

The development of phytosanitary monitoring methods – creating of a schematic scale for assessing the development of Alternariosis on grape leaves

*Natalia Aleinikova**, *Yevgenia Galkina*, *Vladimir Andreyev*, *Elena Bolotianskaya*, and *Sergey Belash*

All-Russian National Research Institute of Viticulture and Winemaking Magarach of the RAS, 298600 Yalta, Republic of Crimea, Russian Federation

Abstract. To ensure consistent good quality grape yields it is necessary to minimize losses associated with affection of grape plants by various diseases. One of the main conditions to ensure the effective control of phytopathogens is a timely monitoring, revealing and assessing of harmful organisms development intensity. In modern research, in order to improve the accuracy and reliability of quantitative characteristics of disease development intensity, as well as comparability of the results obtained, it is proposed to use a “set of standard diagrams” or a “schematic scale” - a set of illustrations depicting an increasing percentage of damage to the surface of one or another plant organ. To date, similar "sets of standard diagrams" were proposed for such grape diseases as mildew, anthracnose and grape leave rust. The article presents the results of creating a new "schematic scale" for assessing the development intensity of grape Alternariosis as a disease with the recorded increasing importance at a recent time of climate change. The scale was developed using colored and black-and-white photographs of grape leaves affected by Alternariosis to varying degrees to determine the real intensity of the disease in digital form. Scale levels were established in accordance with minimum and maximum values of damage degree. Validation was carried out by two groups of assessors with different experience in accounting. The determination coefficient (R^2) of regression line and the absolute error variance demonstrated an increase in accuracy, repeatability and reproducibility of estimates using the developed “schematic scale”. Using of the developed scale made it possible to increase the accuracy of quantitative assessment of intensity of Alternariosis damage to grape leaves.

1 Introduction

To ensure consistent good quality grape yields it is necessary to minimize losses associated with affection of grape plants by various diseases. Global and local climate changes ob-

* Corresponding author: aleynikova@magarach-institut.ru

served in recent years, manifested in an increase in air temperature, frequency of extreme weather events (drought, waterlogging, warm or severe winters, etc.) lead to stress of agricultural plants, changes in their immune status. There is an increase in the importance of pathogens of semi-parasitic and saprotrophic types of nutrition [1, 2].

Similar tendencies are observed for grape agrocenoses in viticulture regions of Russia and worldwide - against the background of climate change, transformations of ampelocenosis pathocomplexes are registered - species composition of phytopathogenic fungi and their harmfulness level change [3]. Under these conditions, the development and implementation of modern methods of diagnostics, monitoring and forecasting of phytosanitary biological objects (molecular genetic identification, mathematical modeling, digital technologies, etc.) are required.

According to the results of a long-term study of phytosanitary situation in vine plantations of main viticultural zones of Crimea, an increase in importance of grape Alternariosis was established. Development of *Alternaria sp.* on leaves of grape plants is mainly associated with a weakened physiological state of grape plants (the formation of leave sun scald and loss of turgor), due to such stressful phenomena as increased solar insolation, high air temperatures, low relative humidity and soil drought. In this case, *Alternaria* transforms from a saprotrophic to a pathogenic form. On the upper side of leaves, at first, single small angular brown spots develop, which later merge into large dark, gray-ashy ones along leave ribs, resulting in complete leave dry out. Such manifestation of Alternariosis is especially intense on 'Aligote', 'Chardonnay', 'Rkatsiteli', 'Cabernet-Sauvignon', 'Saperavi Severny' and 'Bastardo Magarachskiy' grapevine cultivars.

A study of Alternariosis harmfulness show that when 48% of leaves are affected by the disease to an average degree - with an intensity of 23.2%, the amount of crops harvested per bush, as well as the content of sugars in grape juice, is significantly lower than in the variant with effective control of Alternariosis (4.2% of leaves affected with an intensity of 1.4%), quantitative yield losses amount to 11.4%, qualitative (sugar content) - 13.7% [4-6].

