

## Gumisil-D

# Effect of Microfertilizer Gumisil-D for Increasing of Harvest and Quality of Chardonnay Grapes

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Original scientific paper

### SUMMARY

The results of the influence on the yield and quality of the Chardonnay variety of root-dressing by the organomineral microfertilizer GumiSil-D were determined.

It is found that 3-fold spraying of the vineyards increases the growth of the biological mass of the above-ground part of the grape bush. The yield of grapes rises by 15,2-26,8%, while the accumulation of sugar in the juice of berries increases and its acidity decreases. The rate of consumption of microfertilizer GumiSil-D 60 ml/10 l was the most effective, which increased by 44% more control than the rate of 40 ml/10 l. The use of microfertilizer led to an increase in the vegetative mass of the bush. The area of the leaf surface of the bush in the experimental variants reached in comparison with the control by 47,7 - 49,8%. With the use of microfertilizer GumiSil-D, the quality of grapes was improved. Thus, the mass concentration of sugars in the juice of berries increased by 3-9.7% more control.

**Key words:** Chardonnay, grapes, microfertilizers, sugarness, yield

## INTRODUCTION

The lack of nutrients and digestible forms of microelements in the soil leads to a decrease in the crop yield and to a deterioration of its quality, that is the cause of the appearance of various diseases (Klein et al., 2000). In the presence of the necessary amount of trace elements, plants are able to synthesize a full spectrum of enzymes, which will allow more intensive use of energy, water and nutrition (N, P, K), and, accordingly, get a higher yield (Kazemi, 2013).

Biologically substantiated doses of active microelements, introduced independently of the soil composition, do not affect the total content of trace elements in the soil, but have a beneficial effect on the state of plants.

When using them, the state of physiological depression in plants is excluded, which leads to an increase in their resistance to various diseases, which will generally affect the increase in the quantity and quality of the crop (Gilmanov et al., 2013).

The introduction of microfertilizers on vegetative plants is also one of the methods of their application. Getting to the surface of the leaf, microelements penetrate into its tissues and are incorporated into biochemical reactions of metabolism in the plant. This method allows to significantly increase the utilization rate of microelements and provide plants with the necessary set of trace elements during the formation of reproductive organs (Abilfazova and Belous, 2016).

This leads to the enrichment of microelements of plants and allows you to receive a full crop and, importantly, the laying of a future crop.

The numerous works on foliar application of microfertilizers unequivocally show the positive influence of this method on the

(Klein et al., 2000).

(N, P, K)

(Kazemi, 2013).

(Gilmanov et al., 2013).

(Abilfazova

and Belous, 2016).

(Abasheeva et al., 2003; Borovik, 2012; Sima and Mohsen, 2014; Zubkova and Gulidova, 2015; Kameneva and Tkachenko, 2019)

- yield and quality of agricultural crops, grapes, fruit (Abasheeva et al., 2003; Borovik, 2012; Sima and Mohsen, 2014; Zubkova and Gulidova, 2015; Kameneva and Tkachenko, 2019)

- The peculiarities of grape culture impose an imprint on the use of drugs with biological activity.

- The grapes require the greatest need for fertilizing before flowering, during the tying and before the ripening of berries. It is very necessary to fertilize before flowering, when the bush spends most of the nutrients, when forming inflorescences.

2)

GumiSil-D

1)

The aim of research was 1) to determine the effect of organomineral microfertilizer GumiSil-D on the yield and quality of the Chardonnay grape and 2) to investigate the most effective application rates of the studied drug in the conditions of the south of Ukraine.

## MATERIAL AND METHODS

*Materials.* Chardonnay was chosen

- for experiment due to prevalence of variety across Ukrainian wine regions (the 3d place after Aligote and Cabernet Sauvignon). In the year of 2017, the weather conditions in the south of Ukraine were rather complicated.

- The winter was cold, many of the vineyards froze. Summer weather conditions also can not be called favourable for a good harvest in terms of volume, in fact there are actually no normal rains for three months, so the harvest is usually low. Harvest was on August 14, 2017. Grapes were obtained for processing for making white sparkling wines.

2017 .

GumiSil-D (

)

( ) .

"GumiSil-D" (for orchards and vineyards) is a complex organic fertilizer based on the humate of potassium from natural raw materials (low peat). Fertilizer is used for the processing of seedlings, foliar fertilizing and spraying.

( ); 2 -  
 GumiSil-D 40  
 ml/10 l; 3 -  
 GumiSil-D 60 ml/10 l.

GumiSil  
 GumiSil-D 40 60 ml/10  
 : 2  
 - I (01.07.2017),  
 II  
 (20.07.2017)  
 - III (29.08.2017).

20°C.

( )

0,1 N,  
 g/dm<sup>3</sup>

*Scheme of experience:* option 1 - control (water); option 2 - foliar dressing with GumiSil-D drug rate of 40 ml/10 l; option 3 - foliar dressing with GumiSil-D drug rate of 60 ml/10 l.

The bushes were treated by an aqueous solution of organomineral microfertilizers from enterprise "GumiSiL" named GumiSil-D at the rate of 40 and 60 ml/10 liters of water in the time frame: 2 days before flowering - I period (01.07.2017), in the growth phase of berries II (20.07.2017) and at the beginning of ripening berries-III (29.08.2017).

During the growing season, phenological observations were carried out on the sites under study and the onset of phases of flowering, maturation, and technical maturity was recorded. After stopping the vegetative growth of the bushes, biometric measurements are carried out such as the determination of the leaf surface, the annual increment in the linear and volumetric measurement. Terms of harvesting are established, based on the dynamics of indicators of mass concentration of sugars, titrated acids, pH, sensory properties of grapes. The number and average weight of the bunch are taken into account when harvesting.

*Chemical methods.* The hydrometric method for determining the mass concentration of sugar in the juice of grapes is based on the direct dependence of the gravity of grape must on the mass concentration of sugar in it. According to the hydrometer readings, brought to a temperature of 20°C, a mass concentration of sugar is found.

The determination of the titratable acidity is carried out by adding alkali (titration) to the wort or wine sample until the moment of neutralization occurs, as indicated by the colour change of the indicator.

For titration use a solution of caustic sodium. Concentration of alkali 0.1 N. the titratable acidity g/dm<sup>3</sup> is calculated according to the

$T = a \cdot K \cdot 0.0075 \cdot 100$ ,  
 ml,  
 10 ml ;  
 correction to alkali normality.  
 (LSD)  
 $S_x = S^2/n$   
 LSD  
 $S_d = 2S^2/n$   
 d 0,5, d 0,5

formula  $T = a \cdot K \cdot 0.0075 \cdot 100$ , where  
 a is the amount of ml of alkali that has  
 gone to titration with 10 ml of wine;  
 - correction to alkali normality.

*Statistical data.* The Least  
 Significant Difference (LSD) was used to  
 determine the essentiality of the  
 differences between the variants of the  
 experiment.

To assess the significance of  
 particular differences, the following values  
 were calculated:

- the generalized error of the mean:  
 $S_x = S^2/n$

- mean difference error:  $S_d = 2S^2/n$

LSD indicates the maximum error  
 for the difference of the two sample  
 means. If the actual difference is significant,  
 when d 0,5, for d 0,5 is inessential.

## RESULTS AND DISCUSSION

The appropriateness of the  
 application of a given method to the  
 biological characteristics of each variety is  
 determined by the growth force of the  
 bushes, or by the development of a one-  
 year increment, and accordingly the leaf  
 surface, the magnitude and productivity,  
 which ultimately determine the possibility  
 of obtaining a high and conditional harvest  
 this year and the creation of appropriate  
 prerequisites for normal growth and  
 fruiting of the grape plant in the next  
 growing season (Table 1).

1.

GumiSil-D

“, 2017

**Table 1. Effect of organomineral microfertilizer GumiSil-D on the growth and development of the Chardonnay grapes, 2017**

Sample	Number of shoots per bush	Sheet diameter, cm	Area of leaf surface of the bush		Average length of shoots, cm	Diameter of the shoot, mm	Volume of one-year increase	
			m <sup>2</sup>	%			/bush, cm <sup>2</sup>	%
Control (water)	16,5	15,5	8,46	100,0	147,6	9,4	1689,2	100,0
40 ml/10 l	18,1	16,0	12,48	147,7	168,2	10,0	2389,9	141,4
60 ml/10 l	16,2	16,3	12,67	149,8	191,4	10,0	2434,0	144,0
LSD <sub>05</sub>					14,6			

	34-37		27.2	
		50		
19 cm.			10	
16.3 cm,		15.5 cm	16.0	
	60 ml/10 l,		GumiSil-D	
	4.21 m <sup>2</sup>	47.7%.		
D	40 ml/10 l,		GumiSil-	
		4.02 m <sup>2</sup>	49.8%.	
		2.0 m		
			GumiSil-D	
20.6 cm	40 ml/10 l			
	60 ml/10 l,		43.8 cm	
		LSD05 = 27.4 cm.		
			10.0	
9.4 mm				
			GumiSil-D	
744.8 cm <sup>3</sup>	60 ml/10 l,			
	44%			
			GumiSil-D,	

The use of microfertilizer led to an increase in the vegetative mass of the bush. There was an increase in the diameter of the leaf, the number of leaves, the length and diameter of the shoots. The number of leaves in the experimental versions increased to an average of 34-37 pieces to escape. On the control variant, the number of leaves was 27.2 pieces per shoot. In some shoots, the number of leaves reached 50 or more pcs. to escape, this was due to a large number of formed stepsons, which then had to be removed.

The diameter of the sheet in the experimental versions varied from 10 to 19 cm. The average diameter of the sheet in the experimental variants was 16.0 and 16.3 cm, versus 15.5 cm at the control.

The largest area of the leaf surface of the bush was observed in the version where GumiSil-D microfertilizers were used with the norm of 60 ml/10 l, it increased by 4,21 m<sup>2</sup> or by 47,7%. With the application of foliar fertilizing with microfertilizer GumiSil-D, the norm of 40 ml/10 l of leaf area of the bush increased in comparison with the control by 4,02 m<sup>2</sup> or by 49,8%.

An increase in the length of the shoot was noted, individual shoots in the experimental variants reached a length of 2.0 m or more. The average length of shoot with the use of microfertilizer GumiSil-D with a norm of 40 ml/10 l increased by 20.6 cm more than control and at a rate of 60 ml/10 l, respectively, by 43,8 cm more control. The difference in the variants of the experiment is mathematically proven by LSD<sup>05</sup> = 27,4 cm.

The average diameter of the shoot in the experimental variants was the same and was 10,0 versus 9,4 mm in the control.

The greatest volume of one-year increment of the bush was obtained in the version where the GumiSil-D microfertilizer was used with the norm of 60 ml/10 l, it increased by 744,8 cm<sup>3</sup> or by 44% more control. With the application of foliar fertilizing of grapes with micro-

41.4%      40 ml/10 l  
700.7 cm<sup>3</sup>

30% (1-5).

fertilizer GumiSil-D, the norm of 40 ml/10 l increased the annual growth by 700,7 cm<sup>3</sup> or by 41.4% more control.

In the literature, data are given on the increase in yield under the influence of various growth regulators up to 30% (1-5).

Our experiments showed that in the first year the increase in yield occurs only due to an increase in the mass of the bunch.



