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APPLE CULTIVARS SUITABLE FOR ORGANIC PRODUCTION

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Erwinia amylovora (Burrill) –

10

(Borkh),

Erwinia amylovora (Burrill)

: *Malus domestica*

SUMMARY

The paper presents the first results of the study carried out on the susceptibility of 10 apple cultivars, established in Dresden-Pillnitz, Germany, to fire blight caused by the bacterium *Erwinia amylovora* (Burrill), after artificial inoculation of the apple flowers. The infection was performed under controlled conditions following standard methods of testing the susceptibility to two strains of the bacterium, isolated from different host plants in our country.

The best results were obtained in 'Realka', 'Relinda' and 'Reka' cultivars, which are carriers of the gene for scab resistance, responded with the lowest level of susceptibility to the bacterial disease fire blight at the phenological stage of full bloom.

Key words: *Malus domestica* (Borkh), cultivars, susceptibility, *Erwinia amylovora* (Burrill)

INTRODUCTION

Apple is a major fruit crop worldwide, including in our country. World production of apple fruits is

- characterized by steady growth rates. Many chemical means are used for protecting the apple fruit yield from diseases and pests and those chemicals have a negative effect on human health and on the environment.

- One of the most severe diseases in apple with an economic importance is fire blight caused by the bacterium *Erwinia amylovora*. It is a comparatively new disease in Bulgaria, first discovered 25 years ago (Bobev, 1989).

25 (Bobev, 1989).
2003-2007 .

- In the period 2003-2007 the disease spread quickly, inflicting considerable damage in many pear, quince and apple plantations (Bobev & Deckers, 1998). Depending on the susceptibility of a certain cultivar, the injuries could be observed only in the flowers and the annual shoots, or, the whole plant could be destroyed.

Deckers ,1998). (Bobev &

- The selection of the cultivar is of great importance for reducing the plant protection practices to minimum (Dragoyski et al., 2003).

., 2003).

12 .

- About 12 treatments in average are applied in our country against scab and powdery mildew annually. The number of treatments varies significantly depending on the meteorological conditions. Only sanitary pruning and prevention spraying is applied against fire blight, however when the infection has already developed, there are no registered

(A imovi et al., 2015). chemical products (A imovi et al., 2015).

1947 . Among the major aims set in the breeding programmes along with improved fruit quality, is the resistance to that disease. The genetic resistance to fire blight (*Erwinia amylovora*) was set as a goal in the breeding programme in Germany as early as 1947. In that case, the wild forms *Malus robusta*, *M. Prunifolia* and *M. Floribunda* were used as donors of resistance. The resistant cultivars 'Remo', 'Rewena', 'Reanda', 'Rene' and 'Realka' were the results of that programme (Fischer & Richter, 1998).

(Fischer & Richter, 1998). The requirements of ecological fruit production necessitate an accelerated assessment of the breeding material about its response to economically most important diseases and pests. That could be obtained by the method of artificial infection of the cultivars that are most widely spread in practice – new perspective apple cultivars or introduced cultivars.

The same infectious background and the optimal conditions for bacteria emergence and development were maintained with the aim of selecting the cultivars tolerant to the bacterium.

MATERIAL AND METHODS

2015 . Studies were carried out in the period April-May 2015 at the Laboratory of Phytopathology, the

Plant Protection Department of the Fruit-Growing Institute in Plovdiv. The plant material used comprised 10 apple cultivars of the so-called Re-Cultivar selection of the Institute for Breeding Research on Horticultural Crops in Dresden-Pillnitz, Germany – ‘Reka’, ‘Relinda’, ‘Reanda’, ‘Rene’, ‘Regine’, ‘Renora’, ‘Reglindis’, ‘Realka’, ‘Rebella’ and ‘Rewena’, which are genetically scab resistant.

15-20 cm long shoots from each cultivar were cut at the phenological stage of full bloom and a minimum of 10 flowers were sprayed with a suspension containing isolates of *Erwinia amylovora* bacterium. The isolates used for infecting, were:

➤ Isolate 3325 – isolated from apple on 16 May 2013 in Petrich;

➤ Isolate 3345 – isolated from pear on 27 June 2013 in Botevgrad.