According to foreign researchers, the development of Alternariosis on berries of table grapes is especially dangerous during their long-term storage in refrigerators, attaining an unpleasant flavor. This is due to the fact that the product of *Alternaria alternata* metabolism is the mycotoxin alternariol, which has allergenic properties. The results of studies demonstrating high toxigenic potential of *Alternaria* isolates in wine grape varieties and processed products are also known [7].

The obtained results and data from literature sources [8-10] indicate the harmfulness of Alternariosis and the necessity of effective control of this disease in the vineyards in modern conditions, including the developments in phytosanitary monitoring regulations.

Quantitative assessment of plant diseases, including data on distribution and intensity of development, is a mandatory requirement when conducting phytopathological studies, phytosanitary monitoring, studying the disease harmfulness. It is also necessary when making decisions on feasibility of taking protective measures. For a considered sampling unit (a leaf, for example), the disease intensity is described by percentage of the affected part of the total area, and is usually expressed in points. Damage extent can be assessed visually or by image analysis, molecular or immunological methods. Despite the achievements in digital software for image-based automatic or semi-automated assessment of damage intensity of pathogens to plant organs, quantitative visual assessment remains the most commonly used method, both in the field and in laboratory conditions. The variability and inaccuracy of the assessment influence the obtained entries, their subsequent analysis and conclusions, as well as recommendations from the study results. To improve the accuracy and reliability of assessment of disease development intensity, as well as comparability of the results obtained, it is proposed to use in modern researches a "set of standard diagrams" or a "schematic scale" - a set of illustrations depicting an increasing percentage of damage to the sur-

face of one or another plant organ. They are designed in a wide range of styles and are used as tools prior to visual assessment of disease intensity in order to "calibrate" the assessor's eye and improve the accuracy, consistency and reliability of the results obtained. To date, similar "sets of standard diagrams" have been proposed for mildew, anthracnose and grape leaf rust [11–13].

Due to the fact that similar works for Alternariosis of grape leaves are unknown, the purpose of our research is to develop a scale for quantitative assessment of Alternariosis development on grape leaves and ensure its maximum accuracy in the framework of research on the development of regulations for phytosanitary monitoring of this disease in ampeloceneses.

2 Materials and methods

To develop the scale, we used leaves of widely-spread grapevine cultivars 'Aligote', 'Chardonnay' and 'Cabernet-Sauvignon'. Leaves with varying degrees of disease development, from the minimum to the maximum level, were collected from July to September 2020 and 2021. Leaves with symptoms mixed with other grape diseases were not included in the analysis. Every leaf with symptoms was photographed with image resolution of 4000x3000. The obtained images in two versions, black-and-white and colored, were used for estimating the percentage of affected by the disease leaf surface area using ImageScope M software (Fig. 1). The scale levels were set in accordance with the minimum, maximum and intermediate values of damage degree for convenience in further application and training [14, 15].