1. " " " , 2017  
Fig. 1 Bunches of Chardonnay grapes according to experience options, 2017

GumiSil-D 1- 60 ml/10 l, 2- 40 ml/10 l, 3 –  
Concentration of GumiSil-D: 1- 60 ml/10 l, 2- 40 ml/10 l, 3 – control.

	GumiSil-D	
GumiSil-D	40 ml/10 l,	
	198.6 g,	38.9 g
	GumiSil-D	
	60 ml/10 l,	

The experiment variants showed that the quantity of bunches varies was insignificantly, however, the bunch mass in the experimental variants under the influence of the microfertilizer GumiSil-D changed significantly.

When using microfertilizer GumiSil-D with a norm of 40 ml/10 l, the mass of the bunch was 198,6 g, which is 38,9 g more compared to the control. An even greater increase in the mass of the bunch was obtained with an increase in the rate of consumption of the microfertilizer in question. The mass of the bunch with the use of GumiSil-D microfertilizer with a

211.2 g, 51.5 g  
 ( 2, 1).  
 LSD<sub>05</sub> = 27.4 g

norm of 60 ml/10 l was 211,2 g, which is 51,5 g more than the control. The difference in the variants of the experiment was mathematically proven by LSD<sub>05</sub> = 27,4 g (Table 2, Figure 1).

2.

GumiSil-D

„, 2017

**Table 2. Effect of organomineral microfertilizer GumiSil-D on the harvest and quality of grapes of the Chardonnay variety, 2017**

Sample	Number of grapes on a bush	Bunch weight, g	Harvest from bush, kg	Productivity		Sugar content of berries juice g/dm <sup>3</sup>	Titratable acidity of juice, g/dm <sup>3</sup>	
				t/ ha	%			
Control (water)	14,9	159,7	2,38	9,15	100,0	186,2	11,8	3,22
40 ml/10 l	13,8	198,6	2,74	10,54	115,2	192,5	10,6	3,12
60 ml/10 l	14,3	211,2	3,02	11,60	126,8	204,4	9,6	3,10
LSD <sub>05</sub>		27,4				4,8		

-  
 GumiSil-D 60 ml/10 l, 3.02 kg/ kg/  
 26.8%  
 GumiSil-D 40 ml/10 l, kg/ kg/  
 15.2%  
 ( 2).

The highest yield from the bush was obtained in the version where the microfertilizers GumiSil-D were used with a norm of 60 ml/10 l, it was 3,02 kg/bush, which is 0,64 kg/bush more than in the control. In terms of per hectare of vineyards, the yield in this variant increased by 2,45 t/ha or 26,8% more compared to the control. With the use of microfertilizer GumiSil-D, a yield of 2,74 kg/bush was obtained at a rate of 40 ml/10 l, and 0.34 kg/bush more control. In terms of per hectare of vineyards, the yield increased by 1,39 t/ha or 15,2% more than in the control (Table 2).

-  
 16.0 g/100 cm<sup>3</sup>.  
 GumiSil-D 60.4 ml/10 l, g/dm<sup>3</sup>, 18.2 g/dm<sup>3</sup>

The accumulation of sugars in grapes is of great technological importance. This indicator, as a rule, determines the timing of the collection of grapes, and also predicts the volume fraction of alcohol in future wine materials. The minimum value of sugars in accordance with white grape varieties should be at least 16,0 g/100 cm<sup>3</sup>.

The mass concentration of sugars with the use of GumiSil-D microfertilizer was 60,4 ml/10 l at a rate of 204,4 g/dm<sup>3</sup>, which is 18,2 g/dm<sup>3</sup> more than control.



GumiSil-D  
40 ml/10 l,  
6.3 g/dm<sup>3</sup>

LSD<sub>05</sub> = 4.8 g/dm<sup>3</sup>.

GumiSil-D 60 ml/10 l,  
9.6 g/dm<sup>3</sup>,  
GumiSil-D 40 ml/10 l,  
10.6 g/dm<sup>3</sup>.

3.10 3.12 3.22  
GumiSil-D 60 40 ml/10 l.

"GumiSiL"

GumiSil-D TM

60 ml/10 l

38.9-51.5 g

- With the use of microfertilizer GumiSil-D with a norm of 40 ml/10 l, the mass concentration of sugars in the juice of berries increased by 6,3 g/dm<sup>3</sup> more than control. The difference in this variant of the experiment is not mathematically proven by LSD<sub>05</sub> = 4,8 g/dm<sup>3</sup>.

The mass concentration of titrated acids with the use of microfertilizer GumiSil-D at a rate of 60 ml/10 l was the lowest, it was 9,6 g/dm<sup>3</sup>, which is 2,2 g/dm<sup>3</sup> below the control. With the use of microfertilizer GumiSil-D with a norm of 40 ml/10 l, the mass concentration of titrated acids in the juice of berries decreased in comparison with the control by 1,2 g/dm<sup>3</sup> and amounted to 10,6 g/dm<sup>3</sup>.

- The index of active acidity of pH affects further the quality of wines. Wines obtained from processed grapes are potentially less susceptible to oxidation and are resistant to turbidity.

In the experimental variants, the active acidity index of the pH was 3,10 and 3,12 versus 3,22 on the control, respectively, using the GumiSil-D microfertilizer with the norms of 60 and 40 ml/10 l.

The grapes from the experimental bushes were more harmonious in taste, which is natural, because higher sugar and lower acidity were noted in comparison with the control variant.

### CONCLUSIONS

Studies of the new organomineral microfertilizer GumiSil-D TM "GumiSiL" have shown a positive effect of applying three-times foliar treatment of the Chardonnay grapes to obtain higher yields with some improvement in quality indicators.

- A more effective rate of application of this microfertilizer is 60 ml/10 l. The use of microfertilizer led to an increase in the vegetative mass of the bush. The mass of bunches in the experimental variants increased by 38,9-51,5 g more control.
- Also using of microfertilizer GumiSil-D, the

GumiSil-D,

3-9.7%

- quality of grapes has been improved.
- Thus, the mass concentration of sugars in the juice of berries increased by 3-9.7%
- more control, while there was a slight decrease in titrated acids in the juice of berries. It is planned to continue the experiments to determine the effect of the studied organo-mineral microfertilizer on the differentiated inflorescence bookmark, which will affect the future crop.

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## (*Arthrocladiella mougeotii*)

### (*Lycium barbarum* L.)

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## Influence of Powdery Mildew (*Arthrocladiella mougeotii*) on Different Varieties of the Goji Berry Plant (*Lycium barbarum* L.)

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Original scientific paper

2018-2019

- 290 m

2014

4

3 m / 2 m

(JB1, JB2, JB 4 JB10)

*Lycium barbarum* L.

### SUMMARY

The experiment was held in the experimental field of the Department of Fruit Growing at the Agricultural University - Plovdiv in Brestnik village in the period 2018-2019. The area is 290 m above the sea level. The plantation was established in 2014 year with four *in vitro* propagated varieties goji berry (JB1, JB2, JB 4 and JB10) of the species *Lycium barbarum* L. They were planted at distances 3 m / 2 m. The plants are formed as trees on a supporting construction and are grown under drip irrigation.

The aim of the study was to obtain information on the sensitivity to powdery mildew of some of the known in Bulgaria varieties goji berry and to understand how the disease affects the fruiting.

From the results of this study it became clear, that powdery mildew (*Arthrocladiella mougeotii*) is a disease that causes damage to the leaf blade, shoots and flowers. No damage was found to the goji berry fruits. After an

attack of the disease, was observed a strong recovering mechanism of the plants, which manifest in the formation of new young uninfected leaves and shoots.

The studied varieties showed different sensitivity to powdery mildew. The highest degree of attack was reported in the variety JB 2 (up to 95%) and then in JB 1 - up to 30%. In JB 4 the damage reached 13%, and least affected (up to 4%) were plants of JB 10.

From the applied plant protection products, TOPAZ showed no influence against the pathogen of powdery mildew, and the SKOR had temporary effect.

The average yield from a tree for the period 2018-2019 ranged from 0.12 kg to 1.18 kg. It was highest in JB 1 and lowest in JB 4. In this Study in two of the varieties, respectively JB 1 and JB 4 were found symptoms of damage from the mite *Aceria kuko*.

**Key words:** goji berry, *Lycium barbarum* L., powdery mildew, *Aceria kuko*

(*Lycium barbarum* L.)

(Fuxiang and Haiming, 2009).

*Colletotrichum acutatum*, (*Lycium barbarum* L.) (Sun et al., 2008).

2002-2004 .  
*L. Chinense* Medicinal Herb Garden University of Seattle (Washington, USA)

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**Key words:** goji berry, *Lycium barbarum* L., powdery mildew, *Aceria kuko*

## INTRODUCTION

Goji berry (*Lycium barbarum* L.) is a species, which is recently relatively studied. Five types of diseases have been identified. As the most dangerous diseases are mentioned powdery mildew, anthracnose, root rot, gray leaf spots and black fruit disease. Diseases are spreading rapidly at high humidity conditions combined with high temperature. (Fuxiang and Haiming, 2009).

In China for the first time, *Colletotrichum acutatum* as a cause of anthracnose in goji berry (*Lycium barbarum* L.) has been reported (Sun et al., 2008).

During the period 2002-2004, two species of *L. Chinense* plants from the Medicinal Herb Garden of the University of Seattle (Washington, USA) developed symptoms similar to those of powdery mildew. The causative agent is reported

*A. ougeotii*.  
 (Glawe,  
 2004).  
 2012 . Baden-  
 Wurttemberg, Germany -  
 gall midge (*Aceria kuko*),  
 (*Lycium barbarum* L. and *L.*  
*chinense*) (Schrameyr, 2012).  
 2013 . -  
 (Sakalieva and Dzhugalov,  
 2014).  
 2015 (Dzhugalov et al.,  
 2015) 2020 (Lichev et al.,  
 2020).

as (*Arthrocladiella mougeotii*). This was the first report of powdery mildew in this species in the Northwest Pacific (Glawe, 2004).

In 2012 in Germany in Baden-Wurttemberg, Germany was discovered the enemy gall midge (*Aceria kuko*), which causes various infections in goji berry (*Lycium barbarum* L. and *L. chinense*). (Schrameyr, 2012).

In Year 2014 is the first report about the appearance of powdery mildew in two varieties of goji berry grown in Bulgaria (Sakalieva and Dzhugalov, 2014).

This disease in goji berry plants is mentioned In Bulgaria in 2015 (Dzhugalov et al., 2015) and in 2020 (Lichev et al., 2020).

The tolerance to the diseases known in goji berry is essential for what varieties are more suitable for growing in industrial plantings. Organic production of goji berries is also important.

The aim of the study was to determine the varietal susceptibility to the disease *A. mougeotii* in the natural infectious background – under field conditions and find out how the disease affects the fruiting.

## MATERIAL AND METHODS

2018-2019 . -  
 : (*Lycium*  
*barbarum* L.) – JB 1, JB 2, JB 4, JB 10  
 3 m 2 m -  
 2014 -  
 70-90 cm. -

The study was conducted in the experimental base of the Department of Fruit Growing at the Agricultural University - Plovdiv) in the period 2018-2019. *In vitro* propagated plants of four varieties goji berry: JB 1, JB 2, JB 4, JB 10 of the species (*Lycium barbarum* L.) were planted at distances 3 m x 2 m in June 2014 and formed during the years as trees with stem height of 70-90 cm. They have four main fruit shoulders and are on a supporting construction.

