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Development of *Botrytis cinerea* under reduction of pesticides treatments in Macedonian viticulture production

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Abstract

The monitoring of development of *Botrytis cinerea* under reduction of pesticide treatments in Macedonian viticulture production is possible only when introducing a disease forecasting model. *B.cinerea* causes an increase in the number of chemical treatments just before harvest and calls into question the environmental and health value of the product. Therefore, an attempt was made to create a forecasting model for Botrytis which is based on the relationship between relative humidity and the temperature in the vine canopy. The forecasting model for Botrytis was applied at the white varieties Smederevka and Zilavka and based on the data obtained was made ANOVA statistical test which proves the reliability of the model. On the localities, Smilica and Sopot, Kavadarci, Republic of Macedonia, are the experimental fields that were observed for three consecutive years (2017 till 2019).

Keywords: Vine canopy; *B cinerea*; Forecasting model for botrytis; ANOVA statistical test

1. Introduction

Infections caused by *Botrytis* species most often are controlled routine pesticide applications. The biological cycle of *B.cinerea* is already well known, but its adaptive virulence potential to cause crop failure just before grape harvest creates a cause for concern among grape growers manifested by an increasing number of chemical treatments that are sometimes unwarranted. Fungicide application remains the common method to control *Botrytis* [9]. This anti-disease strategy has become increasingly unacceptable for rational disease suppression. It is essentially necessary to apply fungicides only when needed, thereby eliminating unnecessary chemical treatments. Application of disease management measures when they are not needed is inefficient at best, because it results in unnecessary costs to growers, consumers, and environment [11]. During the last decades, restriction in fungicide application became necessary to reduce the impact on the environment [10] and to limit fungicide residues [15].At the same time, acquired resistance to most botryticides arose in many agronomical situations, sometimes impeding field efficacy and leading to additional sprays [4]. Although there are fungicides for its control, many classes of fungicides have failed due to its genetic plasticity [17]. Rational methodology implies avoiding unnecessary sprays. The omission of the unnecessary sprays is related to warning systems for the development of *Botrytis* disease. The warning systems represent a type of forecasting model for disease development. The forecasting system aims to recognize the favorable conditions for *Botrytis* development, and in such a manner, the use of fungicides can be, rationalized. This research represents a forecasting model of the development of *B.cinerea* where accurately microclimatic analyses are embedded. In other words, the microclimatic analyses cover the biological range of development of the pathogen depending on the temperature and humidity that occurred between leaves and bunches in vines canopy, on which the occurrence of the infection depends. In this way, it is possible to predict the incubation period, and just before the onset of the disease and its symptoms at the same time, can be sprayed vines before the pathogen forms spores. Further, this approach prevents

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subsequent dispersion of the spores in the space and are disabled future infections. On the other hand, the forecasting system allows the reduction of the number of sprays by monitoring disease through microclimatic conditions in the field. If the microclimate in vines canopy is unfavorable for disease development then it is postponed the chemical treatments. This warning system has not been tested at other facultative necrotroph fungi which cause plant disease. The species of the genus *Botrytis* are word widespread and are developing in the different climatic zone, hence the approach to making warning systems was different according to the microclimatic facts on the field. Newly developed warning systems should be evaluated in field experiments, by comparing their recommendations with the conventional management practice [13]. Such knowledge was accumulated over the years and the first attempts for developing warning systems for management of *Botrytis*-incited diseases were made in the 1960s-1970s [12]; [8]. Somewhat later, at the beginning of the 1990s, some researchers started making warning systems that serve as forecasting models of *Botrytis* diseases. Almost everyone's warning systems (forecasting models) operate based on the measurement of temperature and, at the same time the moisture content in the habitus of plants. For example, [5] developed a forecasting system of gray mold in vineyards, while in the Netherlands was an established warning system called, BoWaS by [3] who served for prediction of *Botrytis* leaf blight suppression. It is also important to mention a weather-based the predictive system named Blight-Alert to control *Botrytis* leaf blight of onion caused by *Botrytis squamosa* which was developed in New York. The goal by apply warning systems to achieve control as well as reduce selection pressure for the development of *Botrytis* resistant strains towards chemical fungicides.

2. Material and methods

The research was completed in vineyards located at Smilica and Sopot near Kavadarci Republic of North Macedonia on white grape varieties Smederevka and Zilavka (Table 1). The research lasted for three consecutive years (2017; 2018; 2019). The forecasting model on *Botrytis cinerea* is based, on the relationship between relative humidity and temperature between leaves and bunches in the canopy of the vine.

Table 1 Grapevine varieties that were the target of the research

Varieties	Ha	Locality	Years of research
Smederevka	1.7	Smilica	2018-2019
Zilavka	0.5		
Smederevka	1.0	Sopot	2017
Zilavka	0.5		

The aim of the research is prevent development of *B. cinerea* and consequently reduce the number of chemical treatments.At the vineyard, the control was untreated with botryticides whose role should have to be an indicator of the development of the disease. The decision if or when to apply fungicides depended on control (untreated grapes) (table 2).

Table 2 Observation period and number of spaying treatments over the years 2017 till 2019

Varieties	Number of spraying treatments				Observation period against B. cinerea in control (untreated grapes)	Years of research
Smederevka	I	II	III		14.08-18.09	2019
Zilavka	I	II	III		14.08-18.09	
Smederevka	I	II	III	IV	16.08-18.09	2018
Zilavka	I	II	III	IV	11.08-15.09	
Smederevka	no chemical treatment against *B.cinerea*				16.08-18.09	2017
Zilavka					16.08-18.09	

2.1. Chemical control of *B.cinerea*

Preventive protection and disease control rely most on preventing contact between the pathogen and the host. For preharvest treatment, the strategy consists of several applications of fungicides which are allowed per country depending on its legislation (table 3).

