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Impact of disease severity on infected bunches upon a yield of grape variety Vranec, caused by *Plasmopara viticola* (Berk. & M.A. Curtis) Berl and De Toni

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Abstract

Impact of disease severity on infected bunches upon a yield of grape variety Vanec, caused by *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni., every year causes damage to the yield of vines by infection the bunches while they are still unripe. It is essential to assess the severity of Downy Mildew in vineyards to predict yield loss accurately. The software platform 'image J' was used to detect and quantify the disease severity by measuring infected berries relative to healthy tissue. Regression analysis was used as a statistical method to predict yield loss. *Plasmopara viticola* was monitored in 2022 to find a rational solution to build a Yield Loss Forecast Model. The results of theoretical assumptions compared with actual field situations show that Yield Loss Forecasting Model within the allowed statistical range approximately predicts the yield loss of the control variant.

Keywords: *Plasmopara viticola*; Disease severity; Regression analysis; Yield Loss Forecasting Model

1. Introduction

Plasmopara viticola [(Berk. & Curt.) Berl. & de Toni], belonging to the order of *Peronosporales*, is an obligate biotrophic oomycete pathogen of grapevine and causes downy mildew [3]. According to De Simone et al.,[2] viticulture is one of the prime forms of fruit crop cultivation worldwide, and its global diffusion contributes considerably to human nutrition. However, *P.viticola* is the crucial causal agent of agro-economic losses in grape production at the beginning of the annual production cycle. *Plasmopara viticola,* like a causal agent, attacks all green parts of the vine, including unripe bunches, and represents one of the most damaging pathogens to viticulture worldwide. *Plasmopara viticola*, the downy mildew of grapevine (*Vitis vinifera*), is a very destructive pathogen involved in big losses on viticulture [5]. The fungicide treatment against *P.viticola* is the only measure available to control the disease because the grapevine originating from *Vitis vinifera* is highly susceptible to downy mildew. Several fungicide treatments are required each year to enable grape production. However, the main agro-economic damages from downy mildew occur at bunches. Cluster infections are the most important factor for quantitative yield reduction [6]. Lorenz et al., [8] suggest that infections of the grape berries can occur at the early stage of development, when the individual berries are still firm and the stomata still open, accordingly at BBCH 71 phase. Fröbel and Zyprian [4] indicate that the mycelium of *P.viticola* growth proceeds through the complete inside of the berries, with development of numerous haustoria. Older infected “leather berries” occur in BBCH 77 and BBCH 79 stages, where losses are visually most visible, with that infected young berries being colonized off the inside by the mycelium of *P.viticola*. (Figure1). Monitoring the bunches infection and consequently creating yield loss forecasting models represent pivot tools that give us noticeable information about the management of the plantation. The made on yield loss forecasting models are non-functional without so-called digital imaging quantifying techniques. The image sensing techniques have attracted the interest of many researchers and have been incorporated into plant disease study for their advantages in the analysis of automated, low-cost, non-invasive disease capabilities [1].