Before the beginning of the vegetation the branches along the central axis are

5 cm, a  
10cm.

( , )

30 cm.

40 NPK

(14:10:12)  
300 g/

0-5%

1 –  
2 –  
– 6-25%  
3 –  
– 26-50%  
4 –  
50%

+

, kg/

thinned and all branches on the fruiting shoulders (upright, growing downwards and inwards towards the leader) are removed from the bottom. The brunches from the previous year are shortened to 5 cm and the fruiting shoulders elongated by 10 cm. During the vegetation all shoots were shortened to a length of 30 cm. The plants are grown under drip irrigation and in the hottest months July and August were watered with 40 liters per tree every two weeks. Fertilize is given twice a year 300 g/tree NPK (14:10:12), first after pruning and then before flowering.

There have been made reports to the damage on the leaves and skeletal branches of the goji berry plants. The samples were collected from different parts of the plants - leaf blades, leaf stalks, flowers, shoots and fruits. For the diagnosis a microscopic analysis was performed. The degree of the attack of the pathogen was determined using a common universal methodology as follows:

Score 1 – no damage 0-5%  
Score 2 – low degree of damage – 6-25%  
Score 3 – average degree of damage – 26-50%  
Score 4 – high degree of damage over 50%

During the growing season, the experimental plants were treated twice with TOPAZ against the causative agent of powdery mildew and once with SKOR + acarizine.

It is reported the yield of the fresh fruits, kg/tree.

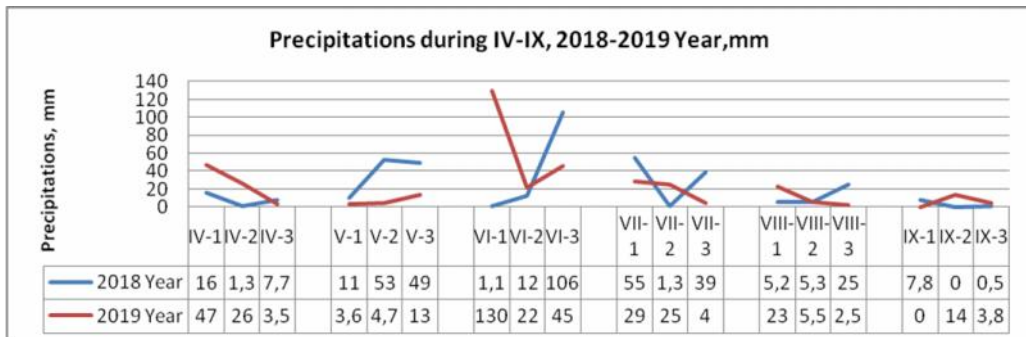
## RESULTS AND DISCUSSION

In Figure 1 is shown the data for the precipitation and in Figure 2 the values of the temperature during the vegetation 2018-2019. Regarding to the amount of precipitation in two years of the study, it can be seen, that there were more rainfall in 2018 in May and August.

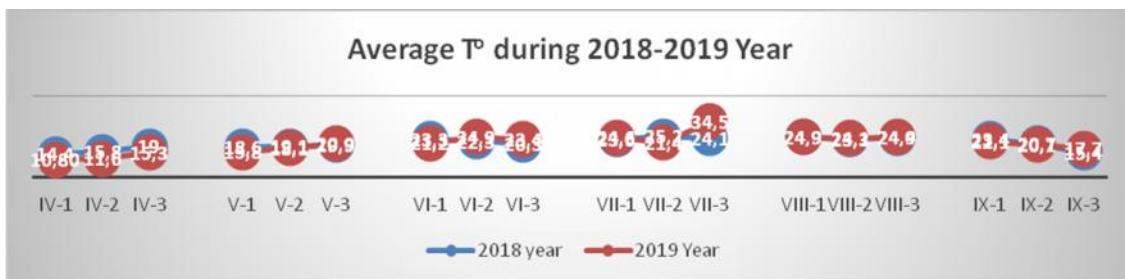
1 -  
2 -  
2018 2019  
2018

2018 . . . . .  
 2019 . . . . .  
 2018 . . . . .  
 2019 . . . . .

- In both years, the highest rainfall was in June, and it was unevenly distributed. In 2018, more rain fell at the end of the month, and in 2019 at the beginning. August also turned out to be a contrasting in terms of this meteorological indicator. In 2019 the precipitation in the first ten days were more, than those in the first year of this study, and in 2018 respectively more in the last ten days of the month.



1. IV-IX 2018-2019  
 Fig. 1. Precipitation during IV-IX in the period 2018-2019



2. 2018-2019 IV-IX  
 Fig. 2. Average T ° during 2018-2019 in the period IV-IX

2018 . . . . .  
 2019 . . . . .  
 2018 . . . . .  
 2019 . . . . .

Immediately before and after sampling, the weather conditions changed intensively. Rainy days were followed by sunny, which was favourable condition for the appearance and development of fungal diseases. In this experiment it was observed an attack of powdery mildew. The results for the spreading of this disease in 2018 are presented in Table 1 and in Table 2 is the information for the Year 2019. In the samples, which were taken for the recognition of symptomatic manifestations and determination of the

2019) - (2018  
 JB 2 ( 95%)  
 JB 1 – 30%. JB 4  
 13%,  
 ( 4%)  
 JB 10.  
 : JB 1 JB4,  
*Aceria*  
 u o.  
 JB 2.  
 %

disease powdery mildew, on the upper and lower side of the old leaves and young shoots were seen deposits of sporulation of the pathogen, which causes powdery mildew. The symptoms were found most commonly on young shoots and plant tops. The damage often penetrates deep in the flower. During the mass flowering, the damage reaches the flowering stems and often the flower dries.

In the established experiment under field conditions, it was observed different sensitivity to the pathogen of powdery mildew. The disease affects not only the leaves and shoots, but also the flower stalks. No damage was observed to the fruit. In both years (2018 and 2019) the highest degree of attack was reported in the variety JB 2 (up to 95%) and then in JB 1 - up to 30%. In JB 4 the damage reached 13%, and least affected (up to 4%) were plants of JB 10.

During the study on two of the varieties goji berry, respectively JB 1 and JB4 were found symptoms of damage from the mite *Aceria kuko*.

From the applied plant protection products, TOPAZ showed no influence against the pathogen of powdery mildew. The treatment with SKOR has an effect of the reduction and development of goji powdery mildew disease only in variety JB 2. In this variety the degree of damage (%) of the attack of powdery mildew decreases after treatment with SKOR almost twice, but with a not lasting effect. Over the time under favourable conditions for the development of powdery mildew, the symptoms appear intensively again. In the other varieties it was not found reduction in the spread of the disease.

1. % 2018  
**Table 1. *Arthrocladiella mougeotii*, % in studied cultivars during 2018**

/ Variety	23.05.	26.06	11.09
JB1	15%	30%	20%
JB2	50%	70%	50%
JB4	6%	4%	3%
JB10	4%	2%	0%



2. % 2019 .  
**Table 2. *Arthrocladiella mougeotii*, % in studied cultivars during 2019**

/ Variety	09.06.19	14.07	4.09
JB1	20%	21%	5%
JB2	40%	56%	20%
JB4	4%	13%	3%
JB10	1%	2%	1%

T kg/ 3. -  
 1. 6 ,  
 JB1 JB2 ,  
 , JB 10 JB 4  
 .  
 0,12 kg 1,18 kg.  
 - JB1 -  
 JB4.

- The results of the analysis of the data on yields kg/tree are presented in Table 3.  
 - The highest yield in each year and on average for the study period was obtained from variety JB 1. At the end of 6 vegetation, the yields in the varieties JB1 and JB2 are still increased, while in JB 10 and JB 4 they decreased. The average yield for the study period ranged from 0.12 kg to 1.18 kg. It was highest in JB1 and lowest in JB4.

3. JB1, JB2, JB 4, JB 10  
 2018-2019 , kg/  
**Table 3. Yield fresh fruits of the cultivars JB1, JB2, JB 4, JB 10 for the period 2018-2019, kg/tree**

/ Variety	/ Yield		
	2018	2019	2018-2019
JB 1	0.66	1.71	1.18
JB 2	0.62	0.71	0.66
JB 4	0.15	0.09	0.12
JB 10	0.18	0.12	0.15

The differences are significant  $P < 0.05\%$

. , ,  
 ,  
 ,  
 ,  
 ( 3).

- As already mentioned, powdery mildew does not affect the fruit. Indirectly, it has an effect by damaging the leaf mass, which after an attack has reduced photosynthetic functions and subsequently falls off. The goji berry plant has a recovery mechanism, which is expressed in the rapid formation of new uninfected leaves and shoots, which leads to normal photosynthetic activity (Figure 3).



3.  
**Fig. 3. Goji berry recovery after arthrocladiella mougeotii infection**

✓  
 (*Arthrocladiella mougeotii*),

✓

✓

✓

JB 2

### CONCLUSIONS

✓ Powdery mildew (*Arthrocladiella mougeotii*), causes damage to leaves, shoots and flowers.

✓ There was no damage to the fruits of the goji berry plant.

✓ It was observed a strong recovering mechanism, which was expressed in the formation of new uninfected leaves and shoots.

✓ The studied varieties have different susceptibility to powdery mildew. The highest degree of attack was reported in the variety JB 2 (up to 95%) and then in

( 95%) JB 1 –  
 30%. JB 4  
 13%, - ( 4%)  
 ✓ JB 10.  
 , JB  
 2, ✓  
 2018-2019 0,12  
 kg 1,18 kg – - JB1  
 - JB 4.  
 ✓ 6 ,  
 JB 1 JB 2  
 JB 4  
 JB 10 ,  
 ✓  
 (Aceria u o)  
 JB 1 JB 4.

JB 1 - up to 30%. In JB 4 the damage reached 13%, and least affected (up to 4%) were plants of JB 10.

✓ From the applied plant protection products, TOPAZ had no effect against the agent caused powdery mildew, and SCOR has an effect in the variety JB 2, but it was temporary.

✓ The average yield for the period 2018-2019 from wood is from 0.12 kg to 1.18 kg. It was highest in JB1 and lowest in JB 4.

✓ At the end of 6 vegetation, the yields in the varieties JB1 and JB2 are still increased, while in JB 10 and JB 4 they decreased.

✓  
 ✓ During the study on two of the varieties JB 1 and JB 4 were found symptoms of damage from mite (*Aceria kuko*)

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– (Venturia inaequalis)  
(Cydia pomonella)

1, 2, 2\*,  
, 3, ,

1 , 5600 ,  
2 , 4000 ,  
3 , 4004 ,

## Problems in the Control of the Main Pests of Apples – Scab (*Venturia inaequalis*) and Codling Moth (*Cydia pomonella*)

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Review paper

### SUMMARY

– The main pests of the apple – scab and codling moth annually cause problems in orchards and reduce the quantity and quality of fruit production.

- After detecting scab in 1819, many researchers studied it, including in our country. Determine biological and ecological characteristics, variety sensitivity, offer systems of control, examine the resistance of the pathogen to the fungicides used. In the world have been found 8 *Venturia inaequalis* races. In our country Penev has established with the help of varieties-differentiators the presence of 1, 2 and 5 races.

