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Determination on Microclimatic Conditions at Vines upon Development on Gray Mold (Botrytis cinerea)

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Abstract

One of the most important plant diseases in viticulture is gray mold caused by Botrytis cinerea Pers. Fr., the anamorph of an ascomycete fungus (Botryotinia fuckeliana Whetzel). Locality Smilica, Kavadarci, Republic of North Macedonia, was the place where experimental fields with white varieties Smedervka and Zilavka were continuously observed. Working hypothesis was to follow development of the disease after increasing glucose over 11% until the time of the grape harvest, and microclimate was monitored at the same time. In both white varieties Smederevka and Zilavka on the control variants weren't used botricide treatments to distinguish between the variants that were conventionally treated against *B. cinerea*. The aim of the research was to determine how microclimatic conditions affect the development of B. cinerea and consequently to create forecasting model for gray mold. The forecasting model for B. cinerea is based on relationship between temperature and humidity in the vines' canopies. The aim of the research is to prevent development of B. cinerea and consequently reduce the number of chemical treatments.

Keywords

Gray Mold, Ascomycete Fungus, Varieties, Working Hypothesis, Forecasting Model, Microclimatic Condition, Chemical Treatments

1. Introduction

Botryotinia fuckeliania (de Bary) Whetzel (anamorf Botrytis cinerea Pers.), is the cause of significant economic losses in viticulture around the world. This cos-

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mopolitan fungus is a pathogen attacking over 200 plant species, including the grapevine [1]. Information on *B. cinerea* epidemics is publicly available for the range of different crops. In grape, the disease frequently occurs on ripe berries close to harvest, following rainfalls or long period of high humidity, and develops into the characteristic gray mold [2]. Gray mold epidemics lead to financial losses for growers, reducing us not only yield, but also the quality of the grapes. Globally, about 15 to 25 million dollars a year is spent on fungicides, to suppress *B. cinerea* [3].

The control of *B. cinerea* in grapes has always been a challenge especially if its adaptability to environmental factors is taken into account. Hence gray mold is a facultative saprophyte which means that in its development the saprophyte and parasitic phases are present, which are an adaptive response of the pathogen to external factors. According to [4], in the parasitic phase, the gray mold kills the living cells of the plant and the colonized them, while in the saprophytic phase it draws nutrients from the dead tissue. The appearance of the saprophytic phase on the flowering elements increases the potential inoculum for more intensive development of B. cinerea in the ripening phase of the grapes. It is quite characteristic that during the pathogenesis, in different periods of time, different tissues of the vine are infected, which serve as a bridge and eventually the infection covers the mature grapes. The gray mold always runs parallel to the ripeness of the grapes and the autumn rainy season before harvest. This pathogen manifests its destructive influence from the veraison phase (onset of ripening) to grape harvest. During the process of grape berry infection by B. cinerea, various biochemical interactions take place. These interactions have been investigated thoroughly with respect to host resistance to the fungus and involve both constitutive factors and induced ones following stress or infection [5].

In varieties with very compact bunch, during maturation, more pressure is created on the grape berries and as result at this growth it happens that one berry suppresses the other and at place of junction with peduncle a small cleavage or separation of the grape berries occurs. A drop of grape juice flows through this small opening, which is at the same time a nutrient base for the development of B. cinerea. Then the gray mold spores germinate in the drop of the grape juice and easily penetrate through the small opening and infect the grape berries. Rainy weather during grape ripening only further stimulates infection. This type of infection that occurs from the inside of the bunch is very difficult to control. In case of favorable weather conditions for the development of gray mold, mycelial structures develop on the surface of the grains, and then the infection quickly spreads to the whole bunch. In our country, during the vegetation, exclusively the conidial stage of B. cinerea development, and its conidia spread with the air and thus a dispersion of the infection occurs in the space. In the vineyards appearance of apothecium development is rare and the biological cycle goes in the direction of creating conidial stage and sclerotium.

