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IMPACT OF AMPELOTECHNICAL MEASURES IN THE GRAPEVINE PROTECTION FROM OCCURRENCE OF GREY MOULD (Botrytis cinerea)

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Abstract

Grey mould (*Botrytis cinerea*) provokes severe damages and yield loses in grapevine (*Vitis vinifera*). This research determined ampelotechnical mesaures can be used to influence the development of the disease by reducing the number of chemical treatments. The black grapevine variety Vranec was continuously observed in the experimental field located at Kraiste, Kavadarci, Republic of Macedonia. The working hypothesis was to follow the development of the disease 14 days after the last treatment, when the impact of the fungicide decreases and by applying agrotechnical measures and green operations to control the development of the disease. Those variants where there was a reduction of irrigation and which they were applied ampelotechnical measures was significantly lower intensity of infection in relation of control.

Key words: *ampelotechnical measures, chemical treatments, agrotechnical measures, green operations, intensity of infection, control*

INTRODUCTION

Grey mould (*Botrytis cinerea*) is one of the most significant disease in grapevine (*Vitis vinifera*) causing not only serious economic losses but the development of the plant pathogen. Consequently, additional chemical treatments must be applied immediately before harvesting the grapes raising concerns regarding human health and environmental pollution. Globally, about 15 to 25 million dollars a year is spent on fungicides, to suppress *B.cinerea* (Elad et al., 2004). For these reasons research is directed towards applying certain mesaures during the cultivation of the grapevine that will lead to less use of fungicides.

The disease occurs in all wine regions except that it is more expensive the production of grapes on a unit area, with its enzyme effect worsens the quality of the wine. Considering that external microclimatic influences dictate biological development, appropriate agrotechnical and ampelotechnical measures could influence the health of the grapes. Previous analysis shows that when cultivating the grapevine, micro-location is also important, which could be a significant factor when it comes to profitable grape production, which means lower costs per unit area, (i.e. less of use of fungicides). Field observations found that the use of fungicides and the combined implementation of the ampelotechnical measures, such as the defoliation of the leaves around the bunches, increases the action of the fungicide and reduces the aggressiveness of the disease.

It is important to minimize irrigation at certain stages of disease development that will not create conditions around the bunches favourable to the development of spores of the disease. The current research was undertaken to determine whether from the time of the last fungicide treatment until the time of harvesting (i.e., the period of the fungicide breakdown), is it possible to keep the disease under control without the use of chemicals. Instead of chemicals, simple agrotechnical and ampelotechnical measures were applied, to obtain an environmentally-justified product that would be less harmful to human health. The level of significance was set at 5%. This level in field conditions is acceptable to see the real effect of the treatments.

Infectious features

Grey mould or *Botryotinia fuckeliana* (de Bary) Whetzel (syn. *Sclerotinia fuckeliana* (de Bary) Fuckel) (anamorph **B. cinerea** Pers.), according to taxonomic characteristics, belongs to the genus Botryotinia (family *Sclerotiniaceae*). As a pleomorphic species, it is highly variable and produces conidiophores, conidia, phialoconidia, microconidia, sclerotia, apothecia, accuses and ascospores. Differences between isolates in terms of the speed of growth, the formation of conidia and sclerotia, production of enzymes and pathogenicity, have been noted by several authors (Di Lenna et al., 1981; Grindle, 1979; Leone, 1990; Movahedi & Heale, 1990), who have explored grey mould. In order to simplify the biological cycle of development to explain the infectious features of grey mould, the following phases are 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).

Attachment of conidia for the surface of the grape berry

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 (Doss et al., 1995). A few hours after inoculation, the conidia germination germ tubes are covered with a fibrillar-like extracellular matrix material (Doss et al., 1995). These adhesive structures are excreted by the spore and consist of carbohydrates and proteins (Doss, 1999). 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.

