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Development of hypothetical infection model based on environmental variables for *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni

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Abstract

Development of a hypothetical infection model for *Plasmopara viticola* based on environmental variables was applied to black grape variety Vranec. The infected leaves were digitally quantified using the software platform 'ImageJ' to determine disease severity. The resulting data, along with inputs of average temperature and leaf wetness duration, were used to establish a regression relationship. Downy mildew infection reaches its maximum when we have an average of 2 hours and 29 minutes of leaf wetness duration and an average daily temperature of 26.8 ° C. The regression relationship then by used to describe the results, and based on this, a hypothetical infection model for P. viticola was developed.

Keywords: *Plasmopara viticola*; Hypothetical infection model; Environmental variables; Vranec; Regression relationship; Disease severity

1. Introduction

Downy mildews are very causative agents in commercial agriculture, particularly for agriculturists of crucifers, grapes, and vegetables. The impact of oomycetes on humankind is well documented as both a persistent threat to subsistence and commercial farming and as destructive pathogens of native plants[1].The cause of grapevine downy mildew is the oomycete fungus *Plasmopara viticola* (Berk.& M.A. Curtis) Berl. & De Toni and belongs to the order *Peronosporales*. The asexual multiplication cycles in *P.viticola* appear throughout vegetative growth, and favorable weather conditions can repeatedly occur throughout the growing season, leading to huge losses at vineyards. *Plasmopara viticola*, the downy mildew of grapevine (*Vitis vinifera*), is a very destructive pathogen involved in big losses on viticulture[2].Downy mildew causes both direct yield losses on inflorescences, clusters and shoots, and indirect losses by reducing photosynthetic activity of the affected leaves and by inciting premature defoliation of vines[8].In this study, a simple hypothetical infection model for *P.viticola* was developed based on data provided by the field, emphasizing environmental conditions such as average temperature and leaf wetness duration compared to the disease severity on leaves. Leaf damage is, on the contrary, responsible for an indirect yield loss through a reduction of the carbohydrate production that negatively influences the grape quality, the reserve accumulation and the plant vigour in the next season. Studies have been undertaken to compare the vine response at various levels of defoliation stress during the season [7] , and how leaf infections caused by *P.viticola* affect the physiology of vines. The model developed in this study can use data inputs an average temperature and the wetness duration requirement, simultaneously compared by the development of disease severity, put them in appropriate regression relations. Further, provided data from the simple correlations of disease observations in the field with environmental variables was used to make hypothetical infection model that can predict infection based only upon estimates average temperature and wetness duration. The model is based upon a temperature response function [10] and foliar wetness duration. For many foliar pathogens, the infection submodel is one of the most critical components for disease forecasting[5].Many infection models use regression

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equations, such as those based on polynomials[9] and logistic equations[6] .However, a few models are also known to have been based on using temperature response equations and wet degree hours. These infection models are created from either laboratory or field observations of resulting disease intensity at multiple combinations of temperature and wetness [5].Predictions from the model were statistically compared with the control vines in favorable conditions of development of the *P.viticola*. The main method for controlling downy mildew is fungicide application [7], and the essential objective is a discovery of the critical timing of fungicide treatments from provided data by the hypothetical infection model. A validated model has the potential to increase the chemical control efficiency, decrease the required number of fungicide sprayings, lower the cost of crop production and reduce the negative impact of chemicals on the environment[7]. The overall objective was to develop a simple hypothetical infection model for *P. viticola*, in geographical areas that don't exist forecasting disease networks.

2. Material and methods

This study aims to discover when the infection of *P.viticola* reaches its peak and accordingly to determine the critical timing of fungicide treatments. In 2023, a hypothetical infection model was applied to the black grape variety Vranec according to data provided by the control plot. At the vineyard, the control was treated just once with active ingredient folpet on 20 May 2023. The climatic conditions were favorable for the development of downy mildew in vines. So according to that, it was possible to notice the strengths and weaknesses of the hypothetical infection model.