The bacterium was cultivated for 48 hours at a temperature of 26° on King's B nutrient medium (King et al., 1954). After that the bacteria were scraped off the agar surface and the suspension was adjusted to a density of 3 × 10⁸ cells/ml. The infection was performed by spraying the cut shoots with a bacterial suspension at the full bloom stage. Untreated flowers kept under the same conditions were used as a control.

The infected plants were placed at

25°	80-90%	a temperature of 25° and a relative humidity of 80-90%. The first report was done after 7 days.
7		
4		The results obtained were classified by the grading scale of disease severity by Pusey (1999):
Pusey (1999):		
0 –	-	Class 0 – no visual symptoms of the infection on the inoculated flowers;
;		
1 –	-	Class 1 – necrosis observed on sepals or flower bottoms;
;		
2 –		Class 2 – necrosis observed on the whole fruit set;
;		
3 –		Class 3 – necrosis reaching to the middle of the flower pedicel;
;		
4 –		Class 4 – necrosis covering the whole fruit set and the flower pedicel.

RESULTS AND DISCUSSION

The difference between the aggressiveness of the pathogen isolated from different hosts, as well as the response of the separate cultivars at the most critical phenological stage of apple vegetation could be traced in the present study. All the studied cultivars responded by localizing the infection to the flower pedicel for both strains and only 'Renora' cultivar reacted by the typical systemic expression of the disease, the necrosis reaching to the annual shoot.

In 'Reka' cultivar the lowest infection rates were reported concerning the infection of flowers: 52% class 0, 44,4% class 1 and

– 52%

0

, 44,4%

1 3,7% 4
3325,
94,4% 4
(1 2).

3,7% class 4 with strain 3325
and with the second strain – 94,4%
class 4 (Table 1 and 2).

1.

3325

Table 1. Level of susceptibility of the cultivars inoculated with strain 3325 isolated from apple

Cultivar	Number of flowers	3325 / Strain 3325									
		0 %	1 %	2 %	3 %	4 %	0 %	1 %	2 %	3 %	4 %
Reanda	20				20	25					55
Reka	27	51,9	44,4	3,7							
Relinda	16			25	50						25
Rene	15					26,7					73,3
Regine	20					30					70
Renora	14					28,6					71,4
Reglindis	16										100
Realka	24	4,2	29,2	4,2	20,8						41,6
Rebella	22					18,2					81,8
Rewena	13			23,1	61,5						15,4

-
3325,
3345

The highest infection rates by both strains were reported in 'Renora' cultivar. Similar results were established in 'Reglindis' cultivar by strain 3325, while its susceptibility to strain 3345 was lower. The cultivars 'Rebella' and 'Relinda' showed moderate susceptibility to both strains.

The differences in the aggressiveness of the studied strains were demonstrated by the

- differences in the results obtained for the separate cultivars and probably those differences were also due to the different hosts, from which they were isolated.

2.

3345

Table 2. Level of susceptibility of the cultivars inoculated with strain 3345 isolated from pear

Cultivar	Number of flowers	3345 / Strain 3345							
		0 Class 0%	1 Class 1%	2 Class 2%	3 Class 3%	4 Class 4%	%	%	%
Reanda	17					47,1		52,9	
Reka	18					5,6		94,4	
Relinda	21			4,8		4,8		90,4	
Rene	31					58,1		41,9	
Regine	13					30,8		69,2	
Renora	20							100	
Reglindis	14		7,1	14,3		64,3		14,3	
Realka	21							100	
Rebella	13			23		38,5		38,5	
Rewena	15							100	

(Fischer and Geibel, 2003),

- Comparing the obtained results to previous studies of other authors (Fischer and Geibel, 2003), it could be mentioned that the cultivars 'Reanda', 'Rene', 'Regine', 'Realka', 'Rebella' and 'Rewena' were reported to be resistant, while in the present study they were allocated to the group of slightly susceptible to the disease (Table 3). The results

were the same for the cultivars 'Reglindis' and 'Renora'. In the present study the cultivars 'Reka', 'Relinda' and 'Realka' reacted as slightly resistant, while in literature they were mentioned as slightly susceptible.

3 .
(Fischer and Geibel, 2003).