Germination of conidia

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 (Williamson et al., 1995). When dry conidia are inoculated on plant surfaces and incubated in the absence of free surface water, the emerging germ tube usually remains short before it penetrates the surface (Cole et al., 1996; Salinas & Verhoeff. 1995; Williamson et al., 1995). Inoculation with conidia in an aqueous suspension requires the addition of nutrients (Harper et al., 1981; Van Den Heuvel, 1981). These food nutrients can mimic the damaged epidermis of the plant, thereby allowing a realistic impression of the infectious characteristics of spores and their behaviour in such a situation. An example of this is the high germination efficiency of spores in an aqueous suspension rich in phosphates and sugars, to which prior primordial incubation was performed at a certain temperature and applied to the leaf tissue on a tomato plant test where it showed a high degree of infectivity. It means that any injury to the surface tissue of grape berry under certain conditions favourable for development produces a specified intensity of infection. Certain gaseous compounds, such as ethylene, can stimulate the germination of conidia. Previously, the germination of conidia on a hydrophobic surface was stimulated exogenously by ethylene, but the length of the germ tube under these conditions remained unchanged (Kepczynski & Kepczynska, 1977). In a hydrophilic environment, ethylene stimulated germ tube elongation without impacting on the percentage of germination of conidia . The production of ethylene in plants increases with the ageing of the tissue and ripening of the fruits, which is a signal for conidial germination and initiation of infection.

Differentiation of infection structures

After germination of the spore in a drop of water, the germ tube grows and extends. The energy necessary for the growth and elongation of the germ tube is supplied in part from the spore contents and partially by the environmental nutrients. 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 appressoria are thick-walled swellings at the end of a germ tube, of non-standard form and heterogeneous appearance. Several authors (Akutsu et al., 1981; Cole et al., 1996) observed the swelling of hyphal tips of germ tubes and interpreted these as appressorium-like structures. 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.

Penetration of the host surface

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 (Cole et al., 1996; Williamson et al., 1995). Most often, penetration is followed by enzyme activity by the pathogen. Hence, the cutinolytic enzyme (18 kDa cutinase) activity was investigated by creating mutants that lacked genes encoding for cutinase activity. Studies carried out on the fruits of tomato and in gerbera flowers have shown that the wild isolates of B. cinerea and its mutants are capable of penetrating through the layer of cuticle, which leads to the conclusion that the enzyme 18 kDa cutinase is not essential for penetration. Another enzyme assumed to be meaningful for penetration is 60 kDa lipase (Comménil et al., 1995). There is an opinion (Comménil et al., 1999) that this enzyme is induced by grape berry cuticle components. The lipase possesses cutinolytic activity with clearly expressed kinetic properties, which differ from the typical cutinase, which was previously mentioned (Comménil et al., 1998). While the use of polyclonal antibodies against the conidia of B. cinerea prevent the germ tube from penetrating the cuticle, the antibodies did not affect the germination of the conidia (Comménil et al., 1998).

Excretion of a spectrum of phytotoxic compounds (necrotrophic activity)

Botrytis cinerea kills host cells before they are invaded by hyphae. The invasion of the plant tissue by *B. cinerea* triggers nuclear condensation and plant membrane damage, indicators for programmed cell death, in a ring of cells around the hyphae (Govrin & Levine, 2000). Culture filtrates of *B. cinerea*,grown on a nutritional basis, may induce toxic effects when applied to plant tissue. Phytotoxic compounds were identified as botcinolide, a highly substituted lactone (Cutler et al., 1993), and botrydial, a tricyclic sesquiterpene (Colmenares et al., 2002). The observation that both secondary metabolites were only secreted by *B. cinerea* in medium with high glucose levels, initially raised doubts about their physiological relevance in plants. Recent analytical chemistry studies have, however, demonstrated that botrydial accumulates in infected tissue under physiologically-relevant concentrations (Deighton et al., 2001; Muckenschnabel et al., 2003). The production of botrydial may be an important factor in the infection of (some) host plants but cannot be confirmed because the genes that participate in its synthesis have not yet been identified and, for this reason, appropriate mutants have not yet been created, which will experimentally confirm their role in the pathogenesis.