Table 3 The recommended active ingredients against *B.cinerea* used during the survey depend on state legislation

No.	Chemical	Dose	Years of research
1.	Pyrimethanil (Pyrus 400 SC)	500 cc/ha	2019
2.	Pyraclostrobin + Boscalid (Signum 33 WG)	1.0 kg/ha	
3.	Cyprodinil+ Fludioxonil (Switch 62,5 WG)	0.6 kg/ha	
4.	Pyrimethanil (Pyrus 400 SC)	500 cc/ha	2018
5.	Fenhexamid (Teldor 500-SC)	1000 cc/ha	
6.	Cyprodinil+ Fludioxonil (Switch 62,5 WG)	0.6 kg/ha	
7	Boscalid (Cantus WG)	0.6 kg/ha	
No pesticides were used, against *B.cinerea* during the survey			2017

Legend:1 cc always equals 1 mL. Milliliter (mL) is a special name for the cubic centimeter (cm3).Technical note: volume [ml] = weight [g] / density, or weight [g] = volume [ml] * density the formula for density is d = M/V, where d is density, M is mass, and V is volume. Density is commonly expressed in units of grams per cubic centimeter (g/sm^3)

2.2. Spraying Technique

The pesticide application was conducted with a conventional sprayer of the type "Panther 400 Mounted Type Turbo Atomize".The sprayer was equipped with 8 nozzles arranged on both side (4 nozzles per side). Only the two lower nozzles on each side are used to adapt the sprayer to the vines in chemical treatments against *B.cinerea* where they are grouped most of the bunches in the vines (figure 1). The spraying of the vines was carried out in several stage such as: BBCH 69 (end of flowering), BBCH 79 (majority of berries touching), BBCH 83 (berries developing colour), and BBCH 85 (softening of berries). Technical note: way of sprayed on the vines (table 4), BBCH- describes the phenological development of vines.

Table 4 The calibration values of the conventional sprayer during research

Forward Speed (Km/h^{-1})	Actual Volume Rate (L/ha^{-1})	Flow Rate (L/min^{-1})	No. of Nozzles
1.5	600	6	2

Figure 1 A) Position on most of the bunches in the vines according to which it was calibrated two lower nozzles at conventional sprayer during chemical treatments (photo of the author, Smilica 2019)

2.3. Variants and calculations

For the research purposes of this manuscript, complete insight is given by algorithmic pattern of warning system (forecasting model), to practical display on which part it refers to ANOVA statistical test (figure 2). The numerical data collected from the field were grouped, into six parts:

- Data for microclimatic conditions in vine canopy
- Formulas for determination and occurrence on first symptoms of the *B.cinerea*. Was used a mathematical-statistical method for measuring microclimatic conditions in the field and vines canopies. The temperatures we take into account in the calculation those range from 1 to 30 ° C because, in this biological range, we have the development of the pathogen. According to [14] conidia germinate at a temperature of 1 to 30C, and most massively at 18 °C. The temperatures that were higher than 30 °Care not taken into account. For this purpose, we use the following formula:Tm=(Tda-Tmin)/(Tmax-Tmin); Tm-temperature development factor for *B.cinerea*; Tmin-minimum temperature; Tmax-maximum temperature; Tda-daily average temperature; The next parameter to be determined is humidity point (Hp). Whereby, the length of retention of the dew on the plant organs of the vine is calculated in hours. FDD= Tm x Hp; FDD- Factor for Development Disease; EFDD= [0, 2 x Tm (1-Tm)] x Hp; EFDD-External Factor for Development Disease. The purpose of these calculations is to derive microclimatic data into numerical values. Two variants were installed for the survey:
  - Chemically sprayed grapes against *B.cinerea* ;
  - Unsprayed grapes against *B.cinerea* -control.
- Creating Botrytis disease forecasting model based on microclimatic conditions in vine canopy,
- Monitoring of the disease until harvest of grape,
- Results,
- Working hypothesis. The warning system refers to the influence of independent variables which are represented through EFDD-External Factor for Development Disease what in the base are the climatic conditions (temperature and humidity) in the field, and the other side is dependent variables, are represented through FDD- Factor for Development Disease what in the base are microclimatic conditions between leaves and bunches in vines canopies, where the disease occurs. The ANOVA statistical test refers to which part of the numeric data is predictions before the symptom of the disease appears. In other words, data analysis is done during the incubation period, which is an essential approach in deciding whether there are conditions for the disease to occur and if there is a possibility of infection to determine the true timing of chemical treatment.

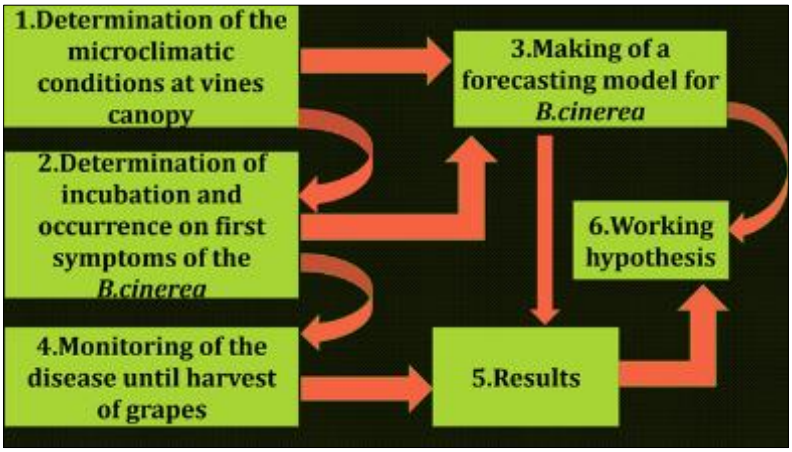

Figure 2 Algorithmic pattern of warning system

3. Results

3.1. Results obtained from the observation of the development of *B. cinerea* at Sopot locality in 2017

The monitoring of gray mold started on 16.08.2017 till 15.09.2017, was followed by very high temperatures and relative humidity that was at a level outside the range of influence to cause the development of *B.cinerea*. Due to such weather conditions, there was no infection of the control (untreated grapes) because measurements of microclimatic conditions in the vine's canopy indicated that there would be no development of gray mold (figure 3)