In practice, the codling moth is

1819 .,  
,  
,  
,  
,  
8 *Venturia*  
*inaequalis*.  
-  
1, 2 5.

maintained in low numbers by using a wide range of insecticides, mainly organophosphorus and synthetic pyrethroids. The disadvantages of this method are the destruction of beneficial entomofauna and pollinators, as well as the ability to quickly build resilience. Thus the control with conventional means is not always effective and does not recommend switching towards integrated pest management.

**Key word:** apple, *Venturia inaequalis*, *Cydia pomonella*, control

## INTRODUCTION

The use of a wide range of pesticides (insecticides and fungicides) in the practice of protecting apples from the main pests - scab and codling moth often have a negative impact on beneficial species, pollinators and are risky for human health.

In this regard, the purpose of the present study is to specify the derivation of plant protection measures against these pests, the choice and method of application of preparations to avoid the emergence of resistance.

Scab - *Venturia inaequalis* Cooke G. Winter is fungal disease of the greatest economic importance to all apple growers in different parts of the world. It was first described in Sweden by Fries in 1819, who defined it as *Spilocaea pomi* Fr.

In our country, the disease was first reported by Malkov in 1903 under the name "apple scab" with the causative agent *Fusicladium dendriticum* (Waarl.). In the United States scab was first registered in New York and Pennsylvania states in 1834, in England in 1845, in Australia in 1862, in South Africa in 1888, and in India in 1930 (Sutton et al., 2014; Penev, 1997).

*inaequalis* Cooke G. Winter - *Venturia*

Fries 1819 ., *Spilocaea pomi* Fr.

1903 . " *Fusicladium dendriticum* (Waarl.).

1834 ., 1845 ., 1862, 1888 ., 1930 . (Sutton et al., 2014; Penev, 1997).

<p>Bubov (1923), „  <i>Fusicladium dendriticum</i> <i>F. pitium</i>.“</p>	<p>- According to Bubov (1923), "the scab spots on the leaves of apple and pear trees are caused by <i>Fusicladium dendriticum</i> and <i>F. pitium</i>.", In our country</p>
<p>16-20 °C,  4  7 9 (Boykov et al,1951).</p>	<p>the most favorable temperature for infection by the fungus is between 16-20 °C, at which the infection lasts for 4 hours, and the incubation period is the shortest from 7 to 9 days (Boykov et al., 1951). For optimal conditions and the rate of infection, Sutton et al., (2014) noted that the infection lasts for 18-23 hours at 6 °C and for 6-9 hours at 16-24 °C, and rarely it can occur when the temperature is above 26 °C. It is also important that the relative atmospheric humidity is above 70%.</p>
<p>Sutton et al., (2014)  6 6-9 16-24  26  70%.</p>	<p>-</p>
<p>Hristov and Krastev (1964)  <i>V. inaequalis</i></p>	<p>- Hristov and Krastev (1964) note that the first infections with the fungus <i>V. inaequalis</i> occur in early spring when the buds sprout. This disease has two distinct phases of development - parasitic and saprophytic. The parasitic phase develops throughout the growing season and attacks all the green parts of the apple, while the saprophytic phase develops from leaf fall to the appearance of new leaves in spring. This cycle of development must be well known in order for the struggle to be successful. These two authors mention that physiological races have been identified in the cause of scab, so there are many cases when apple varieties that are resistant in some areas are strongly attacked in other areas. In addition, in some varieties the fruits are resistant, while the leaves are strongly attacked and vice versa in other varieties.</p>
<p>Hristov and Krastev (1964), Childers (1975)</p>	<p>- In order to protect the crop in the fight against scab, Hristov and Krastev (1964), Childers (1975) recommended that precautionary spraying be carried out to prevent contamination with ascospores. Spraying is carried out in phenophases and begins with two pre-flowering from the opening of the mixed buds to show the color of the flower, ie every 7-8 days.</p>
<p>7-8</p>	<p>- The next flowering and post-flowering</p>

<p>20</p> <p>10</p> <p>1:50 1:100</p> <p>1,6-2.0%</p> <p>1:400</p>	<p>- sprays have an interval of about 10 days at first, and later in 20 days and they continue as long as there are conditions for the development of the disease. At that time they used Bordeaux mixture, colloidal sulfur - 1: 400, liquid lime sulphur - 1.6-2.0% and diluted 1:50 to 1: 100 for flowering and post-flowering sprays. In rainy weather, copper oxychloride sold in powder form was particularly suitable.</p>
<p><i>pomonella</i> L.)</p> <p>(<i>Cydia</i></p> <p>Sutton et al., (2014)</p>	<p>The codling moth (<i>Cydia pomonella</i> L.) is a widespread and most harmful species in apple-growing countries. In some years, it multiplies en masse and is a limiting factor for obtaining quality products. The caterpillars damage the fruits of apple, pear, quince and walnut.</p> <p>They feed on seeds and cause fruit to fall off. According to Sutton et al., (2014), the codling moth originated in Asia Minor and was first found in North America in California and Washington states in 1880.</p>
<p>1880</p> <p>19</p> <p><i>Cydia pomonella</i></p> <p>2-3</p> <p>4-5 (Stanc - Moise, 2015).</p> <p>(Barnes et al., 1992),</p> <p>2 (Stanc -Moise, 2015),</p> <p>- 2 (Barnes et al., 1992),</p> <p>2 - 3 (Miletic et al., 2011),</p> <p>- 3 (Reuveny and Cohen, 2004),</p> <p>- 3 (Fernández et al., 2010).</p>	<p>It was introduced in the early 19th century by European colonists and spread rapidly throughout the United States.</p> <p>Depending on climatic conditions, <i>Cydia pomonella</i> develops a different number of generations. In Europe and North America, the codling moth develops 2-3 generations, and under favorable conditions 4-5 (Stanc -Moise, 2015). In Switzerland, the codling moth develops one complete and partial second generation (Barnes et al., 1992), in the region of Sibiu, Romania - 2 generations (Stanc - Moise, 2015), in Oregon, USA - 2 (Barnes et al., 1992), in Serbia 2 - 3 (Miletic et al., 2011), in Israel - 3 (Reuveny and Cohen, 2004), in Argentina - 3 (Fernández et al., 2010).</p> <p>The codling moth for the conditions of our country develops two generations a year and winters as a caterpillar in a dense cocoon under the old cracked bark, on the thick branches and less often in the</p>

		soil. First-generation butterflies fly from early April to late June - early July.
15-16		The development of this enemy took place in Southern Bulgaria a week earlier than in Northern Bulgaria. Butterflies are active in the evening, laying their eggs in the evening, a few hours after sunset at a temperature above 15-16 °C, mainly on the upper side of the leaves individually. In the Plovdiv region, egg-laying begins in mid-May or a few days later. One butterfly lays an average of 50-60 eggs.
	50-60	
	8-12	The egg stage lasts 8-12 days and the larval stage 30-35 days. One caterpillar damages several fruits by feeding mainly on seeds. Most of the caterpillars pupate, and another smaller part fall into diapause and pupate the following year.
	30-35	
		Second-generation butterflies usually fly in late June-early July to mid-September. They lay their eggs mainly on the fruit.
30-40		One butterfly lays an average of 30-40 eggs. The caterpillars hatch in the second half of July as one caterpillar damages two fruits. Damaged fruits fall off before or during harvest, and losses from caterpillars of this generation are greatest. The fully developed caterpillar remains in the wintering grounds.
( <i>C.pomonella</i> )		Successful control of the codling moth ( <i>C.pomonella</i> ) is essential for obtaining quality products. Its number is difficult to control by entomophagous inhabitants of apple orchards, which requires plant protection measures.
		There is a good set of insecticides to control the codling moth, but their widespread use in practice over the years has led to the development of resistance.



## RESULTS AND DISCUSSION

Soufflet-Freslon et al., (2008)

12-15

44

1970

Purdue, Rutgers Illinois – PRI,

(Ballard, 1998; Moore and Janick, 1983; Dzhuvinov et al., 2016).

1975

(Dimova et al., 2015). 2005 .

- In Europe's apple orchards, according to Soufflet-Freslon et al., (2008), many commercially grown varieties are susceptible to scab and 12-15 treatments are carried out during the season to protect the crop. The problem with this disease is complicated when the spring is rainy and the relative humidity is high, which is especially true for northern countries such as Belgium, the Netherlands, Germany, and for our country when the spring is rainy.

- Scab races have been studied and established by breeders and geneticists of the cooperative program who created the Coop (Co-or) series from 1 to 44 with scab-resistant varieties, the first of which was Prima registered in 1970. These are Purdue, Rutgers and Illinois Universities - PRI selection program, respectively from Indiana, New Jersey and Illinois (Ballard, 1998; Moore and Janick, 1983; Dzhuvinov et al., 2016).

- Repeated use of the same fungicide during the season or years creates a serious problem with the emergence of resistance to a pesticide. For example, in 1975 in the state of Michigan due to the widespread use of Benomilla against scab, it became ineffective for farmers there. This resistance appeared more quickly when used in combination with Kaptan.

- It has also been found that benomyl-resistant pathogens also become resistant to other benzimidazole fungicides such as Topsin M (methyl thiophanate) and Mertect (thiabendazole). Provided that the use of benzimidazoles is discontinued where resistance is established, it reappears even if not used for three years in a row (Dimova et al., 2015).

- In 2005 in France, resistance to scab fungicides has been found to be a growing problem for apple growers. It is

				- based on the use of strobilurins, anilinopyrimidines and inhibitors of sterol biosynthesis. It has been found that after mass introduction into practice of a fungicide with one active substance, in this case strobilurin, after two years of use, resistance of the pathogen to this fungicide is established.
				The same problem has been observed with its use against apple powdery mildew in different parts of Europe (Lesemann et al., 2006).
(Lesemann et al., 2006).				
	Shay and Williams (1956),	1		According to Shay and Williams (1956), race 1 is found in the United States and many other countries, and race 2 in South Dakota. Race 3 was found in the province of Nova Scotia, Canada, while race 4 was found at Pardew University in the Russian seed R12740-7 used in the PRI-Co-op selection program. Following studies by Williams and Brown (1968), 5 breeds were registered that infected varieties derived from <i>Malus micromalus</i> and <i>M. atrosanguinea</i> that carried the Vm resistance gene or the new Rvi5 gene classification ( table 1).
		2		
		3		
		4		
	R12740-7			
	PRI-Co-op.			
	Williams and			
Brown (1968)	5			
	<i>Malus micromalus</i>	<i>M.</i>		
<i>atrosanguinea</i> ,	Vm	-		
	Rvi5 (	1).	6	
	Parisi et al., (1993).			Race 6 was found in Germany, but was first described by Parisi et al., (1993). Race 7 was discovered in England and described by Roberts and Crute (1994) ( table 2). This isolate was found in naturally infected <i>M. floribunda</i> tree, which showed the same symptoms in <i>M. floribunda</i> 821, which are carriers of the Rvi6 resistance gene, but do not infect other varieties carrying the same resistance gene. The discovery of race 8 was the result of an extensive study of the population of <i>V. inaequalis</i> in New Zealand (Bus et al., 2005) ( table 3).
7				
Roberts and Crute (1994) (		2).		
T				
	<i>M. floribunda</i> ,			
	<i>. floribunda</i> 821,			
		Rvi6,		
8				
<i>inaequalis</i>		V.		
2005) (		(Bus et al.,		
3).				

1.