Resistance and susceptibility of the grape bunch and grape berries towards Botrytis cinerea

In grapevine, *B. cinerea* predominantly infects the inflorescences and grape berries. The development of the pathogen in grape clusters begins with the fruit ripening. Before the ripening of the bunches, the green grape berries possess a high amount of fungitoxic or fungistatic compounds known as phytoalexins, which prevent the development of grey mould. The level of resveratrol, a phytoalexin stilbenoid, is correlated with the resistance of grape berries towards grey mould. Besides phytoalexins, green grape berries also contain proteinase inhibitors that prevent cell wall-degrading enzymes. The concentration of polygalacturonase inhibitor proteins decreases during ripening (De Lorenzo et al., 2001). The skin of grape berry, especially the epicuticle and cuticle, play a major role in resistance towards grey mould. Particularly, epicuticle and cuticle waxes were described as very important features of skin grape berry in relation to susceptibility towards grey mould. In this context, warm temperatures, high air humidity and water on the berry surface are known as major reasons for the incidence of microscopic cracks in the cuticle membrane of berries (Becker & Knoche, 2012). The formation of these microscopic cracks largely determines the susceptibility or resistant of the grape berries to disease, because the entry of small quantities of water contribute to the additional cracking of the protective layers of grape berry. Cuticular forms on the surface of grape berry have an additional role as a regulator of molecular diffusion of food nutrients, which can stimulate the development of the conidia. Pores on the surface of grape berry contribute to the diffusion of nutrients, especially in conditions of hot weather and high relative air humidity when the exchange of matter has increased. The intra- and epicuticular waxes form a hydrophobic layer that repels water from the surface of the grape berry, contributing to the rapid drying of isolated water drops. The hydrophobic layer of the grape berry and the fast drying of excess water on the surface of grapes are the main factors in reducing susceptibility towards *B. cinerea*.

Protection measures against grey mould

Reliable methods for prognosis of the occurrence of disease in the vineyard do not currently exist (Leroux et al., 2002). Only with a good knowledge of all factors that directly or indirectly influence the development of the disease, is it possible to apply measures for preventing damage from the disease. By applying adequate agrotechnical and ampelotechnical measures, conditions can be created that endow a disabling attack,via creatingunfavourable conditions for the development of the pathogen or inability to establish the plant–pathogen interaction. Preventive protection and disease control rely mostly on preventing the establishment of contact between the pathogen and the host. In this context, ampelotechnical measures, such as defoliation around the grape bunches, preclude the establishment of micro-conditions that favour pathogen development. Defoliation is of great importance because of the physiology of guttation in grapevine. Since partial exposure of grape bunches and berries to solar insolation leads to rapid drying of the surface of fruits, the surface diffusion of nutrients between the berries and external environment is reduced. Shallow plowing of the soil in summer during the veraison phase (onset of ripening) of the fruit, as an agrotechnical measure,influences the development of the disease because of the rise in the relative humidity in the zone of the grape bunches. Irrigation and its reduction in the vineyard have also shown to have a key role in creating the conditions for the development of *B. cinerea*. From fungicides was used active substance boscalid with which the last treatment was carried out, and we started field monitoring of the disease.

MATERIAL AND METHODS

The research was completed in a vineyard located at Kraiste, near Kavadarci, Macedonia (41°27`14.3676″ N, 22°0`38.5236″ E) on black grape variety Vranec. A double Guyot pruning system was applied in the vineyard. The area of the vineyard was 0.6 ha, the plantation was 13 years old, and soil was degraded clay. The variants set in the experiment considered almost all possible situations encountered in practice during cultivation of grapevine. The goal was to note how the individual variants, during cultivation of grapevine with the application of some agrotechnical and ampelotechnical measures, affect thedevelopment of the 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. Monitoring for development of the disease was carried out every 4 days starting from 10 August 2018.