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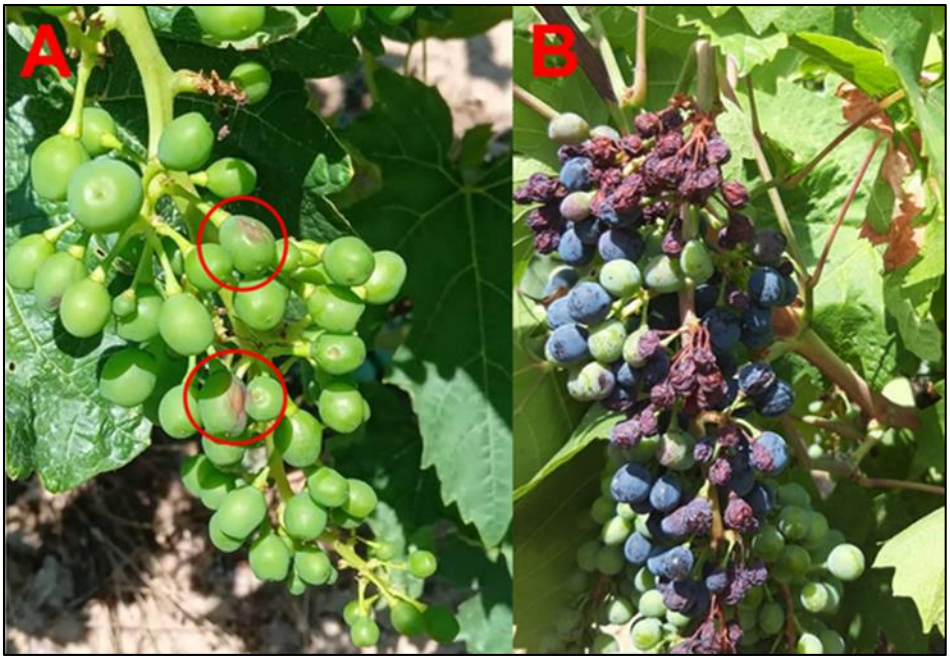

Figure 1 Overview of older infected "leather berries" caused by *P.viticola* where the infection takes place later, and the berries are half-grown, A-BBCH 77 (Berries begin to touch, and symptoms of leather tissue be noticed caused by *P.viticola*.); B-BBCH 79 (The bulk of berries touch, and the *P.viticola* grows mainly internally; the berries become leathery and wrinkled and develop a reddish marbling to brown coloration).Photo of the author, Smilica locality, 2022

The digital images made in the field can be analyzed, with the PCs, Tabs, and Smart Phones provided by different software programs enabling the measurement of disease severity in all green parts of the vine, such as the tool ImageJ. ImageJ is an open-source Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation at the University of Wisconsin. These digital quantifications of the infected tissue provide crucial information for creating yield loss forecasting models, such as the parameter disease severity at bunches and leaves. The software ImageJ tool has a built-in threshold color segmentation method that executes the calculation of the diseased tissue area in the ratio of plants' healthy tissue.

2. Material and methods

The research aimed to determine grape yield loss upon infection development of *P.viticola* on bunches and consequently to create a yield loss forecasting model. In 2022, a forecasting model of yield loss caused by *P. viticola* was applied to the black grape variety Vranec to predict yield loss before executing grape harvesting, with the adoption of "Image J" software.

2.1. Experimental design

The exploration was executed by the time of the period from 18.05.2022 until 21.07.2022 in a vineyard located at Smilica, near Kavadarci, Republic of North Macedonia (41°42`71.4" N, 22°0`10.75" E), planted with Vranec variety. The vines were double cane pruned and vertical trained (double Guyot).The experiment consisted of two variants:

- A-Control canopy;
- B-Standard fungicides treatment (Table 1).

Each variant consisted of 30 vines, and all the bunches were counted at the tested vines. The overview of summarised fungicide applications against *P.viticola* is in Table 1.

Table 1 Overview of variants

Variants	Application date	Fungicide used	Active ingredients	Concentration use/ha	The total number of observed bunches in each of the variants	The average number of bunches per vine
Control canopies	18.05.2022	Folpet 80 WG	80% Folpet	1,5 kg·ha^{-1}	167	5,6
Standard fungicides treatment	22.05.2022	Mikal Premium F WG	50% Fosetil-Al+ 25% Folpet+4% Iprovalicarb	2,5 kg·ha^{-1}	178	5,9
	05.06.2022	Mikal Premium F WG	50% Fosetil-Al+ 25% Folpet+4% Iprovalicarb	2,5 kg·ha^{-1}		
	17.06.2022	Ampexio WG	25% mandipropamid +24% zoksamid	0,8 kg·ha^{-1}		
	04.07.2022	Ampexio WG	25% mandipropamid +24% zoksamid	0,8 kg·ha^{-1}		
	20.07.2022	Comprantol Duo	25% copper oxychloride +24% copper hydroxide	2,5 kg·ha^{-1}		

Calculation of the quantity of fungicide (CQF) to apply in the one hectare area (1000m2) (Equation 1).