2.1. Experimental design

The exploration was executed by the time of the period from 25.05.2023 until 29.06.2023 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 only of one control variant where was measured it several parameters: (i) disease severity on leaves; (ii) average daily temperature; (iii) wetness duration. Upon the provided data from the experimental plot, a hypothetical infection model, compared with real situations on the control vines field, was made. The control variant consisted of 90 vines, where downy mildew monitored on the leaves.

2.2. Disease assessment

The software tool ImageJ plays a significant role in discovering the percentage relationship between healthy and diseased tissue on examined leaves. 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 leaves. Monitoring for the development of the disease was carried out every 7 days starting from 25 May until 29 June 2023 (Table 1). The sample size was 30 leaves, and the analysis of the diseased leaves executed by the software tool ImageJ.

Table 1 Overview of control variant

Period of disease observation and assessment of diseased leaves with software tool ImageJ	Disease severity (%)	Research duration expressed in days
25 May 2023	0	1
1 June 2023	29.5	7
8 June 2023	72.8	14
15June 2023	12.8	21
22 June 2023	16.5	28
29 June 2023	78.7	35

The software tool ImageJ uses fuzzy logic techniques for the analysis of a few parameters as they are: (i) percentage of infections (POI) on leaves; (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 leaves (Figure 1). The calculation of POI is a proportion between DA and TTA (Equation 1). On the other side, the gives result of POI was used to estimation of the disease severity (DS).

$$POI=\frac{DA}{TTA}\times 100 \text{ ..... (1)}$$

In the control variant, average temperature and leaf wetness duration were measured using a digital thermohydrometer in the vine's canopies. During the survey period, the length of leaf wetness duration is measured in hours (LWD; h day^{-1}). According to data from the literature, the leaf wetness duration of less than 2 hour is not considered relevant. For example, in humidity conditions, [4] showed that the germination of sporangia occurred in as little as 2 hours.

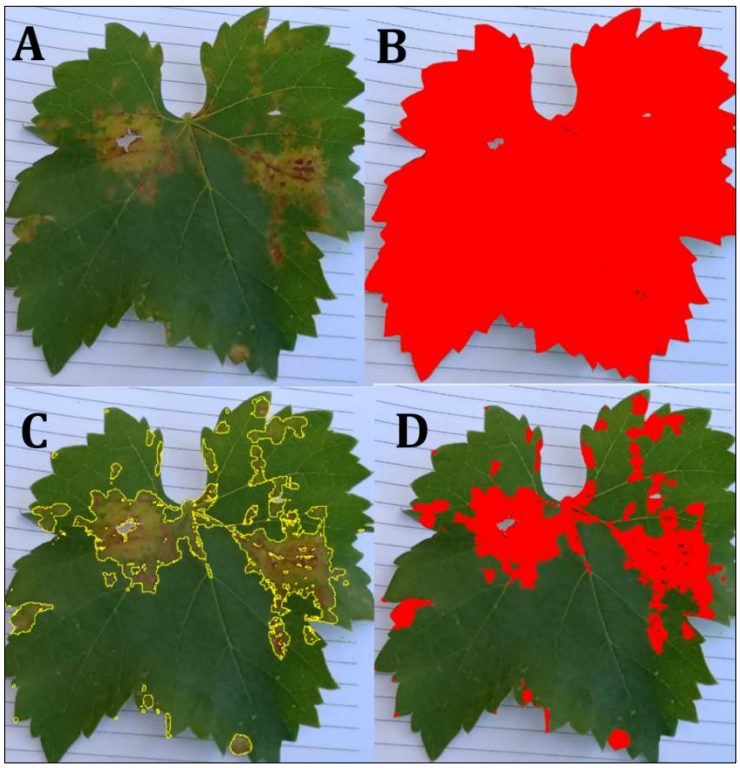

Figure 1 Overview of using the threshold color segmentation method; A- The original image of the diseased leaf; B- total tissue area (TTA); C- The selected diseased area on a leaf; D- Diseased area (DA)