Variants and calculations

Six variants plus control were installed. In each of the variants, the bunches were placed under different microclimatic conditions encountered during the production of grapes, bearing in mind the agrotechnical and ampelotechnical measures undertaken. 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 (Pejchinovski & Mitrev, 2007), and efficiency of fungicide according to Abott's formula (Pejchinovski & Mitrev, 2007).

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 (%)
Without defoliaton around the bunches I. Pyrimethanil II. Boscalid III. Boscalid	Treatment against *B.cinera* under irrigation condition	110	100	90	81,8	20	18,2	28	65,4
	Treatment against *B.cinera* in dry condition	95	100	90	94,7	5	5,3	22,2	72,5
	Treatment against *B.cinera* in dry condition with summer plowing	90	100	78	86,6	12	13,4	27,7	65,8
With defoliation around the bunches I. Pyrimethanil II. Boscalid III. Boscalid	Treatment against *B.cinera* under irrigation condition	110	100	95	86,3	15	13,7	30,8	61,9
	Treatment against *B.cinera* in dry condition	95	100	93	97,8	2	2,2	16,6	79,5
	Treatment against *B.cinera* in dry condition with summer plowing	90	100	81	90	9	10	16,6	79,5
CONTROL		100	100	30	30	70	70	80,8	

Table 2. Formulas for determining the disease parameters.

Category of infection of the bunch	Calculating the index of infection according to Mc-Kynney`s formula	Calculating the efficiency of fungicide according to Abott`s formula
0 – without of infection 1 – rare infection 2 – significant infection 3 – strong infection 4 – whole infection	$I = \frac{\Sigma(n \times k)}{N \times K} \times 100$ I – index of infection n – no. of bunches by category N – simple size (30 bunches) K – no. of categories Σ – sum	$E = \frac{It}{Ik} \times 100$ E – efficiency of fungicide It – index of infection in treated plants Ik- index of infection in untreated plants (control)

To increase the accuracy of the assessment of the infection of the bunches, BRAT (Bunch Rot Assessment Trainer) software was used (http://bunchrot.co.nz/), which measures the percentage of the total area of the bunch that is affected by the disease. The results obtained using this software were compared with statistical analysis of diseased and healthy grape berries. The statistical difference was minimal and amounted to ±1%. The diseased and healthy bunches were categorised as described in Tab. 3.

Table 3. Categorisation of the diseased bunches.

Numerical category of infection	Terminological category of infection	Expressed in %
0	without of infection	/
1	rare infection	1 – 5
2	significant infection	6 – 30
3	strong infection	31 – 70
4	whole infection	71 – 100

The categorisation of the diseased bunches was based on the virulence of *B. cinerea* and the susceptibility of the grapevine variety. At the beginning of infection in the control (untreated plants) when infection was rare, the spread of the disease was checked using arithmetic progression while, in the further stages of the disease, the spread was monitored using geometric progression. This categorisation cannot be used as a general model in determining the diseased bunches,because of heterogeneous structural and mechanical differences in the bunches of different varieties and the natural resistance towards grey mould.

Working hypothesis

Three treatments were carried out against grey mould. The first treatment was performed with the active substance pyrimethanil while, in the other two treatments, boscalid was used as the active substance. The first treatment was executed on 17 June, the second treatment on 4 July, and the third treatment on 27 July (Tab. 4), respectively. On 7 September, when the technological maturity of the grapes was also established, the samples were taken, and their calculations were determined.The working hypothesis was to monitor the development of the disease from14 days after the last treatment against *B. cinerea* and with ampelotechnical measures to control the development of the disease in the period when the activity of the fungicide decreases. The level of significance was set at 5%. Under field conditions, this level is acceptable to see the real effect of the treatments.