Table 3. Level of susceptibility to scab, powdery mildew and fire blight (Fischer and Geibel, 2003)

Cultivar	Scab	Powdery mildew	Fire blight	Fire blight*
Reanda	R	LR	R	LS
Reka	R	LR	LS	LR
Relinda	R	LR	LS	LR
Rene	LR	S	R	LS
Regine	LR	LR	R	LS
Renora	R	LR	LS	LS
Reglindis	R	LR	LR	LR
Realka	R	S	R	LR
Rebella	LR	R	R	LS
Rewena	R	R	R	LS

R- , LR- , LS- , S-

R – resistant, LR – slightly resistant, LS – slightly susceptible, S - susceptible

*Summarized data of the present study are presented in that

3,

In Table 3, along with the results about the studied disease, additional summarized data are presented obtained by the same authors about the level of susceptibility to the other economically most important diseases in apple – scab and powdery mildew.

CONCLUSIONS

- Based on the obtained results and the carried out investigations, the following conclusions and recommendations could be made:
- The cultivars ‘Realka’, ‘Relinda’ and ‘Reka’, which are carriers of the gene for scab resistance, responded with the lowest level of susceptibility to the bacterial disease fire blight at the phenological stage of full bloom.
 - The typical systemic character of the disease was observed only in ‘Renora’ cultivar, while all the other cultivars responded by localizing the infection to the flower pedicel.
 - The studied cultivars ‘Reka’, ‘Realka’ and ‘Relinda’ are suitable for establishing ecological apple plantations, in which the control of the major diseases scab, powdery mildew and fire blight is carried out by a reduced number of pesticide treatments.

/ REFERENCES

1. **A imovi S. G., Q. Zeng, G. C. McGhee, G. W. Sundin, J. C. Wise.** 2015. Supplementary Material Control of fire blight (*Erwinia amylovora*) on apple trees with trunk-injected plant resistance inducers and antibiotics and assessment of induction of pathogenesis-related protein genes (in print).
2. **Bobev S.** 1989. Bacteriosis in quince. Scientific Works of the Higher Institute of Agriculture, 35: 99-101.
3. **Bobev S. & T. Deckers.** 1998. October. Field susceptibility to fire blight of pome fruits in Bulgaria. In VIII International Workshop on Fire Blight 489: 221-224.
4. **Dragoyski K., B. Mihovska, H. Dinkova.** 2003. Development of systems, compatible with the European standards, for maintaining and production of virus-free planting material at the Research Institute of Mountain Stockbreeding and Agriculture-Troyan. Journal of Mountain Agriculture on the Balkans, vol.6, 576-584.

5. **Fischer C., & K. Richter.** 1998. October. Results on fire blight resistance in the Pillnitz apple breeding programme. In VIII International Workshop on Fire Blight, 489: 279-286.
6. **Fischer M. and Geibel.** 2003. The future of disease-resistant apples. Genetics and Breeding of tree fruits and nuts, 622: 329-334.
7. **King E. O., M. K. Ward and D. E. Raney.** 1954. Two simple media for the demonstrating of phycocyanin and fluorescein. J. Lab. Clin. Med., 44, 301-307.
8. **Pusey P. L.** 1999. Effect of nectar on microbial antagonists evaluated for use in control of fire blight of pome fruits. Phytopathology, 89(1): 39-46.

thinning out of clusters. The obtained wines had different chemical composition, as the variant with summer pruning (shoot topping in July) had the lowest average rates of sugar free extract, titratable acids, total phenolic compounds, anthocyanins and color intensity. The wines from the experimental variants were not superior compared to the control concerning these indicators. The samples differed in their tasting characteristics as the control variant revealed the best organoleptic qualities.

Key words: vine, Storgozia variety, summer pruning, grapes, wine

INTRODUCTION

Summer pruning treatments are agronomic practices applied in vineyards as a means of improving the grapes quality and respectively the wines. The impact of shoot topping has been studied by many authors.

Slavcheva et al. (2006) investigated the impact of shoot topping on vines of Cabernet Sauvignon variety. A detailed overview of the impact of this treatment was made by Peykov and Belberova (2012). Shoot topping in vineyards once or twice during the summer improves the exposure of the clusters to light, decreases the yield and slows the rate of ripening.