8

*Venturia inaequalis**Malus***Table 1. Relationship between the different 8 *Venturia inaequalis* races and reference hosts of the genus *Malus***

Host	Genotype	Gene effect	/ Race							
			1	2	3	4	5	6	7	8
h 1	Gala		+	+	+	+	+	+	+	+
h2	Dolgo	V? <sup>y</sup>	-	+	-	-	-	-	-	-
	TSR34T15	Vh2	-	+	-	-	-	-	-	-
h3	Geneva	V?	-	+ <sup>x</sup>	+	-	-	-	-	-
h4	TSR33T239	Vh4	-	-	-	+	-	-	-	-
h5	9-AR2T196	Vm	-	-	-	-	+	-	-	-
	OR45T132	Vm	-	-	-	-	+	-	-	-
h6	Florina	Vf+Vg	-	-	-	-	-	+	-	-
	Prima	Vf+Vg	-	-	-	-	-	+	-	-
h7a		Vg	+	+	+	+	+	+	-	+
	Golden Delicious									
h7b	<i>M. x floribunda</i> 821	Vf+Vfh	-	-	-	-	-	-	+	-
h8	<i>M. sieversii</i> W193B	Vh8	-	-	-	-	-	-	-	+

y

/ not named resistance gene

2.

*Venturia inaequalis*

( Parisi et al. (1993, 1994) Roberts and Crute (1994)

**Table 2. Relationship between *Venturia inaequalis* races and host (according to Parisi et al. (1993, 1994) and Roberts and Crute (1994)**

/ Race/Pathotype	/ Host					
	821	Florina	Liberty	Priam	Prima	Priscilla
1-5	R <sup>a</sup>	R	R	R	R	R
6	R	S	S	S	S	S
<i>M. floribunda</i> 821	S	R	S	R	R	S

<sup>a</sup>R - / resistant, S -

/ susceptible

3.

*(Venturia inaequalis)**Vg, Vf Vfh.***Table 3. Races of apple scab (*Venturia inaequalis*) controlled by the resistance genes *Vg*, *Vf* and *Vfh*.**

Cultivars	Resistance	1	6	7	6+7
		Race 1	Race 6	Race 7	Race 6+7
Gala		+	+	+	+
	<i>Vg</i>	+	+	-	+
Golden Delicious					
Priscilla	<i>Vf</i>	-	+	+	+
Prima	<i>Vf+Vg</i>	-	+	-	+
<i>M. floribunda</i> 821	<i>Vf+Vfh</i>	-	-	+	+

+ /susceptibility

- / no symptoms;

Penev (1997)

-

From his research Penev (1997) has established with the help of differentiators of the races of the collapse the presence of 1, 2 and 5 races in our

1, 2 5

<p>1992-1995 .</p> <p>-</p> <p>4</p> <p>.</p> <p>1994 .</p> <p>Fleckinger (1948).</p> <p>E -</p> <p>1 %</p> <p>,</p> <p>,</p> <p>,</p> <p>.</p> <p>1 , 6</p> <p>,</p> <p>Charmilot and Pasquier, (2003)</p> <p>,</p> <p>,</p> <p>,</p> <p>,</p> <p>,</p> <p>1920 .</p> <p><i>C.pomonella</i></p> <p>(Hough, 1929), 1950 .</p> <p>(Glass and Fiori, 1955).</p> <p>50-</p> <p>-</p> <p>- ,</p> <p>-</p> <p><i>C. pomonella</i>,</p> <p>30 (Croft and Riedl, 1991;</p>	<p>country. He also studied in 1992-1995 the attack of leaf scab reported in July and on fruit - in early September in the varieties Prima and Liberty - resistant to scab and in Cooper 4 and Belgolden - susceptible to the pathogen. The attack on leaves and fruits varied from year to year, with the strongest in 1994.</p> <p>Fungicide treatments against scab and powdery mildew were carried out on the same varieties during the same period according to the phenological phases of development according to Fleckinger (1948). The spraying started from phenophase E - green tips of the buds with 1% Bordeaux mixture and ended before the harvest with Anvil, Ditan, Silit, Kaptan and Perocin in the individual years and the sensitive varieties were also used Punch. In susceptible varieties, it has also been found that 1 to 6 leaves are counted vulnerable to the disease from the top down, after which the leaves acquire ontogenetic resistance.</p> <p>Charmilot and Pasquier, (2003) found that in Western Europe, the effectiveness of conventional plant protection is declining due to the developed resistance of pests to the insecticides used. Resistance to phenoxycarb, tebufenozide, methoxyfenuside and diflubenzuron, as well as deltamethrin, fazalone and azinphos-methyl has been established in Switzerland, Armenia and Bulgaria.</p> <p>In 1920, resistance of <i>C. pomonella</i> to lead arsenate was first reported (Hough, 1929), and in 1950 to DDT (Glass and Fiori, 1955).</p> <p>In the mid of 1950s, the use of organophosphate insecticides, in particular azinphos-methyl, which became the most commonly used means of controlling <i>C. pomonella</i>, resulting in resistance after about 30 years (Croft and Riedl, 1991; Welter et al., 1991).</p>
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Welter et al., 1991).

(Varela et al., 1993; Reuveny and Cohen, 2004; Fuentes-Contreras et al., 2007; Reyes et al., 2007).

15

*C. pomonella*

(Souphanor and Bouvier, 1995; Souphanor et al., 1998; Dunley and Waltre, 2000; Souphanor et al., 2000; Knight et al., 2001; Reuveny and Cohen, 2004; Lori tti et al., 2007; Stara and Kokourek, 2007; Rayes et al., 2007; Mota-Sanchez et al., 2008).

1993-1995 .

(Note National Spv-INRA 2003).

*C. pomonella*

(Souphanor et al, 2006; Rayes et al., 2007).

(Knight, 2010).

Charmillot et al., (2007)

Resistance to azinphos-methyl is now widely documented in various regions of the world apple growers, which is why the use of this organophosphorus insecticide has been discontinued (Varela et al., 1993; Reuveny and Cohen, 2004; Fuentes-Contreras et al., 2007; Rayes et al., 2007).

In the last 15 years, there have been reports of resistance to *C. pomonella* to other classes of insecticides such as synthetic pyrethroids and to the granola virus (Souphanor and Bouvier, 1995; Souphanor et al., 1998; Dunley and Waltre, 2000; Souphanor et al. ., 2000; Knight et al., 2001; Reuveny and Cohen, 2004; Lori etti et al., 2007; Stara and Kokourek, 2007; Rayes et al., 2007; Mota-Sanchez et al., 2008).

In 1993-1995, problems arose in France in the control of codling moth in the main regions producing these fruits, due to the established cross-resistance of this dangerous enemy to the synthetic pyrethroids used (Note National Spv-INRA 2003).

In orchards in southern Europe, *C.pomonella* has also developed resistance to synthetic pyrethroids (Souphanor et al, 2006; Rayes et al., 2007). Cross-resistance to insecticides was discovered in some cases before they were ever used by manufacturers (Knight, 2010).

In laboratory tests, Charmillot et al., (2007) found that in recent years, the codling moth has developed resistance to classical insecticides as a result of their repeated use.

In the US state of Washington, alternative programs have been developed to replace organophosphates with both older and several new insecticides, which have become available to manufacturers. Synthetic pyrethroids are not widely used in this

(Knight, 2010).  
 2005 .  
 90  
 (Knight, 2010).  
*C. pomonella*  
 2005 . 2006 .,  
 39% 53%.  
 90-  
 (Knight, 2010).  
 (Knight, 2010).  
*pomonella*.

region due to the multiplication of mites in integrated fruit production (Knight, 2010).

Since 2005, a slight but gradual increase in the use of lambda-cyhalothrin has been reported. Cross-resistance to synthetic pyrethroids has been reported in both California and Michigan, where these insecticides were more widely used as a substitute for organophosphates in the early 1990s (Knight, 2010).

The resistance of *C. pomonella* to organophosphates was widespread, which is why in 2005 and 2006, studies were carried out in north-eastern Spain in apple orchards treated with Azinphos-methyl and Carbaryl. The result was unsatisfactory due to the low mortality caused by the insecticides used, below 39% and 53%, respectively.

Insect growth regulators, such as chitin synthesis inhibitor novaluron, pyriproxyfen, methoxyfenozide, are commonly included in apple fruitworm control systems mainly against eggs. Unfortunately, cross-resistance among these groups of insecticides is found throughout Europe, where they were first used in integrated control systems developed in the 1990s (Knight, 2010).

Populations of the apple fruit worm with resistance to one or more insecticides in each group of insect growth regulators have also been found in the western United States (Knight, 2010).

Neonicotinoid insecticides, such as thiacloprid and acetamiprid, are relatively effective in controlling *C. pomonella*. Their use is limited due to the creation of conditions for the multiplication of mites in integrated control systems.

Cross-resistance to thiacloprid has been

<p><i>C. pomonella</i></p> <p>(Knight, 2010).</p> <p>2008</p> <p><i>C. omonella</i> (Van Steenwyk and Dunley, 2008).</p> <p>Sansavini, (1997)</p> <p>(John et al., 2001).</p>	<p>found in populations of <i>C. pomonella</i> in Europe but not acetamiprid in orchards in Michigan state. A resistance model similar to neonicotine insecticides for spinosad has been established in both regions. Spinosad can be used in organic production (Knight, 2010).</p> <p>The new insecticide Spinetoram was registered in the USA in 2008 and has been reported to have excellent activity against <i>C. pomonella</i> (Van Steenwyk and Dunley, 2008).</p> <p>According to Sansavini, (1997) Integrated fruit production is widespread in Europe. Compared to conventional, it has higher efficiency and provides higher yields (John et al., 2001).</p>
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## CONCLUSIONS

To reduce the problems with the main pests of apples - scab and codling moth, should not be treated with the same active substances. Do not use pesticides against the two main pests that have shown resistance to them.

Integrated fruit production should take a priority place in Bulgarian agriculture, which will reduce the cost of disease and pest control, and fruit production will be free of pesticide residues.

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**(*Phytophthora cactorum*)  
 (*Corylus avellana* L.)**

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***Phytophthora* Root and Collar Rot (*Phytophthora cactorum*) on Hazelnuts (*Corylus avellana* L.) in Bulgaria**

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Short communication

**SUMMARY**

2016 . -  
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 V-8.  
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 :  
 34.8 27.8 μm, l=1.2.  
 -  
 : 27.11 29.64 μm,  
 - 12-21 μm.

During 2016 a phytosanitary monitoring of hazelnut orchards was performed in the region of Tryavna. Single plants or groups of 2-3 hazelnut trees had low vigor, slow growing and produced no fruits. The buds of the diseased plants arouse later in spring and formed small chlorotic leaves. These symptoms were followed by the sudden wilting of upper leaves and shoots, or the death of the whole plants.

The mycelia colonies of the causal agents on PDA and V-8 are white, with the radial growth. The sporangia are with a prevailing lemon shape, with small papillae. They are caducous, with a short pedicel. Their size is 34.8 27.8 μm, l=1.2. The oogonia are oval-shaped, with smooth walls and sized: 27.11 29.64 μm. The antheridia are paragynous – 12-21 μm. The oospores are oval-shaped, with thick

, 27.03 – 32.06 µm.  
31°C, 25-26°C.

*Phytophthora cactorum*.

ITS 4 ITS 6.  
PCR  
850-900 bp,

*Phytophthora*.