Monitoring of the disease began on 10 August after the initial fungicidal effect of boscalid had passed and when the decomposition period occurs. Decomposition of boscalid requires 28 days (product Cantus WG). The analysis of the samples for each of the variants was consistently performed on the 28th day of the decomposition period, i.e., on 7 September. Boscalid is a mitochondrion respiration inhibitor with a broad spectrum of action that stops spore germination, germ tube extension and attachment of conidia to the surface of the grape berry. It also impacts on the stages of mycelial growth and is water-resistant. The characteristics of boscalid to act against *Erysiphe necator* was of essential importance to the working hypothesis,because its use on the last treatment on 27 July, allowed protecting the grapevine from powdery mildew besides the persistent effect of the decomposing active substance against grey mould. The veraison phase (onset of ripening or colour change of grape berries from green to black) in variety Vranec started on 17 August when the conditions for the development of B. cinerea occur. Defoliation, summer plowing and irrigation were carried out on 15 August.

Table 4. Dates of the chemical treatments.

Number of chemical treatments	Active substance	Date of treatments
I	Pyrimethanil	17 june
II	Boscalid	4 July
III	Boscalid	27 July

Table 5. Schematic presentation of treatments and working hypothesis.

June	July	August	September
		Working hypothesis	
17	4 and 27	10 and 7	

Monitoring of the disease

In order to understand a phenomenon, the tendency of its development should be followed. Observation of the development of the disease was carried out during the working hypothesis. Five vines of each variant were selected, which were marked by six clusters (bunches), to obtain a sample size of 30 clusters while three vines were chosen for the control because it was represented by one row. In the control vines, on average, there were 10 clusters, which again obtained a sample size of 30 clusters. Samples were collected every 4 days to note the change in the development of the disease. Since disease monitoring is a visual method, to avoid the subjectivity of the individual assessment in the categorization of diseased bunches, the BRAT software was used. Following the results of the BRAT software assessment, statistical processing of bunches at the sample level was performed, especially for each observation date. Then, a sample for each of the variants was categorized according to Tab. 3. Observation of the disease began on 10 August and ended on 3 September.

For the 28-day duration, the disease was observed seven times in each of the variants and control and every change in the marked vines (plants) and bunches was recorded. Ampelotechnical measures (defoliation of the leaves around the bunch), irrigation and summer plowing, depending on the variant, was performed on 15 August. The veraison phase (onset of ripening) began on 17 August. On 22 August, it rained up to 20 mm. In addition to the monitoring of the disease, grape sugar was regularly measured. Technological maturity in the variety Vranec was noticed on 1 September. On 7 September, a sample of 30 bunches was taken from each variant, and together with the control, a statistical calculation of the results was carried out.

RESULT AND DISCUSSION

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: (i) infections arising from conidia that first adhere to the surface of the grape berries, build a germ tube, form an appressorium and, finally, hyphae are produced that penetrate the tissue; (ii) formation of mycelium structures by which the infection spreads among the grape berries. The way of infection who make the conidia differ from the infection they make mycelium structures from grape berry to grape berry (Elmer & Michailides, 2007). The infection of grape berry by conidia that arrive by air and stick to the surface of the epicuticle is characterized by a lower speed relative to the infection that occurs with already formed mycelium structures on the bunches. The first mode of contamination that arises from conidia adhered to the surface of the grape berry depends on the micro-conditions of the grapevine, and so certain ampelotechnical measures can be undertaken that influence the speed of development of the disease, i.e., slow down the infectious process. In contrast, in the second mode of contamination when mycelium structures have already formed, the infectious process is very difficult to control only with ampelotechnical measures and can reduce the production of grapes.

Table 6. Development of *Botrytis cinerea* disease during the working hypothesis.