The final effect on the sugars, acids and pH of grapes were minimal, but significant differences were found in wine quality. Wines from shoot topped vineyards were

rated as better compared to the control, due to the weaker “grassy tint” in the taste, more intense colour but slighter flavor (Pszczolkowski et al., 1985). More attention in the literature was paid to the impact of the retained number of clusters (Dhillon and Singh, 1949; Popov et al., 1972). According to the authors the retaining the number of clusters had improved the properties of the raw material without reducing (sensitively) the yield. In a study of Naslada variety, Slavcheva and Purchev (2012) found that by increasing the number of clusters changes occurred in two directions: positive – for the yield, the yield/growth ratio and economic efficiency (yield/yield + growth); negative - on the amount of roots, sugar content and sugar/acid ratio.

The last phase of maturation was determinative of the raw material composition. Upon thinning out the clusters it was obtained maximum level of sugar accumulation, staying longer without finding more rapid decrease in acids (Amati et al., 1994).

The impact of clusters thinning out on the composition of grapes must and wine was confirmed by studies of Amati et al. (1995). It has been observed that the density and the amount of the sugars had increased as a function

of the intensity of thinning out; therefore wine alcohol content was increased in the same way.

The extract was higher in the thinned out samples in comparison with the control, but there was no difference in the function from the intensity of thinning. The content of titratable acids in wines however decreased as a function of the intensity of thinning.

The objective of this study was to investigate the impact of summer pruning treatments – shoot topping and retaining the number of clusters on the composition and characteristics of grapes and the obtained wines.

MATERIAL AND METHODS

The study was carried out in 2009-2011. The object of the study were vines of Storgozia variety, interspecies hybrid, resistant to mildew (*Plasmoparaviticola*), with increased resistance to gray mold (*Botrytis cinerea*) and powdery mildew (*Uncinulanecator*) (Ivanov et al., 1984; Zankov et al., 1985).

The trial plantation was grown in the Experimental Base of IVE-Pleven. The planting density was 2.50 x 1.30 m, the vines were grown on semi-high training system with stem height 1 m. The rootstock was Berlandieri x Ripariya Selection Oppenheim 4 (SO4), pruning was done at spurs. The loading in 2009 was 20 eyes,

2009-2011 .
(*Plasmoparaviticola*),
(*Botrytis cinerea*)
(*Uncinulanecator*) (.,
1984; ., 1985).
-
-
2,50 1,30 m,
1 m.
4
(CO4),
2009 .

20 , 2010 2011 .
 18 . 2009
 .
 50 , 2010 2011 .
 40 .
 :
 V₀ – , –
 ;
 V₁ – () –
 26.06. (2009 .) 28.06. (2010
 2011 .);
 V₂ – (–
)
 1/3 (30 – 35%)
 26.06.
 (2009 .) 28.06. (2010 2011 .);
 V₃ – () –
 20.07. (2009 .), 21.07. (2010 .)
 19.07. (2011 .).

2009 .
 27
 2010 . – 25 ,
 2011 . – 39 .
 () p = 5,0 %,
 p = 1,0 % , p = 0,1 % (, 1999).

and in 2010 and 2011 - 18 eyes per vine. In 2009, 50 vines were left per variant while in 2010 and 2011 they were 40 vines. After counting the flower clusters vines were divided into four variants with approximately the same average number of flower clusters as follows:

V₀ – control – without summer pruning treatments;

V₁ – shoot topping (June) – on 26.06. (2009) and on 28.06. (2010 and 2011);

V₂ – retaining (thinning out) the clusters – 1/3 (30 – 35%) of the clusters were removed totally for the variant on 26.06. (2009) and on 28.06. (2010 and 2011);

V₃ – shoot topping (July) – on 20.07. (2009 .), 21.07. (2010) and 19.07. (2011).

The number of clusters per vine in the experimental variants was defined depending on the average number of clusters per vine obtained after counting in the spring of the respective year of the study.

In retaining the number of clusters in 2009 there were left on the average 27 clusters per vine; in 2010 – 25 clusters, and in 2011 – 39 clusters per vine.