CQF=(recommended dosage ×spray area (in square meters))/(active ingredients ×100) (1)

Folpet=(1,2 kg ×10000)/(80 ×100)=12000/8000=1,5kg/ha explanation: (recommended dosage: in a concentration of 0.15 % (150g/100 L of water or 1,2 kg of 800L water/ha)

Mikal Premium F WG= (2 kg ×10000)/(79 ×100)=20000/7900=2,53 kg/ha explanation: (recommended dosage: in a concentration of 0.25 % (250 g/100 L of water or 2 kg of 800L water/ha)

Ampexio WG = (0,4 kg ×10000)/(49 ×100)=4000/4900=0,8 kg/ha explanation: (recommended dosage: in a concentration of 0.05 % (50 g/100 L of water or 0,4 kg of 800L water/ha)

Comprantol Duo = (1,2 kg ×10000)/(49 ×100)=12000/4900=2,45 kg/ha explanation: (recommended dosage: in a concentration of 0.15 % (150 g/100 L of water or 1,2 kg of 800L water/ha)

Note: doses are by the fungicide manufacturer's recommendation

2.2. Disease assessment

Two parameters were taken into account which are significant to the research to obtain the required results, as they are:

- Disease severity (DS) and
- Disease incidence (DI), which were measured and calculated in assessing the damage from *P.viticola*.

The disease assessment on bunches area was analyzed regularly at each growth stage, beginning May 18 till July 21, 2023, when occurred veraison phase (French: véraison, is the onset of the ripening of the grapes) which marked the end of the possibility of infection of bunches by *P.viticola*. The intensity of *P.viticola* on bunches was evaluated with the

parameter DS using ImageJ software that gives the ratio between infected and healthy tissue. The software tool ImageJ uses fuzzy logic techniques for the analysis of a few parameters as they are: (i) percentage of infections (POI) on bunches; (ii) diseased area (DA); total tissue area (TTA).In this context, use threshold color segmentation method was used to approximate the areas of the infected tissue and the entire healthy tissue to calculate POI on bunches (Figure 2). The calculation of POI is a proportion between DA and TTA (Equation 2). On the other side, the gives result of POI was used to estimation of the DS.

$$POI=DA/TTA\times100 \quad (2)$$

Figure 2 A- the original image that was segmented using the threshold color segmentation method; B- the converted image into the white background to calculate the total bunches area, which is colored black (TTA);C- diseased area (DA)

Disease incidence (DI) can be defined as the number of bunches that are (visibly) diseased, usually relative to the total number of assessed bunches in the sample. Further, the disease incidence parameter shows the percentage of newly diseased bunches in the sample at each measurement (Equation 3), where: x- Number of diseased bunches; N- Total number of units assessed.

$$DI = \frac{\Sigma x}{N} \times 100 \quad (3)$$

The measurements of the parameters DS and DI were always executed after rainfall, during which the amount of precipitation per square meter (mm/m^2) and the average daily temperature during rainy days by recorded.

2.3. Statistical analysis

The obtained statistical results were significant only in the control canopy. However, the yield from the variant with standard fungicide treatment get used solely for comparison with the grapes yield obtained in the control variant and was expressed as kg/ per plant. The IBM® SPSS® Statistics software platform was used for statistical analysis. The overall statistical analysis consisted of several steps: (i) Data collection: this involved gathering relevant data using appropriate methods (Table 2);(ii) Execution of log-log transformation of data obtained in the control variant (Table 2), then fitting a linear regression model to the transformed data;(iii) Data analysis: involved applying appropriate statistical methods to the data to answer research questions or test hypotheses (Table 3) ; linear regression was used as a statistical method for data analysis, (Equation 4) where: ŷ- is the dependent variable; βo- is the intercept; β1- regression coefficient; x- average value of independent variable

$$\hat{y}= \beta o + \beta 1(x) \quad (4)$$

3. Results and discussion

Table 2 Overview of calculation of log-log transformation of data obtained in the control variant