VARIANTS 10 Aug.		Period of disease observation and assessment of diseased bunches with software BRAT (Bunch Rot Assessment Trainer) in %							Statistical calculation of results in %
		14 Aug.	18 Aug.	22 Aug.	26 Aug.	30 Aug.	03 Sept.	07 Sept.	
Without defoliaton around the bunches I . Pyrimethanil II.Boscalid III. Boscalid	Treatment against *B.cinera* under irrigation condition	0	0	1	2,1	4,9	8,7	13,5	18,2
	Treatment against *B.cinera* in dry condition	0	0	0,5	0,7	1,3	2,7	3,5	5,3
	Treatment against *B.cinera* in dry condition with summer plowing	0	0	1,3	2,2	5,7	6,3	9,1	13,4
With defoliation around the bunches I.Pyrimethanil II.Boscalid III.Boscalid	Treatment against *B.cinera* under irrigation condition	0	0	1	2,3	4	7,8	10,3	13,7
	Treatment against *B.cinera* in dry condition	0	0	0	0,5	1	1,2	1,7	2,2
	Treatment against *B.cinera* in dry condition with summer plowing	0	0	1	1,5	3	5,7	6,3	10
CONTROL		0	0	1,7	5,6	12,8	23,7	48,7	70

Control of *B. cinerea* is difficult because the pathogen creates a huge number of conidia, the grey mould attacks the grapevine at various stages of its development, and there is still no reliable method for prognosis of the disease. Consequently, often fungicidal treatments are applied, quite unjustifiably,which pollutes the environmentand places the health of the consumers at risk. It is very difficult for an organism to be placed in a certain frame, such as statistical formulas, and to obtain relevant indicatorssince many unknown factors are likely to act. At the beginning of the infectious process, the rate of infection varied with one arithmetic progression, as can be noted in Tab. 6 variant "Treatment against *Botrytis cinerea* in dry conditions" (0.0, 0.5, 1.0, 1.2, 1.7, 2.2). However, when mycelium structures of the pathogen had formed on the surface of the bunch, the rate of the infection moved according to a geometric progression, and this was most visible in the control (1.7, 5.6, 12.8, 23.7, 48.7, 70.0, Tab. 6). If the control is presented in the form of a graph, the curve displays exponential features (Fig. 1).

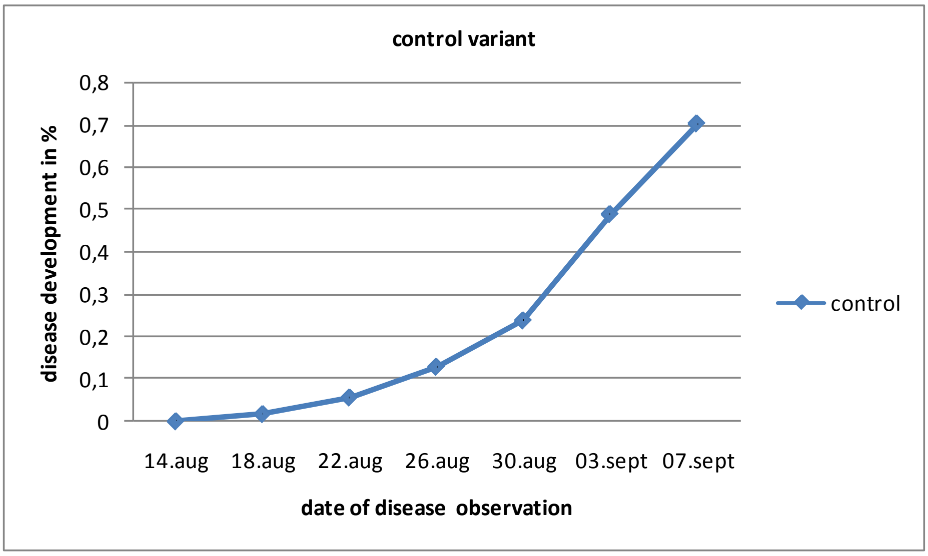

Figure 1. Graphic for disease observation and disease development in %.