The analysis of variance was made by Fisher at confidentiality level of the differences (Student's criteria) p = 5.0 %, p = 1.0 %, p = 0.1 % (Dimova and Marinkov, 1999).

Upon reaching technological maturity, the grapes from the experimental variants was harvested and processed in the experimental winery of IVE under the conditions of micro-vinification by the classical method for red wine making (Yankov, 1982). In 2009 vintage for obtaining wines with normal chemical composition, the sugar content of grape pulp was adjusted by adding sucrose. The alcoholic fermentation was conducted with pure culture of dry wine yeast *Saccharomyces cerevisiae*, in an amount of 20 g/hl, at a temperature of 25-26°C. After completion of the process the samples were decanted and further sulfated.

The chemical composition of grapes and the wines from each variant was determined by the conventional methods in enology (Ivanov et al., 1979). For evaluating the organoleptic characteristics it was used 100-score tasting scale (Tsvetanov, 2001; Prodanova, 2008).

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RESULTS AND DISCUSSION

The quantitative changes in the yield from the experimental vines of Storgozia variety during the study period are presented in Table 1.

2009 (3,501 kg),

- V₀ (5,019 kg)

V₂

V₃

The lowest yield per vine for 2009 was obtained from variant V₂ (3.501 kg), due to the reduced number of clusters, followed by the control - V₀ (5.019 kg) and V₃

(5,202 kg).
 V₁ (5,299 kg),
 -
 V₂
 (, 1999).
 - V₂ (28),
 V₁ (36) V₀ (38).
 - V₃ (39).
 . V₂ -
 .
 - V₂ (125,48
 g), - V₁
 (147,19g).
 2010
 -
 V₃ (7,487 g),
 -
 (182,61 g). (V₀)
 V₁ 6,349 6,399
 kg. -
 V₂ (4,500 kg),
 . -
 V₂ ,
 (,
 1999).
 - V₁
 (168,39 g), a -
 V₃ (182,61 g).

(5.202 kg). The highest yield had V₁ (5.299 kg), as a result of the higher average mass per cluster for this variant. The difference between the variants was statistically proven. Variant V₂ was distinguished for its well outlined difference compared to all other variants (Dimova and Marinkov, 1999).

The average number of clusters was the lowest in V₂ (28), followed by variants V₁ (36) and V₀ (38). The highest average number of clusters had variant V₃ (39). The differences for this indicator were statistically proven. Variant V₂ had proven differences with all other variants in the trial.

The average mass per cluster was the lowest in V₂ (125.48 g), and the highest in V₁ (147.19 g).

In 2010 from the four variants the highest yield was obtained for variant V₃ (7.487 g), mainly due to the higher mass per cluster (182.61 g). From the control (V₀) and variant V₁ the yield was almost equal, respectively 6.349 and 6.399 kg. The lowest yield had variant V₂ (4.500 kg), because of the reduced number of clusters. The analysis of variance revealed that variant V₂ had proven differences compared to all other variants (Dimova and Marinkov, 1999).

The average mass per cluster was the lowest in variant V₁ (168.39 g), and the highest in variant V₃ (182.61 g). There were

			no proven differences for this indicator.
	2011	-	For vintage 2011 the highest
V ₁ ,		-	yield was obtained from variant V ₁ ,
-		.	mainly due to the higher mass per
-		V ₂ ,	cluster. The lowest yield had
		-	variant V ₂ , which was a result of
		-	the reduced number of clusters.
		-	After the statistical processing of
			the data for this indicator there
			were no proven differences
			between the variants in the trial.
	V ₂ (41), a	V ₃	The lowest average number
-		-	of clusters had variant V ₂ (41), and
(57).		-	the highest number - V ₃ (57). The
		-	difference between the variants for
			this indicator was proven. The
	(V ₂)		variants with thinning out of the
			clusters (V ₂) had proven
			differences compared to the
			control and the other variants in
			the trial.
		V ₂	The highest average mass
(171,64 g),		V ₀	per cluster had variant V ₂ (171.64
	V ₁ (161,79 g)	V ₀	g), due to the removal of part of the
(149,12 g),	-	-	clusters. It was followed by
	V ₃ (145,23 g).	-	variants V ₁ (161.79 g) and V ₀
		V ₂	(149.12 g), while the lowest mass
		-	per cluster had V ₃ (145.23 g).
		-	Concerning this indicator there
			were proven differences between
			V ₂ and the control as well as
			between the variant with retained
	(V ₂)	V ₃ .	number of clusters (V ₂) and variant
			V ₃ .
		(V ₂) -	The lowest average yield
5, 021 kg.		V ₃ -	during the three years had the
			variant with retained number of
			clusters (V ₂) - 5,021 kg. The
6,986 kg.			highest average yield from the
			three years had V ₃ - 6,986 kg. The