2.3. Statistical analysis

The essence of the research is to understand the trend of downy mildew development in the control variant and provide data by the field using a mathematical-statistical method for this objective. It used the IBM® SPSS® Statistics software platform to calculate regression models between independent environmental variables (average temperature; leaf wetness duration) and dependent variable (disease severity). The overall statistical analysis consisted of several steps: (i) Data collection: this involved gathering relevant data using appropriate methods (Table 1;Equation 1); (ii) Linear regression was used as a statistical method for data analysis (Equation 2) where: ŷ- is the dependent variable; βo- is the intercept; β1-regression coefficient; x- average value of independent variable;(iii) Quadratic polynomial regression was used as a statistical method for data analysis where the relationship between the variables could not represented by a linear model and instead showed a curved pattern; the quadratic polynomial regression equation takes the following form (Equation 3), which is needed to calculate the vertex of a quadratic equation and can use the formula (Equation 4);where: y- is the dependent variable;x- is the independent variable;a,b,c- are the coefficients that the regression analysis aims to estimate; (iv) The graph represents a hypothetical infection model (H) of *P.viticola* made according to the following form (Figure 4; equation 5); where H- hypothetical infection model for *P.viticola* (equation 5); A- is a probability (P^) where we have two conditions on the field that can occur in case of favorable external conditions for the development of *P.viticola* and that an infection may or may not happen, so according to that $P\hat{} = \frac{1}{2} = 0.5$; t- research duration is expressed in days ; B is a coefficient obtained from the result of the quadratic polynomial equation, and then a log-log transformation is performed; W- is a coefficient obtained from the result of the linear regression equation, and then a log-log transformation is performed.

$$\hat{y} = \beta o + \beta 1(x) \text{ .....(2)}$$

$$y = ax2 + bx + c \text{ ..... (3)}$$

$$\text{x-vertex} = -\frac{b}{2a} \text{ ..... (4)}$$

$$H = A^{-bt} \times \cos(Bt + Wt) \text{ ..... (5)}$$

3. Results and discussion

The correlation coefficient (R) measures the strength and direction of the relationship between the dependent variable and the independent variable. In this case, an R value of both models (LM ,R=0.927; QM, R= 0.840) (Table 2) indicates a strong positive correlation between the variables especially in LM (Figure 2). This means that as one variable increases, the other tends to increase as well. The coefficient of determination (R^2) represents the proportion of the variability in the dependent variable that is explained by the independent variables in the model. An R^2 value of 0.860 in LM (Table 2) and 0.706 in QM (Table 2) means that approximately 86 % of the variance in LM and 70.6% in QM from the dependent variables is accounted for by the independent variables included in the model. The adjusted R^2 takes into account the number of independent variables in the model and adjusts the R^2 accordingly. A value of 0.855 in LM (Table2) and 0.688 in QM (Table2) suggests that about 85.5% in LM and 68.8 in QM of the variances in the dependent variables are explained by the independent variables, accounting for the complexity of the model. At first glance, the values of both regression models showed statistical significance, making it valid for further consideration (Table2). The purpose of the calculation of regression models is to obtain the coefficients that we need for the computation of the hypothetical infection model formula, where the result helped to make a graph by showing the curves of the predictability of the disease and the development of disease severity (Figure 4). In our case, the linear equation gives us the data of average leaf wetness duration (h/ day-1) for the research period in a control variant, which amounts to 2h and 29 minutes. When fitting LM to the data for 2h and 29 minutes all of the estimated coefficients was significant (βo, p<0. 000000000000001; $\beta 1$, p<0.040)(Table 2). Next, the log-log transformation is performed to get the coefficient required in the hypothetical infection model formula.

Table 2 Data analysis

Model Summary	**R**	**R Square**	**Adjusted R Square**
Linear model (LM)	0.927	0.860	0.855
Quadratic model (QM)	0.840	0.706	0.688
ANOVA			
Linear model (LM)	F	Sig.	
	202.069	0.000000000000001	
Quadratic model (QM)	38.486	0.000000003	
Coefficients			
Linear model (LM)	Wetness duration (βo)	Unstandardized Coefficients	Sig.
		13.249	0.000000000000001
	Intercept ($\beta 1$)	-8.879	0.040
Quadratic model (QM)	Temperature (b)	77.400	0.000000001
	Temperature (ax^2)	-1.700	0.0000000008
	Intercept (c)	-807.039	0.00000001

Linear model calculation:

$$\hat{y}= \beta o + \beta 1(x)= -8.879+ 13.249\times 3.8=41.5\approx 2h\ 29\ min.$$

$$41.5/100=0.415$$

$$\log(0.415)\approx -0.38=-(-0.38)=0.38\pm 0.03=0.35$$

is the coefficient representing W in equation 5.