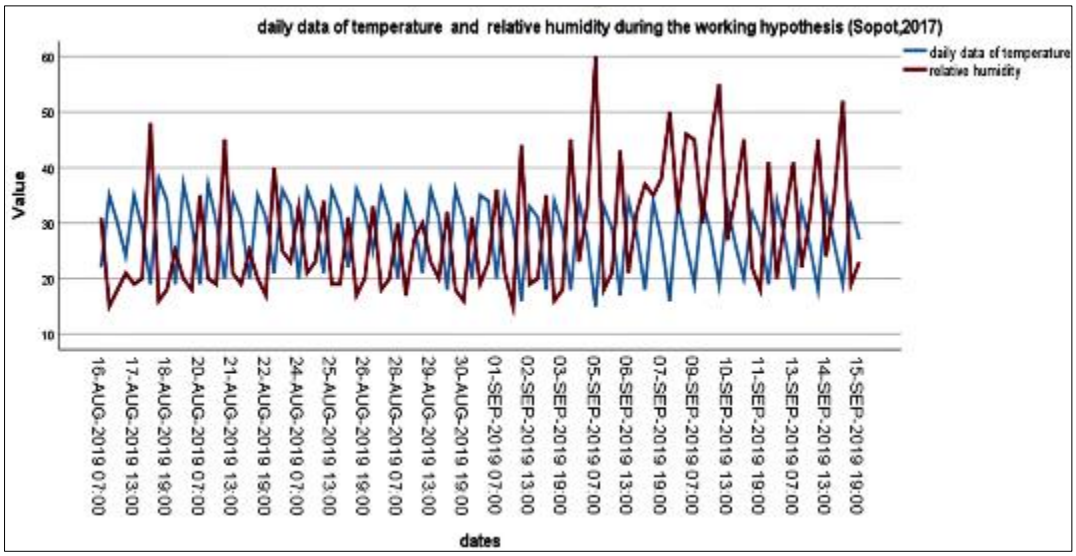

Figure 3 Illustration of temperature variations and relative humidity in the vines canopies during the period of observation (Sopot 2017)

3.2. Results obtained from the observation of the development of *B. cinerea* at Smilica locality in 2018

The measured values for temperature and relative humidity in the vine`s canopies that were later the target of the ANOVA statistical test at white varieties, Smederevka (table 5) and Zilavka (table 6). The microclimatic data in both tables represent the biological range of development of *B.cinerea* (figure 4). It is characteristic that in both controls (untreated grapes) first symptoms of gray mold appeared on 27.08.2018, although the observation period did not start on the same date.

Table 5 Determination of values for temperature and relative humidity in the vine`s canopies in the Smederevka variety in August 2018

Determination of incubation and occurrence on first symptoms of the *B.cinerea*	**Daily Average Temperature (Tda)**				**Hp in hours**	**Tm=(Tda-Tmin)/(Tmax-Tmin) FDD=Hp x Tm EFDD=[0,2 x Tm(1-Tm)] x Hp Middle interval $= \frac{EFDD+FDD}{2}$**			
Dates	0.7	13	19	Tda	Hp	Tm	EFDD	FDD	Mi
16.08.2018	13	25	19	19	4	0.5	0.32	2	1.16
17.08.2018	13	26	20	20	4	0.53	0.18	2.1	1.15
19.08.2018	11	22	19	17.3	4	0.57	0.18	2.2	1.23
21.08.2018	19	29	20	22.6	6	0.36	0.26	2.1	1.21
23.08.2018	14	25	19	19.3	4	0.48	0.18	1.9	1.05
24.08.2018	12	27	20	19.6	4	0.50	0.2	2	1.1
27.08.2018	21	29	24	24.6	8	0.45	0.39	3.6	1.99
28.08.2018	19	25	28	24	4	0.55	0.19	2.2	1.19
29.08.2018	19	29	20	22.6	6	0.36	0.26	2.16	1.21
30.08.2018	21	29	19	23	6	0.4	0.28	2.4	1.34
31.08.2018	24	30	29	27.6	3	0.6	0.14	1.8	0.97
02.09.2018	21	30	29	29.3	7	0.39	0.28	2.73	1.5
03.09.2018	21	29	28	26	6	0.62	0.24	3.72	1.98
05.09.2018	23	30	30	27.6	2	0.65	0.08	1.3	0.69
07.09.2018	19	28	24	23.6	2	0.51	0.09	1.02	0.55

Table 6 Determination of values for temperature and relative humidity in the vine`s canopies in the Zilavka variety in August 2018

Determination of incubation and occurrence on first symptoms of the *B.cinerea*	**Daily Average Temperature (Tda)**				**Hp in hours**	**Tm=(Tda-Tmin)/(Tmax-Tmin) FDD=Hp x Tm EFDD=[0,2 x Tm(1-Tm)] x Hp Middle interval = $\frac{EFDD+FDD}{2}$**			
dates	07	13	19	Tda	Hp	Tm	EFFD	FDD	Mi
11.08.2018	14	22	18	18	6	0.5	0.3	3	1.65
12.08.2018	12	20	17	16.3	5	0.54	0.28	2.7	1.49
13.08.2018	13	25	21	19.6	5	0.55	0.25	2.75	1.5
14.08.2018	10	27	24	20.3	4	0.6	0.19	2.4	1.3
15.08.2018	15	26	21	20.6	6	0.5	0.3	3	1.65
16.08.2018	13	25	19	19	4	0.5	0.32	2	1.16
17.08.2018	13	26	20	20	4	0.53	0.18	2.12	1.15
19.08.2018	11	22	19	17.3	4	0.57	0.18	2.28	1.23
21.08.2018	19	29	20	22.6	6	0.36	0.26	2.16	1.21
23.08.2018	14	25	19	19.3	4	0.48	0.18	1.9	1.05
24.08.2018	12	27	20	19.6	4	0.50	0.2	2	1.1
27.08.2018	21	29	24	24.6	8	0.45	0.39	3.6	1.99
28.08.2018	19	25	28	24	4	0.55	0.19	2.2	1.19
29.08.2018	19	29	20	22.6	6	0.36	0.26	2.16	1.21
30.08.2018	21	29	19	23	6	0.4	0.28	2.4	1.34