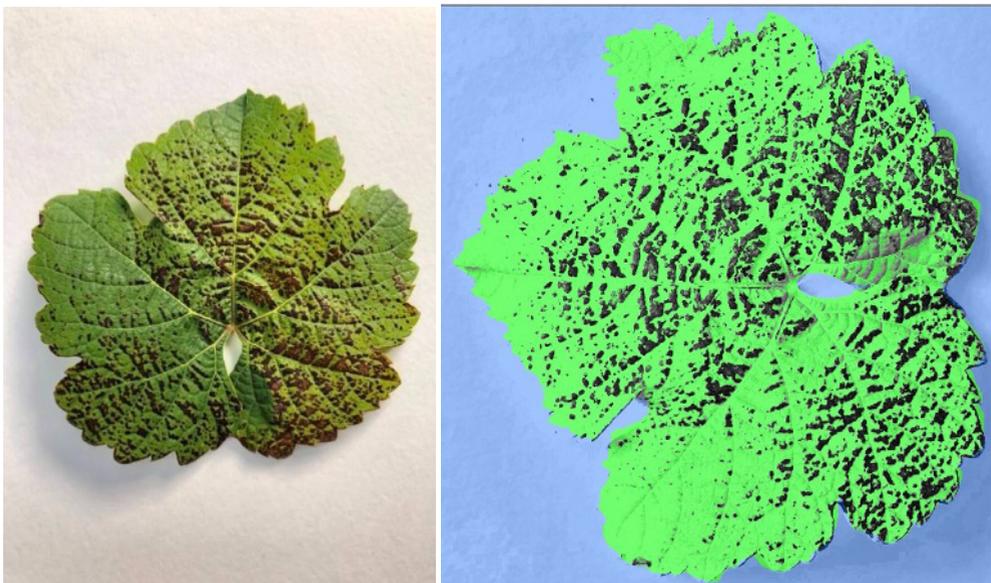


Fig. 1. Grape leaf with symptoms of Alternariosis: a) untouched photo; b) photo, processed with Image Scope M software (disease development level 27%).

Validation and confirmation of accuracy of the developed "schematic scale" was carried out on the basis of assessment of 30 grape leaves with symptoms of Alternariosis with varying intensity by 16 specialists: group 1 - 8 people with no experience in quantitative assessment of diseases; group 2 - 8 people with experience in quantitative assessment of diseases. The assessment was carried out in 2 stages: a sequence of 30 images of leaves with different disease intensity was evaluated without using the developed scale; the same imag-

es were presented in a different sequence and evaluated using the proposed scale. The evaluation results were processed statistically using Microsoft Excel software.

The proposed scale was approved on the basis of analysis of accuracy, reliability and repeatability of the estimates. After receiving from each assessor, the entries were subjected to a simple linear regression analysis between the actual damage degree (independent variable) and the estimated development degree (dependent variable) without and with using the scale. The model accuracy was estimated using the determination coefficient (R^2) of regression line and the absolute error variance. To assess the effectiveness of developed scale, we used the determination coefficient indicators (R^2), presented in Table 1.

Table 1. Coefficient of determination (R^2).

Quantitative measure of correlation ratio	Qualitative characteristic of bond strength
0,1-0,3	Weak
0,3-0,5	Medium
0,5-0,7	Noticeable
0,7-0,9	High
0,9-0,99	Extremely high

3 Results and discussion

As a result of the research, in order to improve the accuracy, repeatability and reproducibility of assessing damage degree to grape leaves by Alternariosis, a “schematic scale” was developed, which included 6 levels: level 1 - up to 5%; level 2 - from 6 to 10%; level 3 - from 11 to 25%; level 4 - from 26 to 50%; level 5 - from 50 to 75%; level 6 - from 75% and above.

As the primary data analysis showed, when accounted without using the scale, most of assessors from both groups noticeably overestimated the level of Alternariosis development on grape leaves [14, 15]. This was due to the difficulty of perception the uneven spreading of disease symptoms on the leaf surface area. When reviewing and using the proposed scale, this tendency was not followed or was observed to a lesser extent.

Data analysis in the first group (assessors without experience) showed that the lowest level of determination (R^2) without using the scale was obtained by assessors 6 and 8 ($R^2=0.52$ and 0.25). However, after determining the intensity of Alternariosis according to the developed scale, the level of determination was 0.63 and 0.65 , respectively.

Noticeable correlation in primary accounting was obtained by assessors 1, 3, 5 and 7 with the level of determination (R^2) - 0.67 ; 0.69 ; 0.62 and 0.68 . Using of the scale made it possible to increase the level by an average of 11.4% , the indicator was 0.74 ; 0.77 ; 0.74 and 0.75 (high dependency, Fig. 2).