Observation dates	Data collection		Log-log transformation of data	
	Disease severity on bunches (%)	Disease incidence on bunches (%)	Disease severity on bunches	Disease incidence on bunches
18-May-2022	1	2,3	0	0,3793435
23-May-2022	5	4,29	0,69897000	0,63291043
24-May-2022	26	5,7	1,41497334	0,76111791
28-May-2022	27	8,8	1,43136376	0,94662601
29-May-2022	29	11,2	1,46239799	1,04898646
31-May-2022	36	7,5	1,55630250	0,87869554
06-Jun-2022	48	9	1,68124123	0,95860731
08-Jun-2022	53	11	1,7242758	1,04139268
09-Jun-2022	76	13,4	1,88081359	1,12979123
10-Jun-2022	79	9	1,89762709	0,95860731
11-Jun-2022	82	5,7	1,91381385	0,75696195
17-Jun-2022	69	13,6	1,83884909	1,13469857
18-Jun-2022	63	7	1,79934054	0,84618513
24-Jun-2022	57	5,6	1,75587485	0,75284538
27-Jun-2022	50	8	1,69897000	0,90308998
01-Jul-2022	25	15,2	1,39794000	1,18234020
04-Jul-2022	29	17,9	1,46239799	1,25403343
05-Jul-2022	35	18,7	1,54406804	1,27300127
09-Jul-2022	55	15,3	1,74036268	1,18708664
14-Jul-2022	43	9	1,63346845	0,95860731
20-Jul-2022	39	5	1,59106460	0,69897000

After a log transformation of data obtained from DS and DI, the histogram becomes more or less symmetric (Figure 3), performing a statistical analysis that assumes normality to help meet the assumption of constant variance in linear modeling. The histogram of standardized residual coefficients formed follows a normal distribution (Gaussian distribution) representing the difference between an observed value and a predicted value in our linear regression, enabling to fit of a linear regression model that accurately captures the relationship between dependent (DS) and independent (DI) variables, thus allowing approximate accuracy of the model (Figure 4).