There are still dilemmas about the epidemiological features of *B. cinerea*, the

epidemiology of bunch rot of grape is not well understood. Most studies have generally focused on mature grape berries. According to [6] B. cinerea may infect grape flowers and remain latent in these tissues until grape berries begin to mature. Infection of bunches resulting from infected floral parts and aborted berries trapped within the grape bunch ("bunch trash") is also a potential infection mechanism. The authors [7] found that 28.6% of the bunch trash recovered from within bunches at veraison was infected with B. cinerea; 95.5% of this infected material was aborted berries, 3.6% was calyptrae and the remaining material was made up of leaf, stem and tendril pieces. According to [2] studied the infection of grape flowers by B. cinerea and found that floral parts were heavily colonized after 72 h but this study did not make a link between this fungal colonization and gray mold symptoms at harvest. Based on how the gray mold infects the bunches, two models of infection of grapevine can be distinguished: 1) the spores are dispersed through the vineyard and grape berries become infected by conidia; 2) in favorable condition occurs berry to berry infection with mycelium structures. Questions still exist about these two models of epidemiological development of gray mold. The authors [8] reported that removing the leaves near the bunches could successfully control the disease. Reliable methods for prognosis of the occurrence of disease in the vineyard do not currently exist [9]. For these reasons development of B. cinerea was monitored inside of canopy microclimate condition.

2. Materials and Methods

The research was completed in a vineyard located at Smilica near Kavadarci Republic of North Macedonia (41°42'71.4"N, 22°0'10.75"E) on white grape varieties Smederevka and Zilavka. A double Guyot pruning system was applied in the vineyard. The Smederevka variety was present on an area of 1.7 ha while Zilavka variety was present on an area of 0.5 ha. The variants set in the experiment considered consisted of treated and untreated grapes by simultaneously measuring microclimatic conditions and monitoring disease. Each variant was placed in an area of two rows, and the samples were taken from the middle of the variant, to prevent any external influence. Except for the control (no treatments against grey mould), which was represented by only one row, treatments against downy mildew and powdery mildew were regularly performed, but no active substances were used which could have a side effect against grey mould. The development of the grey mould was followed during the working hypothesis. From each variant, five plants were marked, and from each plant, six bunches were selected, which were marked on the rachis (handle) of the bunches with red tape. In the control, the disease was monitored in three plants.

2.1. Variants and Calculations

The essence of the initial observation is to understand the trend of the disease. For this purpose, in the first part of the field analysis, when the incubation pe-

riod and the appearance of the first symptoms should be determined, a mathematical-statistical method was used. This method involves daily measurement of temperature and relative humidity in the habitat of the vine while the working hypothesis is in progress. The aim of the research was to determine how the climate change impacts upon development of the *B. cinerea* and consequently to create forecasting model for gray mold. The temperatures we take into account in the calculation are those that range from 1°C to 30°C because in this interval we have the development of the pathogen. According to [10] conidia germinate at a temperature of 1°C to 30°C, and most massively at 18°C. Temperatures higher than 30°C are 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). Where is the length of retention the dew on the plant organs of the vine expressed in hours.

$$FDD = Tm \times Hp$$

FDD—Factor for development disease;

$$EFDD = [0.2 \times Tm(1-Tm)] \times Hp$$

EFDD—External factor for development disease.

From each variant, a random sample of 30 bunches was taken. For each of the variants, the following were calculated: average number of grape berries in the bunch, average number of healthy grape berries in the bunch, average number of diseased grape berries in the bunch, infection index according to Mc-Kinney's formula [11], and efficiency of fungicide according to Abott's formula [11].

2.2. Working Hypothesis

The working hypothesis or observation period of gray mold started when the bunches reaches a sugar content of more than 11% until the grape harvest. At each varieties had two variants: treated and untreated grapes. In both white varieties Smederevka and Zilavka on the control variants wasn't used botricide treatments to distinguish between the variants that were conventionally treated against *B. cinerea* From each variant, five plants were marked, and from each plant, six bunches were selected, which were marked on the rachis (handle) of the bunches with red tape. In the control, the disease was monitored in three plants. For that time, temperature and humidity were measured using a digital thermohydrometer in the vines habitat. The length of moisture retention was measured in hours. Humidity of less than half an hour was not taken into account. Also not considered are very low they intensity rains up to 0.2 mm/h, when we have long lasting rains of more than half an hour. Then we measured

the length of the rain and the period of water drop retention on the vines. The forecasting model for *B. cinerea* based on relationship between temperature and humidity in the vines canopies. The aim of the research is prevent development of *B. cinerea* and consequently reduce the number of chemical treatments.