When the variants with and without defoliation were compared (Tab. 6) where identical agrotechnical measures were assumed (i.e., treatment against *B. cinerea* in dry conditions, summer plowing in the veraison phase (onset of ripening) and irrigation), although there were no drastic differences in the percentage of numerical values of diseased bunches, the infectious process was somewhat faster in the variants without defoliation, possibly due to the higher epercentage of relative air humidity around the bunches. The assumption determined by the working hypothesis that it is possible in field conditions to control the disease using ampelotechnical measures proved accurate in variants with defoliation: treatment against *B. cinerea* in dry conditions (96.1%), and treating in dry conditions with summer plowing in the veraison phase (onset of ripening) (96.1%), at the 5% level of significance (Tab. 7).

Table 7. Sum of data between variables for determining the working hypothesis.

VARIANTS		Infection index according to formula of Mc-Kinney(%)	Efficiency of fungicide according to formula of Abott (%)	Allowed a level of significance of 5%
Without defoliaton around the bunches I . Pyrimethanil II.Boscalid III. Boscalid	Treatment against *B.cinerea* under irrigation condition	28	65,4	93,4
	Treatment against *B.cinerea* in dry condition	22,2	72,5	94,7
	Treatment against *B.cinerea* in dry condition with summer plowing	27,7	65,8	93,5
With defoliation around the bunches I.Pyrimethanil II.Boscalid III.Boscalid	Treatment against *B.cinerea* under irrigation condition	30,8	61,9	92,7
	Treatment against *B.cinerea* in dry condition	16,6	79,5	96,1
	Treatment against *B.cinerea* in dry condition with summer plowing	16,6	79,5	96,1

CONCLUDING REMARKS

The most spreaded and scientific observed plant disease, grey mould (*Botrytis cinerea*) provokes severe damages and yield loses in grapevine (*Vitis vinifera*). This is first research determined that ampelotechnical measures that can be used to influence the development of the disease by reducing the number of chemical treatments. The black grapevine variety Vranec was continuously observed in the experimental field located at Kraiste, Kavadarci, Republic of Macedonia. The working hypothesis follow the development of the disease 14 days after the last treatment, when the impact of the fungicide decreases and by applying agrotechnical measures and green operations to control the development of the disease. Those variants where there was a reduction of irrigation and which they were applied ampelotechnical measures was significantly lower intensity of infection in relation of control.

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ВЛИЈАНИЕ НА АМПЕЛОТЕХНИЧКИТЕ МЕРКИ ВО ЗАШТИТА НА ВИНОВАТА ЛОЗА ОД ПРИЧИНИТЕЛОТ НА СИВО ГНИЕЊЕ *(Botrytis cinerea)*

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Резиме

Сивото гниење кај виновата лоза (*Botrytis cinerea*) предизвикува сериозни оштетувања и големи економски загуби во производството на винова лоза (*Vitis vinifera*). Ова истражување стави посебен акцент на влијанието на ампелотехничките мерки кои може да се користатат да влијаат на спречување на развојот на болеста со намалување на бројот на хемиски третмани.

Црнатавинскасортавранецбешепредметнапостојанонабљудувањевоексперименталното поле лоцирано во Краиште, Кавадарци, Република Северна Македонија. Поставената работна хипотеза имаше за цел да го следи развојот на болеста 14 дена по последниот третман, кога влијанието на фунгицидот се намалува и преку примена на агротехнички мерки и зелени операции, да се изврши контрола на развојот на болеста. Оние варијанти каде што имаше намалување на наводнувањето и за кои се применуваа ампелотехничките мерки значително го намалија интензитетот на инфекција во однос на контролата.

Клучни зборови: *ампелотехнички мерки, хемиски третмани, агротехнички мерки, зелени операции, интензитет на инфекција, контрола.*