: (*Corylus*  
*avellana* L.),  
*Phytophthora cactorum*

19  
200  
600  
-  
(Mitov et al., 1996).  
21

200  
10 000  
ha  
(*Corylus avellana* L),

:  
*Phytophthora* sp. ( ),  
*Armillaria mellea* ( ),  
*Cylindrocarpon* sp. ( ),  
*Macrophomina phaseolina* ( )  
*Sclerotinia minor* ( )  
*Phytophthora*, *Phytophthora*  
*cinnamomi*,

(Guerrero et al., 2014).

walls, sized: 27.03 – 32.06 µm. The mycelia growth of the isolates was registered between 2 and 31°C, with optimum temperatures of 25-26°C. The morphological and cultural characteristics of the isolates are identical to those described for the specie *Phytophthora cactorum*.

The molecular analysis using the PCR amplification protocol with ITS4 and ITS6 primers pair gives a band with a size of 850-900 bp, for all the isolates confirming that they belong to genus *Phytophthora*.

**Key words:** hazelnut (*Corylus avellana* L.), root rot, *Phytophthora cactorum*

## INTRODUCTION

Bulgaria is a country with traditions in fruit growing but the hazelnut is not wide spread. At the end of 20<sup>th</sup> century the hazelnuts areas were 200 ha on agricultural lands and 600 ha in the forests, mainly in the regions of Burgas and Haskovo (Mitov et al., 1996). At the beginning of the 21<sup>st</sup> century the structure of the fruit crops was changed and the hazelnut area was increased. Industrial hazelnut plantations with the size of 200 or more hectares were planted with a Bulgarian or an imported planting material.

In Chile, in a region with around 10 000 ha hazelnut orchards planted with the European hazelnut (*Corylus avellana* L), the following pathogens were detected as causal agents of the root rot: *Phytophthora* sp. (root rot), *Armillaria mellea* (white root rot), *Cylindrocarpon* sp. (root rot), *Macrophomina phaseolina* (carbonaceous rot of roots) and *Sclerotinia minor* (root rot in nursery). From the genus *Phytophthora*, the pathogen *Phytophthora cinnamomi*, was identified as the causing root and neck rot on the hazelnut plants (Guerrero et al., 2014).

*Phytophthora citricola* (Khosrowfar et al., 2005).  
*(Corylus cornuta var. californica)* P.  
*ramorum* (DiLeo et al., 2008).  
*Phytophthora* sp.  
 (Pscheidt and Ocamb, 2019).

In Iran the pathogen *Phytophthora citricola* was isolated and identified from dying hazelnut roots (Khosrowfar et al., 2005).

In California (USA) on the California hazelnut (*Corylus cornuta var. californica*) the quarantine specie *P. ramorum* was isolated and identified (DiLeo et al., 2008). In Oregon state *Phytophthora* sp. was isolated and identified on the fine roots of young dying hazelnut plants. *Phytophthora* is considered to be a rare problem on that crop. In general, it is found on different crops on soils having poor drainage (Pscheidt and Ocamb, 2019).

## MATERIAL AND METHODS

In Bulgaria in the region of Tryavna (in the village of Chernovrah; the Balkan range (Stara planina)) a survey about the phytosanitary status of a hazelnut orchard, sized 300 ha, was carried out. Some hazelnut plants in small groups showed low vigor and slow growing. These symptoms were followed by dying of the plants. Samples were randomly taken from the crown and the roots of the diseased hazelnuts.

Isolations were done on PDA and PARP selective media for isolation of *Phytophthora* pathogens. Also "baiting bioassay" was performed using young apple fruits (Erwin and Ribeiro, 1996).

The pathogenicity of the isolates was tested through inoculation of hazelnut shoots with well-developed leaves and small apple fruits, "walnuts size".

Eight shoots were inoculated with mycelia plugs of each isolate (covered with wet cotton and parafilm). In control shoots an un-inoculated (sterile) agar plugs were used instead. The shoots were kept in a growth chamber at 25°C and 85% relative humidity, 12 hours light/12 hours' darkness. With each isolate 3 apple fruits were also inoculated.

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 (PARP).  
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 (Erwin  
 and Ribeiro, 1996).  
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 25°C 85%  
 12/12  
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2 : V-8, 4  
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24

V-8,

0-1 36°C

Excel 2010 SPSS 21.  
, 10-12-

EZNA  
Fungal DNA Mini Kit (Omega Bio-tek).  
PCR ITS  
(internal transcribed spacer),  
(TCCTCCGCTTATTGATATGC) ITS4  
(GAAGGTGAAGTCGTAACAAGG). ITS6  
PCR  
- 2 µl; - 10  
(Fermentas dream taq buffer + KCl +  
MgCl - 5 µl); dNTP - 4 µl;  
ITS1 - 2.5 µl; ITS4 -

The morphological and cultural characteristics of the pathogenic isolates were studied on 2 media: PDA and V-8, 4 replicas, at 25°C, in dark. The type of the colonies was described and the mycelia growth was measured on a daily base. The mycelia hyphae, the formation of the sporangia, the oogonia, the antheridia, and the oospores were observed under the microscope.

In order to see the type of the sporangia and measure their size, a sporangia formation was induced on mycelia plugs in rain water, under laboratory conditions, in Petri plates. The microscope observations were carried out after 24 hours.

On PDA and V-8 observations about the formation of the oogonia, the antheridia and the oospores were done under the microscope daily. Their size was measured.

The influence of the temperatures, in the range from 0-1 to 36°C, on the mycelia growth was studied in thermostats in dark. The minimum, the maximum and the optimal temperatures were registered.

The statistical analyses were performed on the data received from the size of the sporangia, the oogonia, the antheridia, and the oospores, applying Excel 2010 and SPSS 21 programs.

From the mycelia colonies on PDA, 10-12 days old, a fungal DNA was extracted. The mycelium was frozen in liquid nitrogen and the DNA was extracted using EZNA Fungal DNA Mini Kit (Omega Bio-tek). In the PCR reaction the ITS (internal transcribed spacer) region was amplified using ITS4 and ITS6 primers pair. Primer sequences are, as follows: ITS4 - TCCTCCGCTTATTGATATGC; ITS6 - GAAGGTGAAGTCGTAACAAGG. In the PCR reaction the following quantities were used based on the protocol: fungal DNA – 2 µl; buffer - 10 (Fermentas dream taq buffer + KCl + MgCl -5 µl); dNTP - 4 µl; forward primer ITS4 - 2.5 µl; reverse primer ITS6 - 2.5 µl;

2.5 µl; Taq polymerase – 0.4 µl;  
 - 33.6 µl. PCR  
 : 94<sup>0</sup> - 3'; 35 x  
 (94<sup>0</sup> - 45"; 57<sup>0</sup> - 30"; 72<sup>0</sup> - 1').  
 PCR

Taq polymerase – 0.4 µl; sterile water -  
 33.6 µl. The conditions for the PCR  
 reaction are: 94<sup>0</sup> -3'; 35 x (94<sup>0</sup> -45";  
 57<sup>0</sup> -30"; 72<sup>0</sup> -1'). After the PCR reaction  
 the products were added to Agarose gel.  
 The gel was visualized with ethidium  
 bromide.

## RESULTS

During April - May 2016, when the  
 phytosanitary monitoring of the hazelnut  
 orchards in the region of Tryavna was  
 carried out, the diseased plants were  
 clearly visible against the background of  
 the healthy ones. They had low vigor and  
 slow growing in spring. The buds of the  
 infected plants arouse later and formed  
 small chlorotic leaves, slightly curled up.  
 Some buds on such plants did not  
 develop at all. During the summer months  
 of July and August when temperatures  
 are high the symptoms intensify. The  
 leaves on the diseased hazelnut plants  
 are chlorotic to pale yellow with a reddish  
 discoloration. They curl upside, wilt and  
 drop off. On some leaves necrotic spots  
 are formed, with the size of 8-10 mm.  
 Later in the season a sudden wilting was  
 found on the twigs and leaves, and after  
 that some branches or whole trees dye  
 back.

The symptoms are visible on the  
 roots and the crowns of the plants where  
 wet dark grey spots are formed. A reddish  
 brown discoloration due to the infection is  
 observed under the bark on the cambium  
 tissues. The infected tissues clearly  
 differentiate from the healthy ones (Figure  
 1).

( 1).



. 1.

**Fig 1. Symptoms at the roots and stem base (crown) of hazelnut**

PARP  
10<sup>-</sup>  
( 2).

Isolations were done from the diseased plants with the symptoms on PDA and PARP media.

Pathogenicity tests were performed. The symptoms on the hazelnut shoots in the growth chamber were seen on the 10<sup>th</sup> day after the inoculation. On the hazelnut leaves close to the infection point systemic chlorosis on the veins were expressed first (Figure 2). Chlorotic and later necrotic irregular scattered spots were formed on the leaves after that. These symptoms first appear on the leaves close to the inoculation point and later on the upper leaves.



. 2.

**Fig 2. Symptoms on hazelnut branches and leaves after artificial inoculation**



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V-8, 25°C. 7  
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V-8  
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34.8 x 27.6  
µm; l = 1.2.  
3,47 µm.  
27.11-29.64 µm,  
28.37 µm.  
12-21 µm.  
27.03-32.06 µm, 29.54 µm,  
( 1).  
25°C 6  
Tr 3 2  
31°C.  
25 - 26°C.

The inoculated apples show symptoms – brown, dry rot around the infection plug, on the 7<sup>th</sup> day after the infection. The causal agent identical to initial isolate was re-isolated from inoculated shoots and apple fruits.

Morphological and cultural characteristics of the pathogen were described on PDA and V-8, at 25°C. On the 7<sup>th</sup> day the colonies on PDA have a radial growth and whitish, fluffy, not very high mycelia, with colorless base. They are broadly petaloid, with a slightly curved periphery. The mycelia hyphae are hyaline, non-septate, with protuberances. On V-8 the colonies show a radial growth and a whitish base. They are also broadly petaloid, with a slightly curved periphery. The mycelia hyphae are curly-like.

The sporangia vary in shape: the lemon-shaped ones with small papillae are the prevailing shapes but the obpyriform or wide ovate sporangia are also found. They are caduceus, with a short pedicel. The size of the sporangia is 34.8 x 27.76 µm; l=1.2. The papillae have the size of 3.47 µm. The sporangiophores are branched sympodially.

The isolates are homothallic. The oogonia are oval-shaped, with smooth walls and the size of 27.11-29.64 µm, average 28.37 µm. The antheridia are paragynous, 12-21 µm. The oospores are oval-shaped, with thick smooth walls, sized 27.03-32.06 µm, av. 29.54 µm, plerotic (Table 1). They are formed in the media after 6 days, at 25°C.

The growth of the mycelia of the isolate Tr 3 was registered between 2 and 31°C. The optimum temperatures for the mycelia growth are 25 - 26°C.

1.