3. Results

This analysis aimed to create a model where the infectious characteristics of the grey mould would be elaborated. In developing this model, the infectious properties of grey rot depended on external factors. Base on the way in which the B. cinerea infection occurs, the working hypothesis can be roughly divided into two parts: 1) the aim was to determine the incubation period and to predict the appearance of the first symptoms based on the measurements of microclimatic and phillospheric conditions inside of the vines canopies; 2) with the appearance of the first symptoms to determine the dynamics of development of *B. cinerea* by calculating: average number of grape berries in the bunch, average number of healthy berries in the bunch, average number of diseased berries in the bunch. The determination of the incubation period started for each of the varieties from the moment when 11% sugar content was measured. At the Smederevka variety, this percentage of sugar was measured on 16.08. And results were taken on a daily basis and statistically were processed to create a graph that will provide insight into the situation on the field. The incubation period of the gray mold in the Zilavka variety was quite characteristic and unusual, and it started on 11.08.2018 when 11% sugar content was measured. According to the observations and calculations, the length of the incubation in this case lasted until 24.08.2018 but the first symptoms of B. cinerea appeared on 27.08.2018. while latent period was registered on August 25 and 26, 2018. The curve determined the incubation period and the onset of symptoms. After the first symptoms of the control appeared on August 27, 2018 at the Smederevka and Zilavka varieties, with visual observation starting to follow the dynamics of the disease development, while in the treated grapes there was still no symptoms of the disease. The appearance of symptoms of B. cinerea in the treated grapes was noticed on September 11, 2018, i.e. 16 days after the last chemical treatment. Between the last chemical spray on 27.08 at the treated grapes and the appearance of the first symptoms on 11.09 was followed and calculated with Mc-Kinney's and Abott's formulas (Table 1). In this way, the relevance of the research study was proved by analyzing the treated and untreated grapes at the same time while monitoring the development of the disease.

4. Discussion

The model describes two key stages of the *B. cinerea* life cycle in vineyards: 1) infection of mature berries by conidia; 2) development of mycelium when it occurs berry to berry infection. In order to simplify the biological cycle of development to explain the infectious features of grey mould, the following phases are

Table 1. Overview of variants and the calculated results.

Variants	Average number of grape berry in the bunch	%	Average number of healthy grape berry in the bunch	%	Average number of disased grape berry in the bunch	%	Infection index according to formula of Mc-Kinney (%)	Efficiency of fungicide according to formula of Abott (%)	Allowed a level of significance of 5% (p < 0.05)
Smederevka (chemically treated)	156	100	149	95.5	7	4.5	5	94.1	99.1
Smederevka-control (untreated)	149	100	30	20.1	119	79.9	84.2	/	/
Zilavka (chemically treated)	113	100	104	92	9	8	7.7	90.2	97.9
Zilavka-control (untreated)	121	100	18	14.9	103	85.1	78.3	1	/

Smederevka (Mc kinney)
$$I = \frac{\Sigma(n \times k)}{N \times K} \times 100 = \frac{(27 \times 0) + (1 \times 3)}{2 \times 30} \times 100 = 5$$
; Smederevka-control (Mc kinney)
$$I = \frac{\Sigma(n \times k)}{N \times K} \times 100 = \frac{(1 \times 1) + (2 \times 2) + (12 \times 3) + (15 \times 4)}{4 \times 30} \times 100 = 84.2$$
; Smederevka (Abott) $E = 100 - \frac{It}{Ik} \times 100 = 100 - \frac{5}{84.2} \times 100 = 94.1$; Zilavka (Mc kinney)
$$I = \frac{\Sigma(n \times k)}{N \times K} \times 100 = \frac{(26 \times 0) + (1 \times 1) + (3 \times 2)}{3 \times 30} \times 100 = 7.7$$
; Zilavka-control (Mc kinney) $I = \frac{\Sigma(n \times k)}{N \times K} \times 100 = \frac{(2 \times 1) + (4 \times 2) + (11 \times 3) + (13 \times 4)}{4 \times 30} \times 100 = 78.3$; Zilavka (Abott) $E = 100 - \frac{It}{Ik} \times 100 = 100 - \frac{7.7}{78.3} \times 100 = 90$.