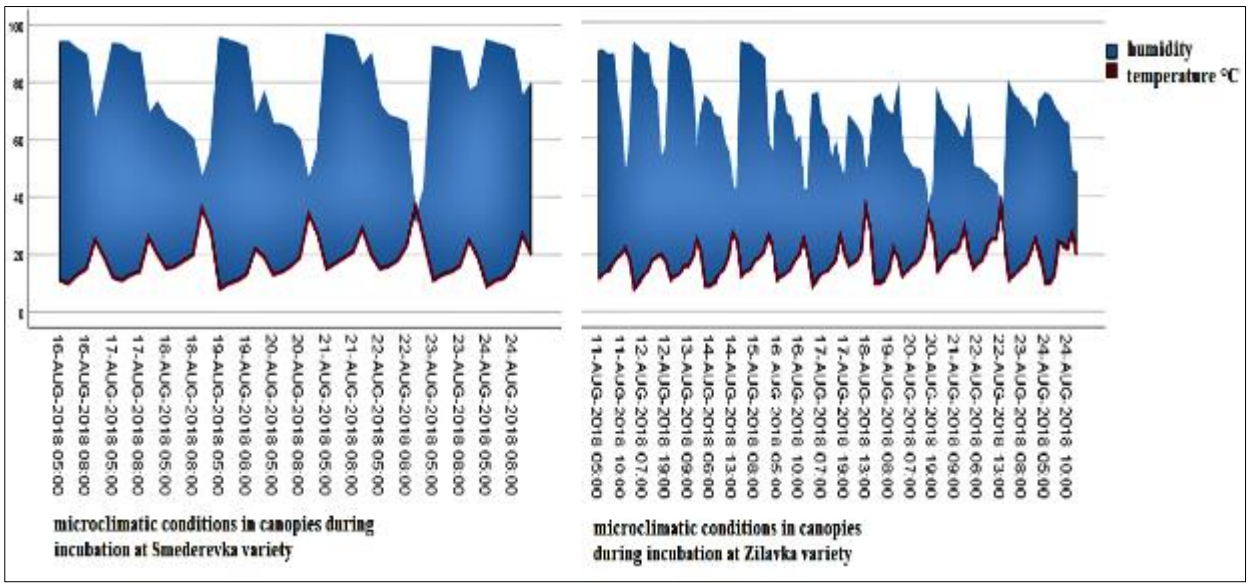

Figure 4 The biological range of development of *B.cinerea* during incubation period according to tables 5 and 6

3.3. Results obtained from the observation of the development of *B. cinerea* at Smilica locality in 2019

During the observation period, the disease has not appeared, i.e., at the untreated grapes, the percentage of diseased bunches was low, ranging from 1 to 1.5%. Symptoms of *B. cinerea* appeared seven days before grape harvest in controls (untreated grapes) with very slow pathogenesis, while in the conventional plantation (treated grapes) where the chemical treatments have performed, the disease did not appear at all. The retention of water droplets in the morning, on average, lasted about an hour for the entire duration of the period of observation while the maximum daily temperatures were above 30 ° C, which conditioned haven't development of *B. cinerea* which would cause major damage to the grapes and yield (figure 5). As the impact of *B.cinerea* on grape damage was insignificant, due to these facts did not continue statistical calculations.

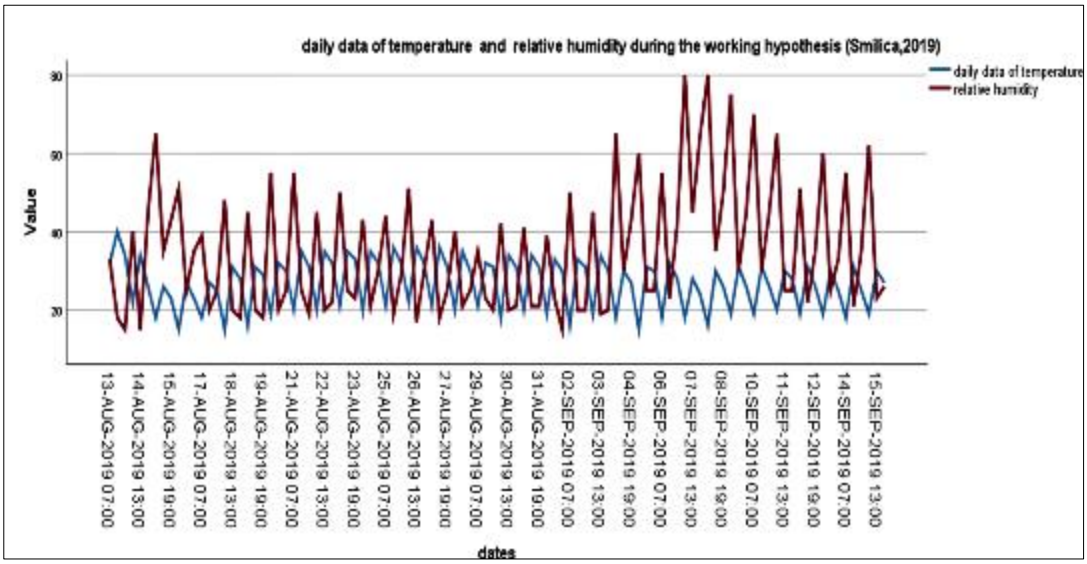

Figure 5 Illustration of temperature variations and relative humidity in the vines canopies during the period of observation (Smilica locality, 2019)

4. Discussion

Table 7 List of the fungicide used against *Botrytis cinerea* during the survey in the corresponding phenological stages of vines development

Pre-infection chemical sprays against Botrytis disease in 2018			
Period	**Organ**	**Active ingredients**	**dates of spray**
End of flowering (BBCH 69)	Reproductive organs	Pyrimethanil	18.05.2018
Young berries begin to swell (BBCH 71)	Berries	Fenhexamid	07.06.2018
Majority of berries touching (BBCH 79)	Berries	Cyprodinil+ Fludioxonil	11.07.2018
Chemical spraying after occurred of infection at the bunches in 2018			
Softening of berries (BBCH 85)	Berries	Boscalid	27.08.2018
Pre-infection chemical sprays against Botrytis disease in 2018			
Period	Organ	Active ingredients	dates of spray
End of flowering (BBCH 69)	Reproductive organs	Pyrimethanil	25.05.2019
Young berries begin to swell (BBCH 71)	Berries	Fenhexamid	12.06.2019
Majority of berries touching (BBCH 79)	Berries	Cyprodinil+ Fludioxonil	08.07.2019