A quadratic model was derived from the relationship between disease severity as the dependent variable and average temperature as the independent variable (Figure 3). In the following, in the quadratic equation (y= ax2+bx+c=-1.700x2+77.400-807.039) are noted the legality where ax2<0 ; bx>0 . According to this principle, the curve forms a peak on the graph, reaching the highest point to indicate the most optimal temperature for the development of the infection (Figure 3). The highest point of the curve is called a vertex. The determination of this value is significant. Additionally, all of the estimated coefficients were significant (ax2, p<0. 0000000008; b, p<0. 000000001; c, p<0. 00000001) (Table 2). Further, the value is log-log transformed into a coefficient that becomes a term in the hypothetical infection model formula. It is also significant to mention the unstandardized coefficient that represents the slope of the line between the predictor variable and the dependent variable. In this case, the coefficient of intercept of the curve has a negative sign (c= -807.039) (Table2), and shows that any increase in temperature above 26.8 ° C decreases infection.

Quadratic model calculation:

$$\text{x-vertex}=-\ b/2a=-77.4/(2\times(-1.7)\ )=26.78201\approx 26.8\ C;\ 26.8/100=0.268$$

$$\log(0.268)\approx -0.57187=-(-0.57187)=0.57\pm 0.03=0.54$$

is the coefficient representing B in equation 5

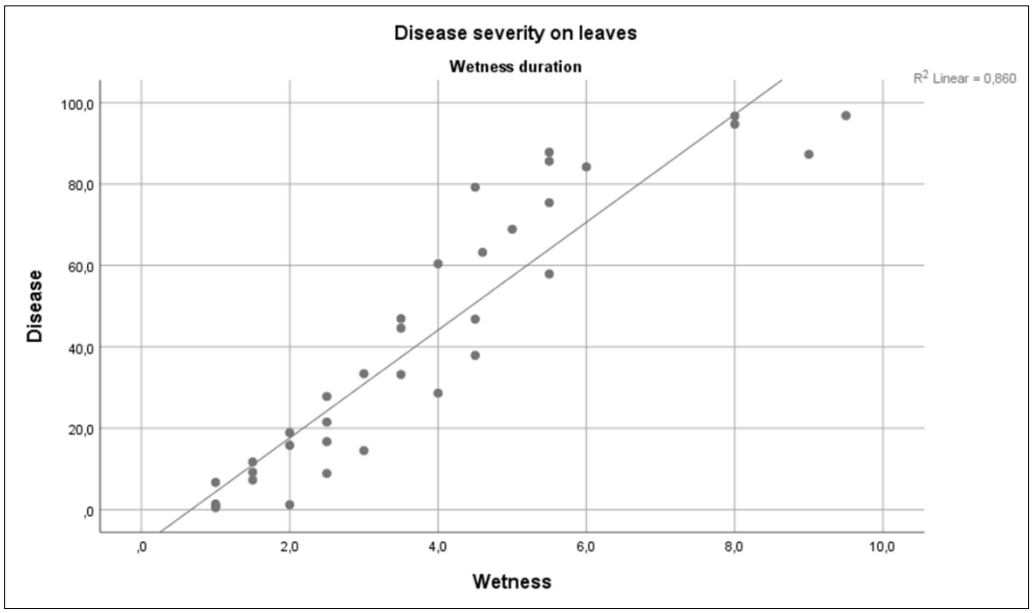

Figure 2 The relationship between x-variable (wetness duration) and y-variable (disease severity) of *Plasmopara viticola*