emphasised: attachment of conidia to the surface of the grape berry, germination of conidia, differentiation of infection structures on the host surface, penetration of the host surface and excretion of a spectrum of phytotoxic compounds (necrotrophic activity). Spores are dispersed in the wind. Once deposited on the surface of the plant, the spores adhere to the surface tissue of the grape berries. This phase represents the interaction of the spore with the cuticle surface. Initially, hydration of conidia occurs, which typically involves weak adhesive forces, resulting from hydrophobic interactions between the host and conidial surfaces [12]. A few hours after inoculation, the conidia germination germ tubes are covered with a fibrillar-like extracellular matrix material [12]. These adhesive structures are excreted by the spore and consist of carbohydrates and proteins [13]. The spores are attached to the surface of the cuticle by the fibrillar extracellular matrix material, which also protects it from dehydration and the various defence mechanisms of the plant cells. Several factors influence the germination of a conidium. Free surface water or high relative humidity (>93%) is essential to germinate and penetrate the host epidermis [14]. After germination of the spore in a drop of water, the germ tube grows and extends. When the tip of the germ tube touches the surface of the plant tissue, the wax of the cuticle degrades forming a recess or hole for appressorium formation. The invasion of the plant tissue by B. cinerea can involve active penetration or penetration over natural openings and plant wounds of the tissue. The wounds of the tissue can be caused by an abiotic or biotic agent. Exclusively physical damage or brutal mechanical penetration through the cuticle by B. cinerea has not been seen [15]. Most often,

penetration is followed by enzyme activity by the pathogen. The infectious forecasting model for botrytis is essentially based on the relationship between relative humidity and temperature inside of the canopies of vines and the goal was to create a graph based on the parameters listed earlier (FDD, EFDD). A phenomenon can be understood only in the phase of the tendency of its development. In developing this predictive infectious model for gray mold, only the microclimatic parameters representing the biological range for B. cinerea development, which are characteristic of the geographical area where the research was conducted, were taken into account. The incubation period of B. cinerea at the Smederevka variety according to this analysis is considered to have lasted a total of 8 days from 16.08.2018 to 24.08.2018 (Figure 1). The first symptoms appeared on 27.08.2018. The days just before the onset of the first symptoms on 25.08.2018 and 26.08.2018 are considered as latent period, taking into account the measured microclimatic parameters inside of the canopies of vine. Gray mold shows a degree of adaptability just before the onset of the first symptoms in the control variant that was not treated with botricides. Recent microscopic, histological-chemical researches and gene function analysis indicate that these structures act as functional appressoria, useful for attaching the pathogen to the host surface before penetration of the tissue, due to a fibrillar-like extracellular matrix material covering, which retains water while the polysaccharide component is extremely hygroscopic, allowing the pathogen to adapt to external factors. According to [16] latency, once described as an enigmatic aspect of *Botrytis* ecology, has been the focus of many research studies in order to define epidemiological role and relationship to crop loss. The incubation period of the gray mold at Zilavka variety was quite characteristic and was accompanied by discontinuities as a result of variable microclimatic conditions in the canopies of the vines (Figure 2). In general, the incubation period can be divided into two parts: 1) the period when there were favorable conditions for the process of installing

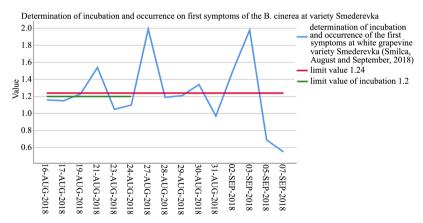


Figure 1. View of the Smederevka variety control. Data above the limit value (1.24) indicate a possible onset of symptoms, except for the incubation period. Where the blue line intersects the incubation limit value (1.2) indicates the favorable conditions for incubation period development.