Table 7 shows the active substances used during the research at variant chemically sprayed grapes in the corresponding phenological stages of vines development.Both active ingredients pyrimethanil, and cyprodinil belong to the chemical family of Anilino-Pyrimidines. AP-fungicides inhibit methionine biosynthesis and the secretion of hydrolytic enzymes

in gray mold. The enzyme cystathionine-β-lyase catalyzes the production of homocysteine, which is a direct precursor to methionine amino-acid. Fludioxonil interferes with the signal transduction in fungi, however, the mode of action exact is not known and belongs to the Phenyl Pyrroles chemical family.Mode action of these pesticides is mainly on the cell membrane. For successful control of B. cinerea, a fungicide mixture (cyprodinil and fludioxonil), was applied. The mode of action of the boscalid active ingredient is to inhibit the mitochondrial respiratory chain. An active ingredient that inhibits its electron transport chain of complex III is pyraclostrobin. Fungicides that inhibit mitochondrial respiration by binding to cytochrome b, a part of the cytochrome bc1 complex are known as inhibitors of complex III. The pyraclostrobin was used to control *B. cinerea* did not achieve the required effect, due to low intrinsic activity and expression of the terminal alternative oxidase (AOX). The alternative oxidase (AOX) allows maintaining needed metabolic homeostasis in fungal cells. For this reason, especially on the grapevine, the control of gray mold is very problematic with this active ingredient and therefore was applied fungicide mixture (pyraclostrobin and boscalid). Most studies on host resistance, timing of fungicide applications, biological control, control by cultural practices and disease prediction models of *B. cinerea* on grapevines were based on assumptions and conclusions made on mature berries [1];[5];[7]. Because of these reasons, the survey was set when the bunches increase sugar content by more than 11% (onset of ripening or color change of grape berries from green to yellow) and, at the same time, the incubation period also occurrence. Hence, the goal is to implement a model that relies on the rational assessment in the vine canopies microclimatic conditions. The monitoring in the vines' canopies showed that in 2017 (figure 3) and 2019 (figure 5), there were no favorable conditions for more intense infection of *B.cinerea*. Unfavorable conditions for the development of the disease led to a reduction of chemical treatments, in that in 2017, no active ingredients against *B.cinerea* were used at all, while in 2019, there were only three sprays (table 7). The use of fungicides in the vineyard in 2019 allows pre-infection disease control (table7). Unfavorable climatic conditions in 2019 for the development of the disease and the method of pre-infection disease control provide preserving the grape harvest from gray mold. In 2018, microclimate conditions was favorable and caused the development and emergence of *B.cinerea*,immediately before the onset of the first symptoms of the disease i.e., during the incubation period,the values for temperature and humidity were calculated and converted into coefficients (tables 5 and 6) that served to perform the ANOVA statistical test. Four chemical treatments were performed that year, as field results indicated (table 2). The figures (6 and 7) for linear regression analysis connect the interrelationships of two or more phenomena ie these figures answer the interdependence of the factors for disease development (FDD) and the external factors for the development of disease (EFDD). To explain the forecasting disease model, we determine the phenomenon which represents the dependent variable. In this case, that is FDD. While EFDD represents the second phenomenon that is an independent variable and that affects the dependent variable FDD. The values of the independent variable EFDD allow us to explain the variations of the dependent variable FDD. The essential benefit of regression analysis is determining how changes in the independent variables are associated with shifts in the dependent variable, which can be visually seen, in the variation in temperature and humidity that give the biological range of development of *B.cinerea* during the incubation period (figure 4). In linear regression, coefficients are the values that multiply the predictor values. The sign of each coefficient indicates the direction of the relationship between a predictor variable and the response variable. A positive sign indicates that as the predictor variable increases, the response variable also increases and vice versa. The correlation between these values is strong which, can be seen from the calculated Pearson coefficient (Multiple R) according to Tab.8 at Smederevka variety which is r= 0.736855, while at Zilavka variety is r= 0,710804 Tab.9 The results showed that in every case (at both varieties) there, was a high correlation between FDD and EFDD. The coefficient of determination (R2) or R Square for the two different cases was R2= 0,542955 at Smederevka Tab.8 and R2= 0,505242 at Zilavka variety Tab.9 This value (R^2) is an indication of how much changes in one variable (EFDD) cause changes in the other variable (FDD) and the convection is expressed in percentage, respectively R^2=0,542955 x 100 =54% at Smederevka and R^2=0,505242 x 100 = 50,5% . This means that the other 46% of Smederevka and the remaining 49.5% at Zilavka variety belong to the category of unknown factors. Adjusted R Square typically lower than the R Square in both variety, respectively Adjusted R Square =0,542955 at Smederevka Tab.8 and Adjusted R Square =0,467184 at Zilavka variety Tab.9 Frequently R-squared values range from 0 to 1 and are commonly stated as percentages from 0% to 100%. In essence, Adjusted R-squared is a modified version of R-squared and decreases when a predictor improves the model by less than expected. It has indicated, that the development of the *B. cinerea* largely depends on the influence of microclimatic conditions that create the possibility of its prognosis. The determination of linear regression model i.e. its significance we consider the data for F-statistic along with the corresponding p-value.

Hence F-statistic given in the ANOVA tables (Table 8 and Table 9) as well as the p-value which is labeled as Significance F.

- F-statistic: 15, 44361; Tab.8
- F-statistic: 13, 27548 Tab.9

Technical note: The F-statistic is calculated as MS regression divided by MS residual. MS regression / MS residual =3, 877664/0, 251085 = 15, 44361 Tab.8

MS regression / MS residual = 1, 596855/0, 120286 = 13, 27548 Tab.9

The Significance F in fact is p value for the regression model. The Significance F in fact is p value for the regression model. The alternative hypothesis cannot be tested directly; it is accepted by exclusion if the test of statistical significance rejects the null hypothesis. In this case null hypothesis suggests that no linear relationship between the EFDD and FDD vs alternative hypothesis which assumes linear relationship between the EFDD and FDD. A good hypothesis must be based on a good research question. It should be simple, specific and stated in advance [6]. The null hypothesis is rejected in favor of the alternative hypothesis if the P value is less than alpha (α type I error), the predetermined level of statistical significance [16]. Nonsignificant results are those with a P-value greater, than alpha (α type I error).In this case, the alpha value is 0.05 this means that it is rejected null hypothesis and accepted alternative hypothesis if the p-value was less than or equal to P≤ 0.05. As you can see in both tables (table 8 and table 9) the p-value for this forecasting model was considerably lower than alpha value of 0.05. It can be concluded that the linear regression model is significant. The intercept is the point where the function crosses the y-axis. With intercept coefficient (Y_{ij} shows the point where the line of the best fit or regression line crosses y axis when the value x is zero, respectively *Yi* = 0,848502 (Table 8) and Y_i = 1,104838 (Table 9**)**. The second value is the coefficient on EFDD as a result of the slope. For a simple linear regression model the most basic version of the equation is:

$$Y=m\times X + b$$

Y – Predicted value
m- Slope of the line of the best fit
X- Value of independent variable
b- Intercept
A need arises again to interpret this p -value only with little more detail because of our hypotheses.