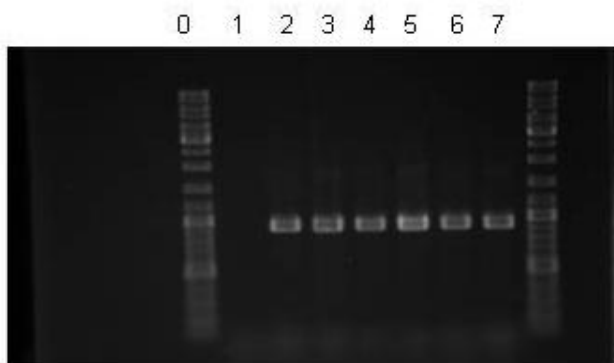
Tr 3,  $\mu\text{m}$

**Table 1. The size of oogonia and oospores, isolate Tr 3, in  $\mu\text{m}$**

Media PDA								
Isolate	Oogonia				Oospores			
Tr 3		d	length	width		d	length	width
	Average	28.37	27.11	29.64	Average	28.54	32.06	27.03
	Cv%	2.09	2.7	3.46	Cv%	18.52	2.86	9.16
	S2	0.35	0.54	1.05	S2	38.73	0.84	6.13
	S	0.59	0.73	1.03	S	6.22	0.92	2.48
		30.03	28.00	32.6	max	38.00	34.00	42.00
	min	27.5	26.00	29.00	min	26.00	31.00	22.00

Phytophthora cactorum,  
Oomycetes.  
Phytophthora cactorum,  
( 3).  
e , 1 -  
, 2 7  
Phytophthora cactorum.

The data received about the morphological and cultural characteristics of the mycelia colonies, the mycelia, the sporangia, the antheridia and the oogonia, and the oospores are identical with the characteristics of the Oomycete specie *Phytophthora cactorum*.  
From the isolates showing morphological characteristics typical for *Phytophthora cactorum*, a high quality DNA was extracted and amplified using the PCR method (Figure 3). On the gel the line 0 is DNA ladder, 1 – negative control, from 2 to 7 are the isolates morphologically identified as *Phytophthora cactorum*.



. 3.

**Phytophthora cactorum**

**Fig. 3. DNA bands of the isolates morphologically identified as *Phytophthora cactorum***

0 – DNA ladder; 1 - negative control; 2 - Tr 1; 3 - Tr 2; 4 - Tr 3; 5 - Tr 4; 6 - Tr 5; 7 - Tr 6

PCR  
ITS4 ITS6,  
850-900 bp,  
*Phytophthora*.

1.  
2.  
(  
)  
*Phytophthora*  
*cactorum*.

3.  
PCR  
850-900  
bp,  
*Phytophthora*  
4.  
*cactorum*,

The molecular analysis using the PCR amplification protocol with ITS4 and ITS6 primers pair gives a band with the size of 850-900 bp, for all the tested isolates. That band size is characteristic for the species from the genus *Phytophthora*.

## CONCLUSIONS

Based on experimental data received the following conclusions can be made:

1. Monitoring of hazelnut orchards have been performed with the aim to determine their phytosanitary status.

2. *Phytophthora* pathogens have been isolated from hazelnut plants showing root and crown rot symptoms. Morphological and cultural characteristics of the isolates (mycelia colony type, mycelia hyphae, sporangia, antheridia and oogonia, oospores), and the cardinal temperatures for mycelia growth, compared with data published in the literature, let us conclude that the causal agent of the disease on hazelnuts in the region of Tryavna is *Phytophthora cactorum*.

3. The molecular analysis based on PCR amplification gives a fragment with a size 850-900 bp for all the isolates tested – a confirmation that they belong to genera *Phytophthora* from *Oomycetes*.

4. On the hazelnut plants in the region of Tryavna the specie *Phytophthora cactorum* was found. To our knowledge this is the first report of *Phytophthora cactorum* on hazelnuts in Bulgaria.

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## N, P, K

\*

5600

”281,

# Impact of Soil Maintenance Systems on the Export of N, P, K with Surface Runoff and Lysimetric Waters in Young Plum Orchard

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Original scientific paper

### SUMMARY

The study was carried out in RIMSA, Troyan in the period 2013-2015, in a plum plantation with Katinka cultivar, under non-irrigated conditions, on pseudo-podzolic light gray forest soils, characterized by an acid reaction and poorly stored with nutrients.

The aim of the present study is to determine the effect of different soil surface maintenance variants (fallow, natural grassland, artificial grassland) on the concentration of nitrogen, phosphorus ( $P_2O_5$ ) and potassium ( $K_2O$ ) in the surface runoff and lysimetric waters.

As a result of the conducted researches it was found that:

The studied nutrients in the surface water runoff and lysimetric waters are within the maximum allowable concentration and do not pose a risk of environmental pollution.

(27,0 mg/l)

(26,0 mg/l),  
(0,05 mg/l).

The highest degree of migration was reported for nitrogen in nitrate form (27.0 mg/l) and potassium (26.0 mg/l), and the lowest one was found in phosphorus (0.05 mg/l).

The maintenance of the soil surface in natural and artificial grassland reduces the exported amounts of nitrogen (ammonium and nitrate).

**Key words:** nutrients, surface runoff, lysimetric waters, soil maintenance, plum

## INTRODUCTION

Clean water is extremely important as a source for drinking, irrigation, industry, transport, recreation, fishing. Imports of pollutants have increased in recent decades, resulting in degradation of water quality (Carpenter et al., 1998).

1998).

(Carpenter et al.,

The same authors found that increased runoff of P and N to surface water, measured after application of fertilizer or tillage to agricultural land, caused losses of P and N fertilizers (on run-off) usually less than 5% of the applied amount.

P N ( )  
5%

( 20%,

).

Manure losses can be slightly higher (up to 20% if precipitation falls immediately after application). However, these percentages do not estimate the total flow of N to aquatic ecosystems, as they do not include infiltration and leaching, which ultimately transfer N to groundwater and surface water.

N

N

The amount of nitrates reaching surface waters in plantations varies greatly, depending on the type of crops, drainage practices, cultivation technology, soils, climate, geology and other factors.

Most of the rainwater in the plantations is absorbed into the ground, and some of the nitrates are deposited, as a result of which less nitrates reach the surface waters. The options for controlling this type of nitrate pollution below the soil

surface are few.  
 Nitrogen, phosphorus and potassium are the main nutrients that, together with the organic matter in the soil, determine soil fertility. Under the influence of water erosion, they are the main object of losses, depending on the terrain, the amount of precipitation, fertilizer application, soil fertility and others. They are exported with both solid and liquid runoff (Pimentel and Kounang, 1998).  
 (Pimentel and Kounang, 1998).  
 N, P, K, Ca  
 Mg  
 - Mg  
 Ca Mg  
 N, P, K,  
 P -  
 (Bertol et al., 2003; 2005)  
 For the most part, plum orchards in the Troyan region are located on light gray, low-yielding soils, low in essential nutrients, on sloping terrain and are at constant risk of water erosion.  
 Erosion is a major factor in soil degradation and surface water pollution with nutrients readily available to cultivated plants in the forms of nitrogen, phosphorus and potassium.  
 Numerous experiments have been carried out in Bulgaria and abroad in order to establish the export of nutrients from imported mineral and organic fertilizers with surface runoff and lysimetric waters. According to a number of scientists (Mihailova, 2000; Oenema et al., 2005; Lockhart et al., 2013), the pollution of surface and lysimetric waters with nitrogen and phosphorus is a consequence of the intensification of agriculture. This is associated with a risk

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The concentrations of N, P, K, Ca and Mg in the water, due to erosion, are strongly influenced by the adopted soil maintenance system and increase with increasing fertilization and tillage intensity.

The highest concentrations of N, P, K, Ca and Mg in water, from water erosion, occur in conventional tillage, and in the case of P - in uncultivated land in natural fields (Bertol et al., 2003; 2005).

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(Aarts, 2003; Christou et al., 2005; Chica-Olmo et al., 2014).

Totev (1998)

Mihaylova and

(K<sub>2</sub>O)

(P<sub>2</sub>O<sub>5</sub>)

2013-2015

2010

4-5°

30 kg

400 g

1.

2.

3.

of high soil load and high concentrations of residual nutrients that are a potential risk of groundwater contamination with nitrates (Aarts, 2003; Christou et al., 2005; Chica-Olmo et al., 2014).

RIMSA Troyan has been working for years on a complex approach to the care of plum trees, with anti-erosion effectiveness. The studies are aimed at combining rational soil surface maintenance systems with measures aimed at environmentally friendly use of soil resources.

Studies of Mihaylova and Totev (1998) show an anti-erosion effect and runoff-reducing activity of some types of forage grasses, which could be used for grassing between rows in orchards.

The aim of the present study is to determine the effect of different soil surface maintenance treatments (fallow, natural grassland, artificial grassland) on the concentration of nitrogen, phosphorus (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) in the surface runoff and lysimetric waters.

## MATERIAL AND METHODS

The experiment was conducted in 2013-2015 in RIMSA Troyan in a plum plantation of cv. Katinka, established in 2010, with northwestern exposure of the slope, at 4-5° slope. The trees are planted in planting pits loaded with 30 kg of manure and 400 g of superphosphate. The study was carried without the introduction of additional fertilizers.

The soil surface is maintained in three variants:

1. Fallow - the interrows are maintained as a fallow by disking;

2. Natural grassland - the interrows are covered by turfgrass of natural perennial grasses;

3. Artificial grassland - interrows are covered by turfgrass of grass mixture from



(1:1), :  
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 ,  
 ,  
 ,  
 ,  
 ( )  
 ( ),  
 ,  
 ,  
 (mg/l)  
 - NANOCOLOR  
 - MACHEREY-NAGEL GmbH &  
 Co.KG

ANOVA Excel,

1 ,  
 2013 .

legume and grasses in ratio (1:1) with bird's-foot-trefoil and red fescue at a seeding rate of 5 kg/da;

- In order to monitor the concentration of nutrients in the surface runoff and lysimetric waters, three facilities were built, one for each variant, representing a trench. The trench is built perpendicular to the slope, covers three rows and three rows.

- The effluent is collected in polyethylene containers located in a pit in the lowest part (lysimetric water) and the highest (surface runoff), from where the samples are taken.

- The concentration of nitrogen, phosphorus, potassium in surface runoff and lysimetric waters (mg/l) was determined spectrophotometrically with NANOCOLOR from - MACHEREY-NAGEL GmbH & Co.KG.

- By mathematical data processing with the ANOVA program in Excel, correlation and regression dependences between some parameters of nutrients considered as environmental pollutants were calculated.

## RESULTS AND DISCUSSION

- Water erosion causes serious damage to orchards in mountainous regions. Which in turn leads to erosion washability and contamination of surface water with essential nutrients. The different systems of soil surface maintenance are of decisive importance.

- Table 1 shows that when betting on the experience in 2013, there are no clear differences in the concentration of nitrogen depending on the soil maintenance systems.

1.

(mg/l) (2013-2015)

**Table 1. Average annual concentration of nitrogen in surface runoff and lysimetric waters (mg/l) (2013-2015)**

Treatment	NH <sub>4</sub> N		NO <sub>3</sub> N		NO <sub>2</sub> N	
	/Surface runoff	/Lysimetric waters	/Surface runoff	/Lysimetric waters	/Surface runoff	/Lysimetric waters
2013						
1. Clean cultivation	0,27	0,50	1,6	27,0	1,000	0,200
2. E Natural grassland	0,90	0,19	0,9	25,0	1,100	0,085
3. Artificial grassland	0,60	0,12	2,9	15,0	1,400	0,100
2014						
1. Clean cultivation	1,98	0,49	8,4	6,6	0,025	0,048
2. E Natural grassland	0,14	0,27	0,3	0,6	0,011	0,016
3. Artificial grassland	0,07	0,49	2,7	4,0	0,014	0,015
2015						
1. Clean cultivation	0,51	0,50	11,7	7,1	0,032	0,055
2. E Natural grassland	0,32	0,35	1,6	1,4	0,170	0,240
3. Artificial grassland	0,30	0,05	1,2	1,5	0,060	0,100

( )  
 - 2014 2015 . -  
 ,  
 ( 9 16  
 2001) ( )  
 e 50 mg/l. (NO<sub>3</sub>N)  
 -  
 -  
 -  
 -  
 ,  
 ( )  
 ),  
 2,9 mg/l ( )  
 27,0 mg/l ( )  
 2013 1,2 mg/l 4,0 mg/l 2015 .  
 ( 1).  
 -  
 4,49  
 mg/l,  
 ,  
 Mihailova Totev (1998).  
 (NO<sub>4</sub>N)

Nitrogen (nitrate form) has a much lower concentration in the grassy variants in 2014 and 2015 and with higher exports while maintaining the soil surface in set-aside, both in surface runoff and in lysimetric waters.