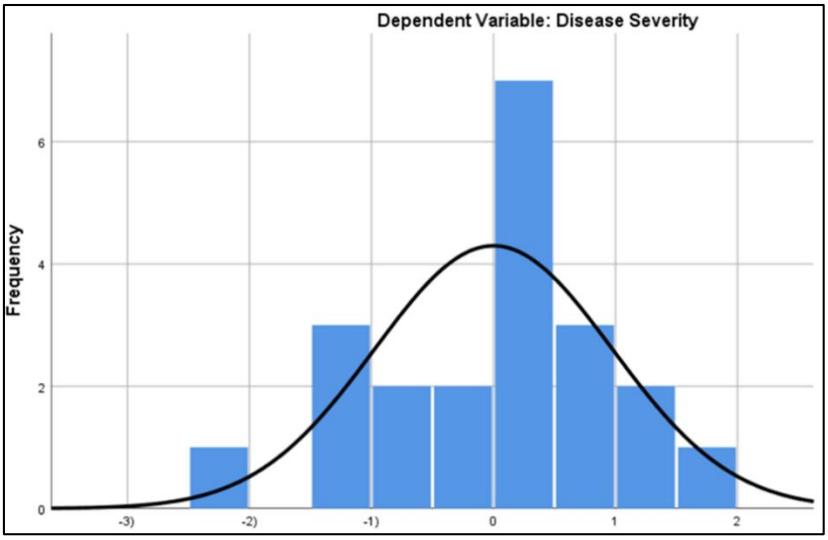

Figure 3 Standardized Residual Histogram

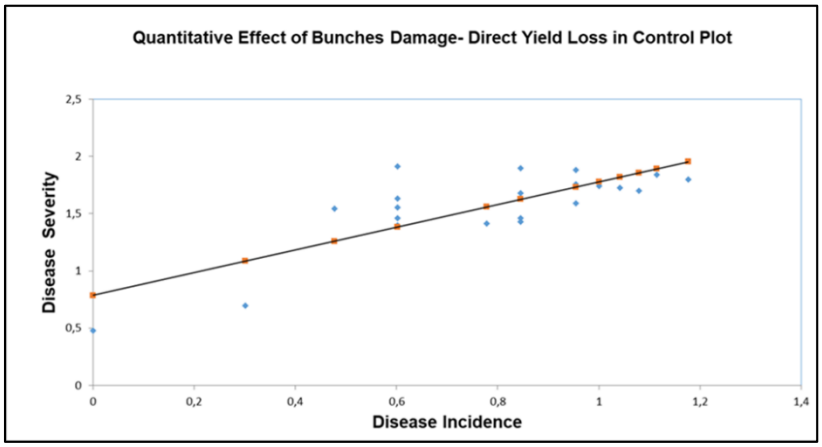

Figure 4 Relationship between disease incidence and disease severity of *Plasmopara viticola*

Table 3 Data analysis

Regression Statistics		
Multiple R	0,571714152267586	
R Square	0,326857071903044	
Adjusted R Square	0,291428496740047	
Standard Error	0,369528881724758	
Observations	21	
ANOVA		
F	9,2258034764102	
F-Significance	0,00677591982902827	
Coefficients		**P-value**
Intercept	0,481574707	0,190080866176687
Disease Incidence	1,118225989	0,00677591982902824

$$\hat{y} = \boldsymbol{\beta o} + \boldsymbol{\beta 1}(\boldsymbol{x}) = \mathbf{0,481574707} + \mathbf{1,118225989} \times \mathbf{0,93} = \mathbf{1,5}\text{kg/per vine in control (average value)}$$

Since the standard error (SE- 0,226135) is higher than usual, we form a confidence interval (Equation 5).

$\hat{y} \pm \boldsymbol{t}\frac{\boldsymbol{SE}}{\sqrt{\boldsymbol{n}}}$ (5) Where: t- is value of the Student's t-distribution as a function of the probability and the degrees of freedom; SE-standard error; n-number of observation

$$\hat{y} \pm \boldsymbol{t}\frac{\boldsymbol{SE}}{\sqrt{\boldsymbol{n}}} = \mathbf{2,093} \times \frac{\mathbf{0,226135}}{\sqrt{\mathbf{21}}} = \mathbf{1,5} - \mathbf{0,103} = \mathbf{1,39}\text{ Low Confidence Interval}$$

$$\hat{y} \pm \boldsymbol{t}\frac{\boldsymbol{SE}}{\sqrt{\boldsymbol{n}}} = \mathbf{2,093} \times \frac{\mathbf{0,226135}}{\sqrt{\mathbf{21}}} = \mathbf{1,5} + \mathbf{0,103} = \mathbf{1,6}\text{ Upper Confidence Interval}$$

ŷ=1,39 to 1,6 kg/ per vine in control-Yield Loss Forecast Model Caused by *Plasmopara viticola* (theoretical assumptions).

4. Conclusion

The expected grape yield in the control variant is between 1,39 and 1,6 kg/per vine; when subtracted from the variant with standard fungicides treatment (where vines give an average of 4,3 kg/per vine), the resulting difference (from 2,7 to 2,9 kg/per vine) represents the lost yield caused by infection from *Plasmopara viticola* or per hectare the loss ranges from 10800 to 11600 kg. The results of field measurements are obtained about 50 days before the grape harvest by theoretical assumptions (calculations) on control canopies allowing us to calculate in advance the potential monetary losses it caused *Plasmopara viticola*.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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