In this case the null hypothesis is that the intercept or slope is zero (*b=0*), while alternative hypothesis is that the intercept or slope is not zero (*b≠0*), as you can see the both values are less than alpha (α type I error), respectively p-value (intercept) = 0,039639; p-value (EFDD) = 0,001726 Tab.8 and p-value (intercept) =0,011959 ; p-value (EFDD) =0,002974 Tab.9 This means the EFFD is a significant variable that impact FDD. From each observation from data that was entered into regression test we get a predicted value of FDD (table 8 and table 9) based on the regression model.

Table 8 Statistical analysis of *Botrytis* disease model at Smederevka variety

Summary output	
Regression	**Statistics**
Multiple R	0.736855
R Square	0.542955
Adjusted R Square	0.507798
Standard Error	0.501084
Observations	15

ANOVA				
	df	**SS**	**MS**	**F**
Regression	1	3.877664	3.877664	15.44361
Residual	13	3.264109	0.251085	
Total	14	7.141773		

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%
Intercept	0.848502	0.371093	2.286493	0.039639	0.046804	1.650199	0.046804
EFDD	6.26987	1.595453	3.929836	0.001726	2.823103	9.716638	2.823103

Residual output				Probability output		
Observation	Predicted FDD	Residuals	Standard Residuals	Percentile	FDD	dates
1	2.85486	-0.85486	-1.77042	3.333333	1.02	07.09.2018
2	1.977078	0.122922	0.254572	10	1.3	05.09.2018
3	1.977078	0.222922	0.461673	16.66667	1.8	31.08.2018
4	2.478668	-0.37867	-0.78422	23.33333	1.9	23.08.2018
5	1.977078	-0.07708	-0.15963	30	2	16.08.2018
6	2.102476	-0.10248	-0.21223	36.66667	2	24.08.2018
7	3.293751	0.306249	0.634244	43.33333	2.1	17.08.2018
8	2.039777	0.160223	0.331823	50	2.1	21.08.2018
9	2.478668	-0.31867	-0.65996	56.66667	2.16	29.08.2018
10	2.604065	-0.20407	-0.42262	63.33333	2.2	19.08.2018
11	1.726283	0.073717	0.152668	70	2.2	28.08.2018
12	2.604065	0.125935	0.260812	76.66667	2.4	30.08.2018
13	2.35327	1.36673	2.830507	83.33333	2.73	02.09.2018
14	1.350091	-0.05009	-0.10374	90	3.6	27.08.2018
15	1.41279	-0.39279	-0.81347	96.66667	3.72	03.09.2018

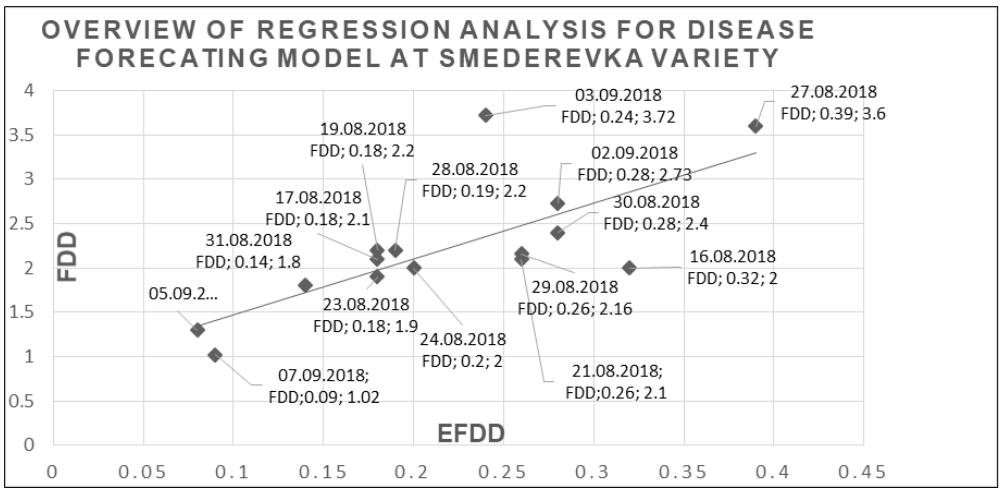

Figure 6 LEGEND: 16.08.2018-onset of incubation; 17.08.2018, 19.08.2018, 21.08.2018- favorable conditions for incubation; 23.08.2018, 24.08.2018-latency period; 27.08.2018-first symptoms on control (untreated grapes); 28.08.2018, 29.08.2018,30.08.2018-reduction of infection; 31.08.2018-latency period of infection;02.09.2018-onset of second infection;03.09.2018-second infection;05.09.2018, 07.09.2018-reduction of second infection

Table 9 Statistical analysis of *Botrytis* disease model at Zilavka variety

Summary output	
Regression	**Statistics**
Multiple R	0.710804
R Square	0.505242
Adjusted R Square	0.467184
Standard Error	0.346823
Observations	15

ANOVA				
	df	**SS**	**MS**	**F**
Regression	1	1.596855	1.596855	13.27548
Residual	13	1.563718	0.120286	
Total	14	3.160573		

	Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**	**Lower 95,0%**
Intercept	1.104838	0.378472	2.919203	0.011959	0.287198	1.922478	0.287198
EFDD	5.345062	1.466991	3.643554	0.002974	2.17582	8.514304	2.17582

Residual output				Probability output		
Observation	**Predicted FDD**	**Residuals**	**Standard Residuals**	**Percentile**	**FDD**	**dates**
1	2.708356	0.291644	0.872644	3.333333	1.9	23.08.2018
2	2.601455	0.098545	0.294862	10	2	24.08.2018
3	2.441103	0.308897	0.924268	16.66667	2	16.08.2018
4	2.1204	0.2796	0.836609	23.33333	2.12	17.08.2018
5	2.708356	0.291644	0.872644	30	2.16	21.08.2018
6	2.815258	-0.81526	-2.43938	36.66667	2.16	29.08.2018
7	2.066949	0.053051	0.158737	43.33333	2.2	28.08.2018
8	2.066949	0.213051	0.637483	50	2.28	19.08.2018
9	2.494554	-0.33455	-1.00104	56.66667	2.4	14.08.2018
10	2.066949	-0.16695	-0.49954	63.33333	2.4	30.08.2018
11	2.17385	-0.17385	-0.52019	70	2.7	12.08.2018
12	3.189412	0.410588	1.228545	76.66667	2.75	13.08.2018
13	2.1204	0.0796	0.238177	83.33333	3	11.08.2018
14	2.494554	-0.33455	-1.00104	90	3	15.08.2018
15	2.601455	-0.20146	-0.60279	96.66667	3.6	27.08.2018

