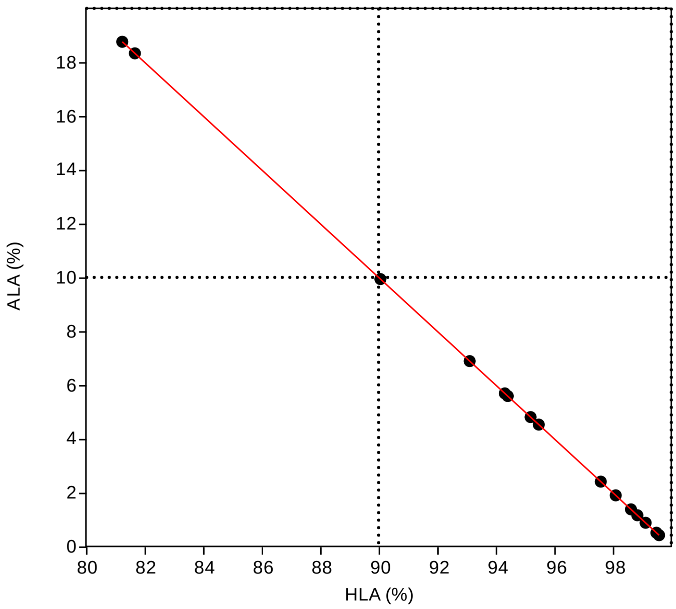


Fig. 8. Graphical distribution of experimental values for ALA (%) relative to HLA (%)

Imaging analysis is increasingly used in the study of vegetation and agricultural crops, from large scale (HERBEI and SALA, 2015, 2016), to small scale (MAHLEIN, 2016), and the imaging methods for phenotyping of plants, according to health status, are very useful (MAHLEIN, 2016).

The multispectral images very accurately express the condition of the vegetal carpet and of the cultivated plants in relation to the state of plants hydration, plant nutrition, vegetation stages of plants or crops, biomass quantity (BENDIG et al., 2014; CANDIAGO et al., 2015; HERBEI et al., 2015).

Various applications, including for portable devices, have been developed for the purpose of diagnosing plant diseases (RENUGAMBAL and SENTHILRAJA, 2015; Petrellis, 2019). The new generations of CNNs (convolutional neural networks) have facilitated obtaining high accuracy results in the field of image classification for plant diseases diagnosis (SLADOJEVIC et al., 2016; WALLELING et al., 2018; TODA and OKURA, 2019). But at the same time, other approach have been studied and developed on this direction. Similar results in terms of accuracy have been described in the present study for assessing bacteriosis (*Xanthomonas juglandis*) attack in walnut (*Juglans regia* L.).

**CONCLUSIONS**

The Scan Sick & Healthy Leaf application has facilitated the analysis and classification of the leaf surface in walnut folioles attacked by bacteriosis. It was possible to estimate the degree of attack with accuracy, both in absolute values and as a percentage.

The 15 case studies were accurately evaluated both in terms of total leaf area (TLA), and healthy leaf area (HLA), respectively of the affected leaf area (ALA).

The Scan Sick & Healthy Leaf application can be extended to evaluate other attacks by plant pathogens, at different plant species, cultivated or in spontaneous flora.

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