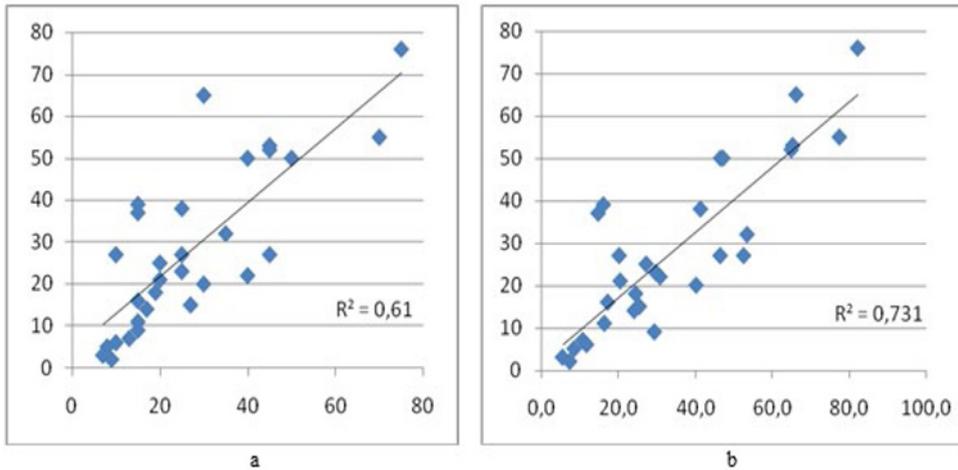


Fig. 2. Coefficient of determination (functional linkage): a) the first group, primary accounting without using the scale; b) the first group, repeated accounting using the scale.

The highest assessment values of Alternariosis development intensity on grape leaves in the first group before getting acquainted with the scale were obtained by assessors 2 and 4 - 0.71 and 0.75, respectively. When assessing the level of disease development using the studied scale, the coefficient of determination (R^2) amounted to 0.8. (Fig. 3)

Therefore, we can conclude that using of the developed scale by a group of people without experience in accounting the disease development made it possible to increase the accuracy of quantitative assessment of Alternariosis damage intensity to grape leaves.

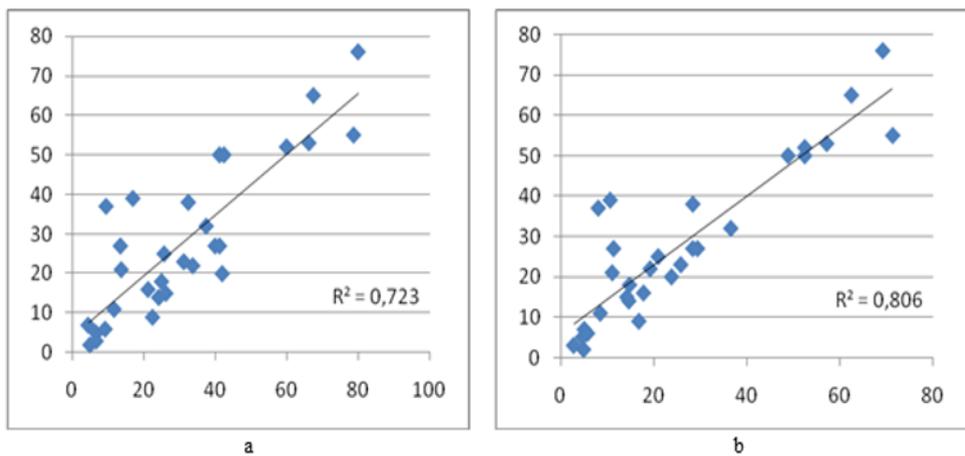


Fig. 3. Coefficient of determination (functional linkage): a) the second group, primary accounting without using the scale; b) the second group, repeated accounting using the scale.

In the second group, the determination coefficient (R^2) in primary accounting without using the scale for 50% of assessors averaged 0.67 (noticeable dependency). Using of a color scale made it possible to increase the accuracy of assessment by 14.1%. Data analysis of assessors 2, 4, 6 and 7 from the group experienced in primary accounting showed high correlation of 0.76; 0.76; 0.71 and 0.74. After assessing the disease development using the scale, this indicator increased by an average of 9.8% and amounted to 0.81; 0.86; 0.82 and 0.81 (high dependency, Fig. 3).

Thus, using of the developed schematic scale, both in the first and in the second group of specialists, made it possible to increase the accuracy, repeatability and reproducibility of quantitative assessment of Alternariosis damage intensity to grape leaves.

4 Conclusion

According to the data obtained, the developed scale for assessing Alternariosis development on grape leaves improved the results of researchers, and also proved to be easy for understanding and using. The proposed six-level scale was well accepted in the field and proved to be useful for short-term training. The developed scale has increased the accuracy and reliability of assessing the degree of Alternariosis damage to grape plants. It can be used for phytosanitary monitoring of this disease and judging about the necessity of protective measures.

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