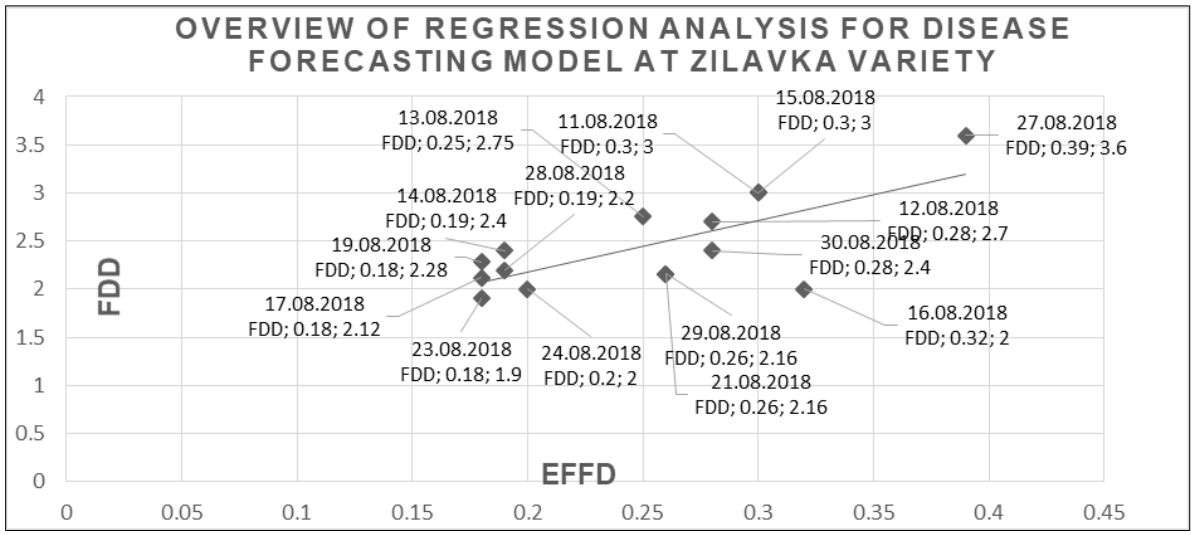

Figure 7 LEGEND: 11.08.2018-onset of incubation; 12.08.2018, 13.08.2018- favorable conditions for incubation; from 14.08.2018 to 24.08.2018-latency period; 27.08.2018-first symptoms on control (untreated grapes); 28.08.2018-duration of first infection; 29.08.2018, 30.08.2018-reduction of infection

The forecasting disease model is a technique that uses past data as inputs to make estimates that are predictive in determining the direction of future trends of *B.cinerea* based on a calculation of ANOVA statistical test. The forecasting disease model is based on the calculated values for predicted FDD data (tables 8 and 9) obtained to ANOVA statistical test at controls variants (untreated grapes) (figures 8 and 9). Further, predicted FDD assumes the trend of development of the disease that occurrence after the measured values for microclimate conditions in vine canopies. Therefore, in practical terms depending on the microclimate conditions, the occurrence of the disease can be expected or not.

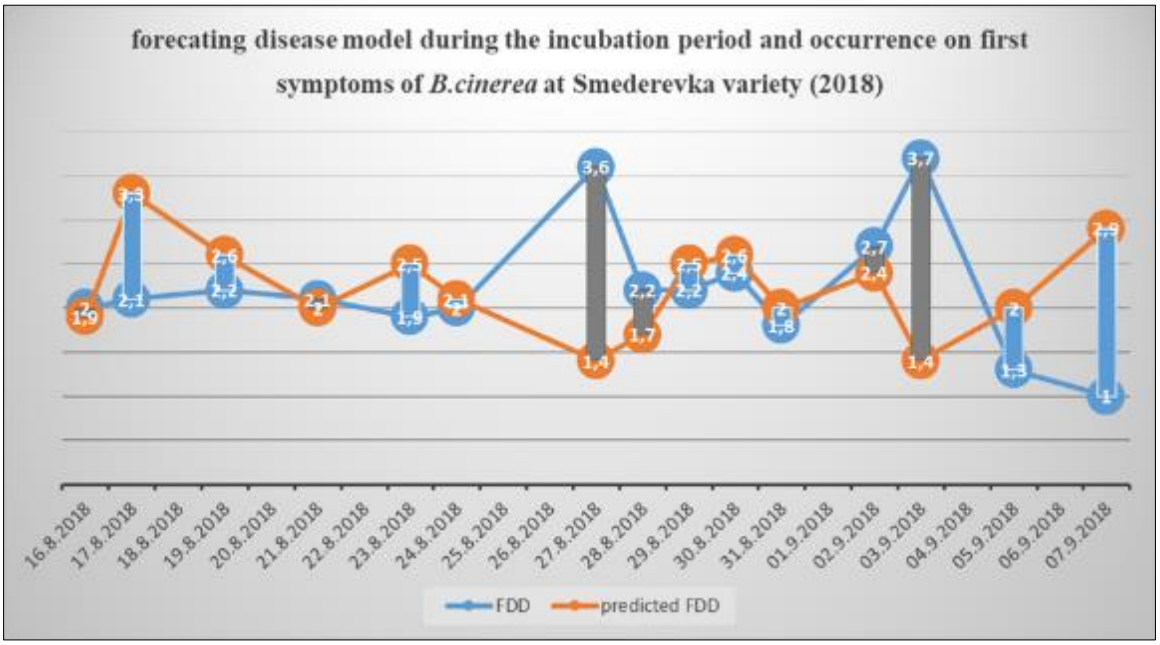

Figure 8 Forecasting disease model based on ANOVA statistical test to controls (untreated grapes) at Smederevka variety