According to (Ordinance 9 of 16 March 2001), the maximum permissible concentration of nitrates (NO<sub>3</sub>N) in drinking water is 50 mg/l. This is the most dangerous pollutant in the natural environment. The concentration of nitrates in most natural sources is less than 10 mg/l.

For the three years of the study, in dynamics, together with the development of grass species in the third variant (bird's-foot-trefoil and red fescue), the amount of nitrate nitrogen decreased progressively from 2.9 mg/l (surface runoff) and 27.0 mg/l (lysimetric waters) for 2013 to 1.2 mg/l and 4.0 mg/l in 2015 (Table 1). Similar results for the lowest concentration at bird's-foot-trefoil 4.49 mg/l, relative to other grass species, in nitrate exports were summarized by Mihailova and Totev (1998).

The same trend is observed for ammonium nitrogen (NO<sub>4</sub>N). There is high

(1,98 mg/l -  
 (0,50 mg/l),  
 (1).

2014  
 ); 2015 .

(NO<sub>2</sub>N) 2015

(NO<sub>3</sub>N),  
 (NO<sub>4</sub>N)

(NO<sub>2</sub>N)

2015 ..

0,3 mg/l  
 (1),

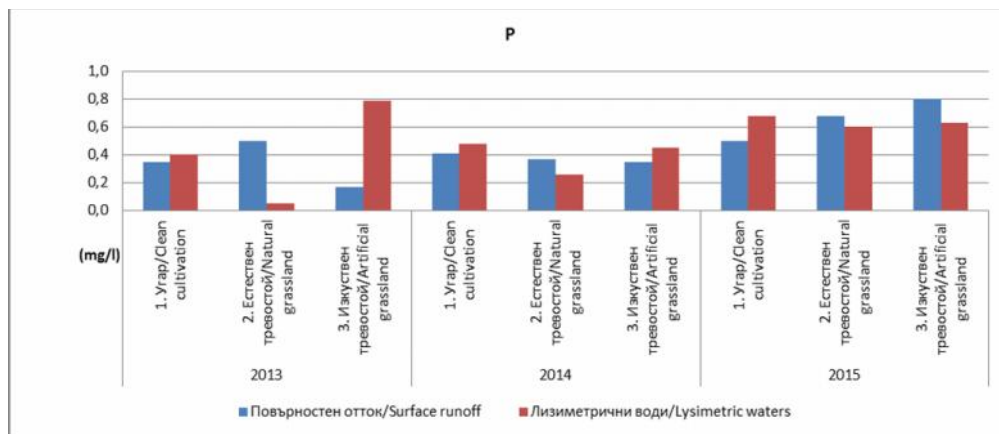
concentration and export when maintain-  
 ing the soil in fallow in 2014 (1.98 mg/l -  
 surface runoff); 2015 (0.50 mg/l), compared  
 to the grassed treatments (Table 1).

Nitrite nitrogen (NO<sub>2</sub>N) for 2015  
 also has a lower concentration in grassy  
 treatments.

The high concentrations of nitrogen  
 in the nitrate form (NO<sub>3</sub>N), together with  
 the other forms of ammonium (NO<sub>4</sub>N) and  
 nitrite (NO<sub>2</sub>N) are below the threshold of  
 the maximum permissible concentration  
 for drinking water and do not pose a risk  
 of water and environmental pollution.

In our study, the concentration of  
 phosphorus is not affected by the treatments  
 soil surface maintenance systems. It is  
 present in small amounts in both surface  
 runoff and lysimetric waters. This is due to  
 the low solubility of soil phosphates and the  
 fixation of its compounds in the soil. In all  
 three treatments, no quantities dangerous  
 for environmental pollution have been  
 reported.

A high concentration was reported  
 in 2015, where the maximum permissible  
 concentration for drinking water was  
 exceeded by 0.3 mg/l in the surface runoff  
 (Figure 1), which is not a risk value.

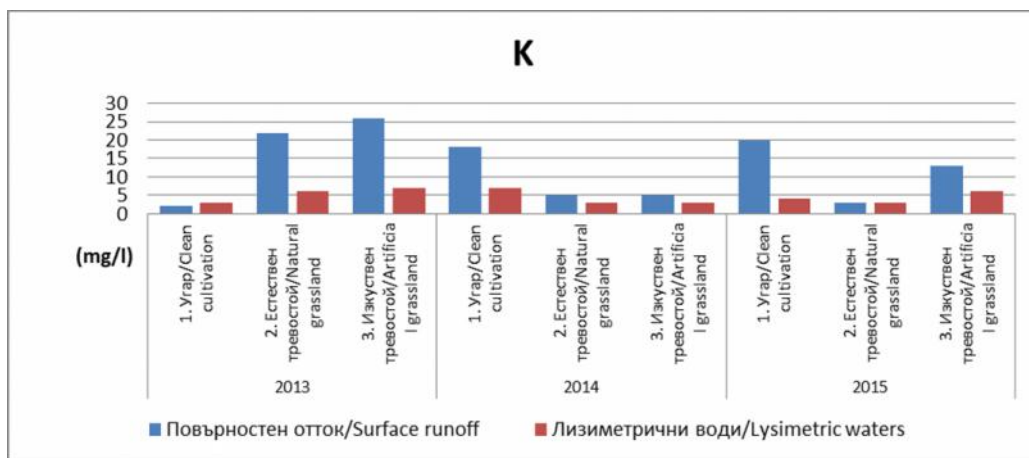


1.  
 (mg/l) (2013-2015)  
**Fig. 1. Average annual concentration of phosphorus in surface runoff and lysimetric waters (mg/l) (2013-2015)**

2013 (26 mg/l -  
; 22 mg/l -  
2014-2015 (

Potassium is exported in larger quantities by surface runoff than in lysimetric waters. When setting the trial in 2013, its concentration is the highest (26 mg/l - artificial grassland; 22 mg/l - natural grassland). In the following years 2014-2015 there was a sharp decline (Figure 2). High concentration is maintained only when keeping the soil surface set aside.

Under the influence of the anti-erosion vegetation in the natural and artificial grassland, the export of potassium with the surface runoff is several times smaller.



2.

(mg/l) (2013-2015)

Fig. 2. Average annual concentration of potassium in surface runoff and lysimetric waters (mg/l) (2013-2015)

(r= 0,8399)  
(R<sup>2</sup>=0.7011)

(NO<sub>3</sub>N)  
(NO<sub>2</sub>N)

3).

$$y=12.532x+3.2451$$

NO<sub>2</sub>

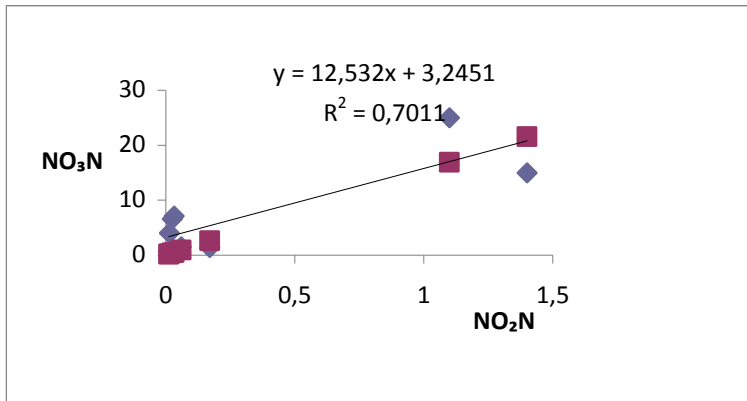
There is a high correlation (r=0.8399) and regression (R<sup>2</sup>=0.7011) between the content of nitrates (NO<sub>3</sub>N) in the lysimetric waters and nitrites (NO<sub>2</sub>N) in the surface runoff, in the three treatments of soil surface maintenance (Figure 3). This function  $y=12.532x+3.2451$  makes it possible to regulate the content of nitrates in the lysimetric waters by acting on NO<sub>2</sub>.

The amount of exported K with the lysimetric waters and the surface runoff is

$r=0,8157$   
 $R^2=0,6655,$   
 ( 4).

$y=3.9781x-5.3934.$

- correlated with  $r = 0.8157$  and a  
 - coefficient of determination  $R^2 = 0.6655,$   
 - in the three treatments (Figure 4). If the  
 - content of K in the surface runoff is  
 - affected, its content in the lysimetric  
 - waters is controlled by the function  $y =$   
 $3.9781x-5.3934.$

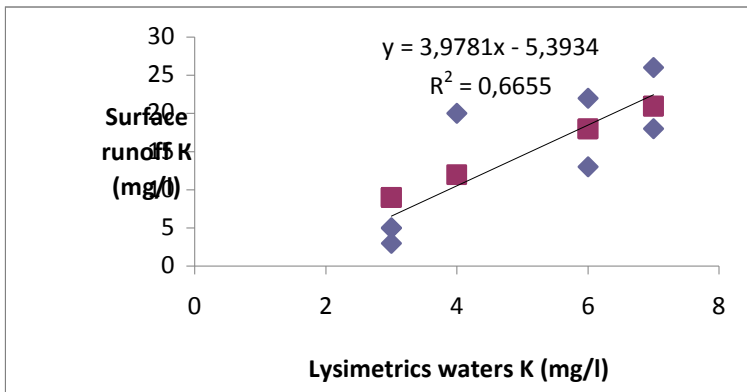


. 3.

(NO<sub>3</sub>N)

(NO<sub>2</sub>N)

**Fig. 3. Graphical model of the regression relationship between the content of nitrates (NO<sub>3</sub>N) in the lysimetric waters and nitrites (NO<sub>2</sub>N) in the surface runoff**



. 4.

**Fig. 4. Graphical model of the regression dependence between the content of K in the surface runoff and its content in the lysimetric waters**

## CONCLUSIONS

Maintaining the soil surface in natural and artificial grassland reduces the export of total nitrogen and favours the protection of the environment from pollution.

The chemical elements (nitrogen, phosphorus and potassium) exported with surface runoff and lysimetric waters are in small quantities and do not pose a threat to environmental pollution.

Nitrate and potassium have the highest migration rate and phosphorus the lowest. A low concentration of nitrates has been established, below the threshold of the admissible concentration for environmental pollution under Ordinance 9 of 16 March 2001 on water quality.

The high degree of correlation and regression dependences between the content of nitrites and nitrates, as well as the same dependences in Potassium, allow us to influence and regulate concentrations, indicators of the mathematical model, through certain forms of fertilization, to control pollution surface runoff and lysimetric waters.

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