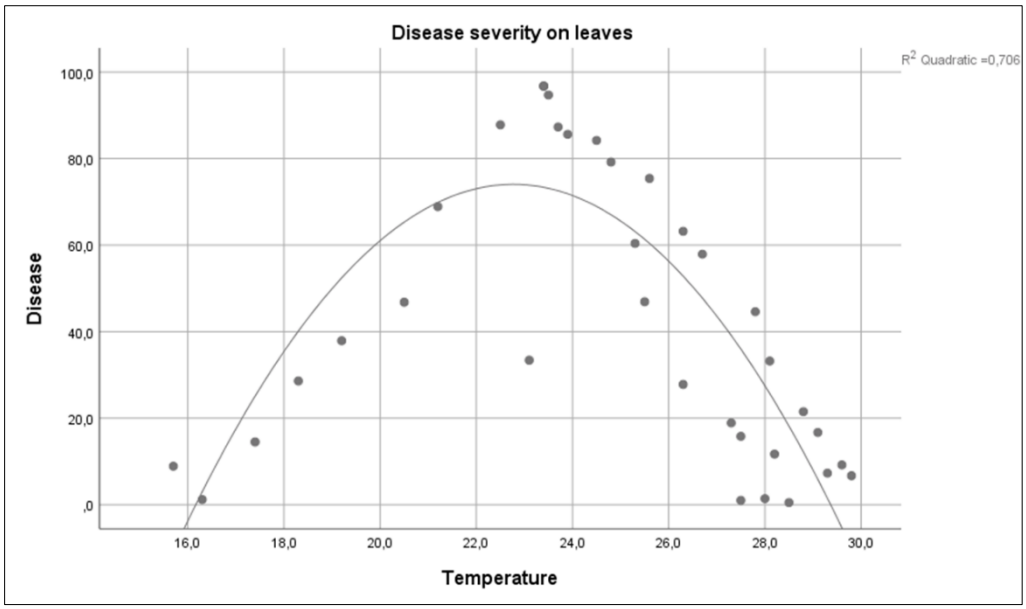

Figure 3 The relationship between x-variable (average temperature) and y-variable (disease severity) of *Plasmopara viticola*

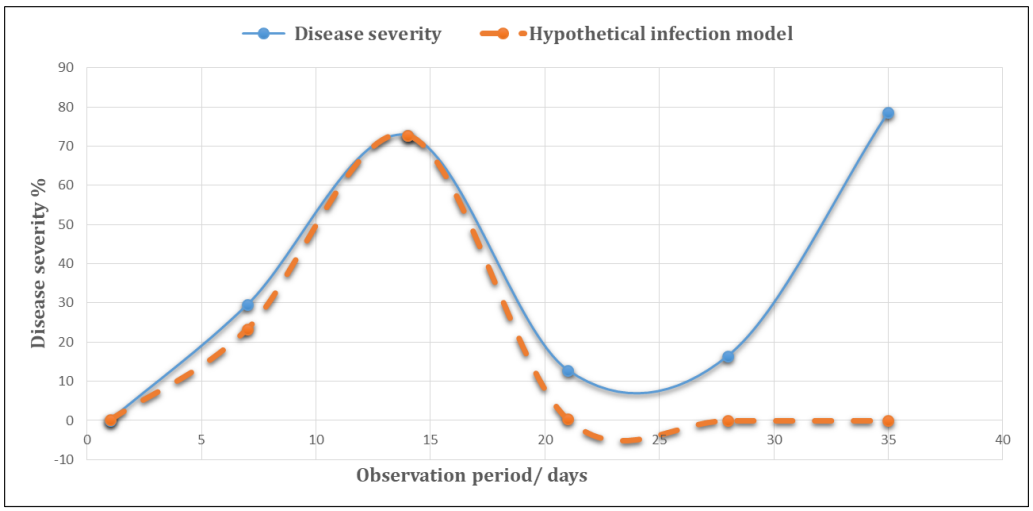

Figure 4 Overview of infection of *P.viticola* in the control variant*;* the continuous line shows the infection dynamics; the dashed line represents hypothetical infection model

4. Conclusion

Our observations showed that the infection reaches its maximum when we have an average of 2 hours and 29 minutes of LWD and an average daily temperature of 26.8 ° C. In those environmental conditions, it has been achieved maximum infection by *P.viticola*. The hypothetical infections model shows that it detects the moment when the infection reaches its maximum, which is a positive aspect. However, when the curve shows decreasing values, it tends to overpredict. Furthermore, when the infection gains momentum, it becomes less predictable.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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