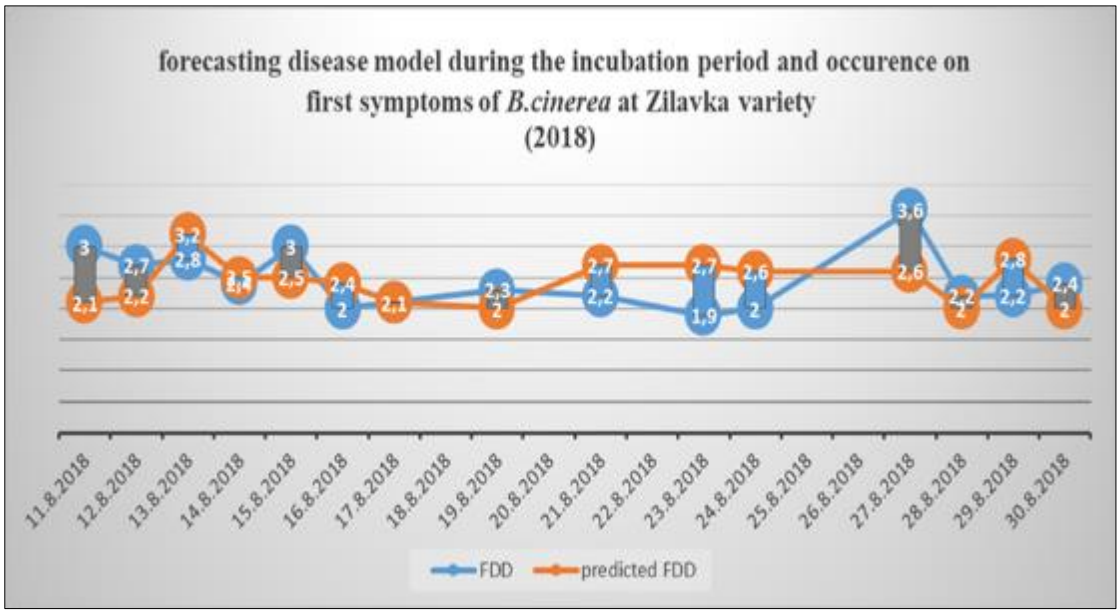

Figure 9 Forecasting disease model based on ANOVA statistical test to controls (untreated grapes) at Zilavka variety

5. Conclusion

The influence of unfavorable external conditions causes the disease to be unable to adhere to the spores on the surface of the grape berries, thus stopping its further development, as happened in 2017 due to which there were no chemical treatments against gray mold. If there is a drop in relative humidity below 90% and an increased temperature above 30°C cause the incubation process stops. The deteriorated microclimatic conditions during incubation lead to a resistance reaction. In other words, occurrence stronger an attachment of the appressorium to the grape berries surface. If the incubation phase of the pathogen was finished, the infection will depend on the moment when favorable conditions occur regardless of the interruptions in the incubation process that occur as a result of unfavorable microclimatic conditions. This situation in the field sometimes leads us to the wrong conclusion that there are no conditions for the development of gray mold, and if precipitation occurs with an intensity of more than 0.2 mm / h, the disease appears whose infection potential, in this case, will depend on the duration of detention of water droplets on the organs of the vine and the temperature factor which should be below 30 °C. The forecasting disease model is correlated with microclimatic conditions and the biological range for the development of *B.cinerea*. The insight in determining the incubation period of *B. cinerea* is the basis for reducing the last chemical treatments just before the grape harvest. If a noticed relative humidity of 60 to 80% and a temperature not exceeding 30°C is observed continuously for one week in vine canopies, in the case of precipitation with a higher intensity of 0.2 mm / h, are recommended to be sprayed prophylactic against *B. cinerea* before precipitation occurs.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

References

[1] Avissar I, Pesis E. The control of postharvest decay in table grapes using acetaldehyde vapours. Annals of Applied Biology. 1991; 118(1): 229-237.

[2] BanerjeeA, Chitnis UB, Jadhav S L, Bhawalkar J S, Chaudhury S. Hypothesis testing, type I and type II errors. Industrial psychiatry journal. 2009; 18(2): 127–131.

[3] Bastiaansen C, Koster ATJ, Van der Meer LJ, Van den Ende JE, Pennock I, Buurman FPM. A disease forecasting system for Botrytis blight ('fire') in lily. Acta Horticulturae. 1997; 430(1): 657-660.

[4] Brent KJ, Hollomon DW.Fungicide resistance: the assessment of risk. vol FRAC Monograph 2th ed.Brussels: Croplife International. 2007.

[5] Broome JC, English JT, Marois JJ, Latorre BA, Aviles JC. Development of an Infection Model for Botrytis Bunch Rot of Grapes Based on Wetness Duration and Temperature. The American Phytopathological Society. 1995; 85 (1): 97-102.

[6] Browner SW, Newman BT, Hulley BS. Getting ready to estimate sample size: Hypothesis and underlying principles. In: Hulley SB, Cummings SR, Browner WS, Grady D, Hearst N, Newman TB, eds. Designing Clinical Research-An epidemiologic approach. 2th ed. Philadelphia: Lippincott Williams & Willikins. 2001.

[7] Chardonet C, L'Hyvernay A, Doneche B. Effect of calsium treatment prior to *Botrytis cinerea* infection on the changes in pectic composition of grape berry. Physiological and Molecular Plant Pathology. 1997; 50(4): 213-218.

[8] Coley-Smith JR, Verhoef K, Jarvis WR. The Biology of Botrytis. London: Academic Press; 1980.

[9] Dean R, van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster DG. The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathology. 2012; 13(4): 414–430.

[10] Fenner K, Canonica S, Wackett LP, Elsner M. Evaluating pesticide degradation in the environment: blind spots and emerging opportunities. Science. 2013; 341(6147): 752-758.

[11] Fry WE. Principles of Plant Disease Management. London: Academic Press. 1982.

[12] Jarvis WR. Botryotinia and Botrytis species – taxonomy, physiology and pathogenicitiy. 15th ed. Harrow: Research Branch. 1977.

[13] Kraker J de, Ende JE. Van den, Rossing WAH, Bastiaansen C, Werf W. van der. Computer aided design of improved warning systems – a case study for Botrytis control in flower bulbs. EPPO Bulletin. 2000; 30(3): 105-113.

[14] Pejchinovski F, Mitrev S. Agricultural Phytopathology (general part). Stip: University Goce Delchev. 2007.

[15] Verger PJ, Boobis AR. Global food supply. Reevaluate pesticides for food security and safety. Science. 2013; 341 (6147): 717-718.

[16] Wayne WD. Biostatistics. 7th ed. New York: John Wiley and Sons, Inc. 2002.

[17] Williamson B, Tudzynski B, Tudzynski P, Van Kan JAL. *Botrytis cinerea:* the cause of grey mould disease. Molecular Plant Pathology. 2007; 8(5): 561-580.