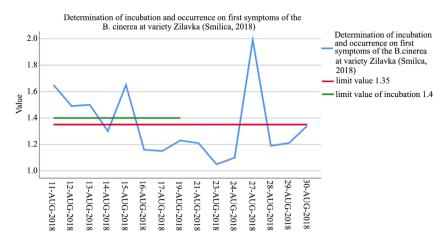


Figure 2. View of the Zilavka variety control. Data above the limit value (1.35) indicate a possible onset of symptoms, except for the incubation period. Where the blue line intersects the incubation limit value (1.35) indicates the favorable conditions for incubation period development.

the infection starting from: 11.08.2018, 12.08.2018, 13.08.2018, and 15.08.2018, with the exception on 14.08.2018 there was a slight decline in the incubation process due to the variation of external factors and thus short discontinuity; 2) latency period lasting from 16.08.2018 to 24.08.2018. In addition to the unfavorable microclimatic conditions that affect the occurrence of latency, immature grape berries also play a role. First symptoms of gray mold at Zilavka variety at same time appeared as in the Smederevka variety on 27.0.2018 indicating the fact that the occurrence of the disease depends on favorable microclimatic factors. From the moment of the appearance of the first symptoms of the disease on 27.08.2018 to 11.09.2018, there was a great trend of increasing the infection rate in the control bunches and during that time, the infection increased with geometric progression ($r = \frac{a_{n+1}}{a_n}$); gray mold infection at Smederevka variety in

control variant expressed in % (2.1; 7.5; 18.9; 36.1; 79.9); gray mold infection at Zilavka variety in control variant expressed in % (3.4; 9.3; 21.9; 43.9; 85.1) from 27.08.2018 to 11.09.2018; (Table 2). The last fungicide spray in the treated grape variant was executed on 27.08.2018 at the same time when the first symptoms appeared in control grape variant (Table 3). The first symptoms in treated grapes appear 16 days after last fungicide spray in the treated grape variant more precisely on 11.09.2018. Then from 12.09.2018 until that time of grape harvesting there was certain stabilization of gray mold development, this slow development of the disease was partly caused by the unusually high temperatures which were above 30°C (86°F) and the low relative humidity. Considering the state of the research fields and the complexity of variable factors that influenced the development of *B. cinerea* the forecasting model for gray mold proved to be functional. Because it clearly foresaw the need for the latest chemical treatment, testing this infectious model for gray mold on the other hand was comparable to the control variants.

Table 2. Development of *Botrytis cinerea* disease during the working hypothesis.

Variants	Period of disease observation								
	27.08.2018	30.08.2019	03.09.2018	07.09.2018	11.09.2018	15.09.2018	19.09.2018		
	Expressed in %								
Smederevka (chemically treated)	0	0	0	0	4.5	5.8	7.1		
Smederevka-control (untreated)	2.1	7.5	18.9	36.1	79.9	82.5	87.6		
Zilavka (chemically treated)	0	0	0	0	8	11.3	13.2		
Zilavka-control (untreated)	3.4	9.3	21.9	43.9	85.1	87.8	89.2		

Table 3. View of the working hypothesis with dates for chemical treatments.

variety -	Numb	er of cher	nical trea	tments	Working hypothesis		<i>c</i> 1	
	I	II	III	IV	start	end	year of research	
Smederevka	10.05	07.06	11.07	27.08	16.08	19.09	2018	
Zilavka	18.05				11.08	19.09		

5. Conclusion and Recommendations

As a result of monitoring the microclimatic conditions for the development of *B. cinerea*, it can be concluded that they are an essential denominator for the development of the disease. If the incubation phase of the pathogen was completed, 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. Deteriorated external conditions during incubation in *B. cinerea* can cause a resistance reaction which sometimes leads us to the erroneous conclusion that there are no conditions for the development of the disease. The insight in determining the incubation period of *B. cinerea* is the basis for reducing the last chemical treatments just before the grape harvest.

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Conflicts of Interest

The authors declare no conflicts of interest about publication of this paper.

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