- V₂ (32),
 - V₃ (46).
 - V₁ (159,12 g),
 V₂ (156,73 g)
 151,30 g. (V₀) –

average number of clusters per vine for the three years was the lowest in variant V₂ (32) and the highest in V₃ (46). The mass per cluster on the average for the three years was the highest in variant V₁ (159.12 g), followed by V₂ (156.73 g) and the lowest mass per cluster had the control (V₀) – 151.30 g.

1.

2009-2011

Table 1. Qualitative changes of the main indicators, characterizing the yield of Storgozia variety, during the period 2009-2011

		/ Indicators		
Variants	Year	Yield per vine (kg)	Average number of clusters per vine	Mass per cluster (g)
V ₀	2009	5,019	38	133,20
	2010	6,349	37	171,59
	2011	8,176	55	149,12
	Average	6,515	43,33	151,30
V ₁	2009	5,299	36	147,19
	2010	6,399	38	168,39
	2011	8,717	54	161,79
	Average	6,805	42,66	159,12
V ₂	2009	3,501	28	125,48
	2010	4,500	26	173,08
	2011	7,063	41	171,64
	Average	5,021	31,66	156,73
V ₃	2009	5,202	39	132,70
	2010	7,487	41	182,61
	2011	8,268	57	145,23
	Average	6,986	45,66	153,51

It should be noted that the impact of the applied treatments was highly dependent on the climatic factors. Weather conditions during the year had

()

(2009, 2011)

(2010)

2.

2011

2009

20,9 %

V₂

V₃ (

modified the impact of the studied factor. The effect of shoot topping was different for the separate years. In dry years the variant of shoot topping in June (2009, 2011) had better result for the yield quantity. In terms of yield in years with more rainfall, the shoot topping in July gave better result (2010).

That could be explained by the prolonged vegetation season and delayed vine development phases.

The chemical composition of Storgozia variety must for the period of the study is presented in Table 2. The data revealed that the year 2011 was the most favorable for the production of red wines with optimal chemical composition and organoleptic properties.

That vintage grapes for all variants, was characterized by the highest sugar accumulation and optimal titratable acids. The lowest sugar content was found in the raw material of 2009.

During the study period the lowest concentration of sugars had the control – 20.9% on the average, as all other variants exceeded it. The lowest amount of titratable acids in V₂ was found for the three years. From all experimental variants, the raw material of V₃ (shoot topping in July), was characterized by higher

titratable acidity, as the average value of the indicator exceeded that of the control.

The differences in the main chemical indicators of the grapes from the trial variants were primarily due to the applied summer pruning treatments in the vineyard but also to the weather conditions of the year.

2.
2009-2011

Table 2. Chemical composition of grape must of Storgozia variety, during the period 2009-2011

Variants	Year	Sugars %	Titratable acids g/dm ³	
V ₀	2009	19,3	6,30	3,29
	2010	20,6	6,60	3,14
	2011	22,8	5,93	3,38
	Average	20,9	6,28	3,27
V ₁	2009	20,6	5,80	3,25
	2010	21,4	6,18	3,25
	2011	23,0	5,60	3,43
	Average	21,7	5,86	3,31
V ₂	2009	19,8	5,60	3,25
	2010	21,1	6,08	3,23
	2011	23,6	5,48	3,48
	Average	21,5	5,72	3,32
V ₃	2009	20,1	6,00	3,24
	2010	20,9	6,45	3,2
	2011	23,3	6,60	3,44
	Average	21,4	6,35	3,29

The differences in the raw material composition were reflected in the chemical composition of the experimental

(3).
 2009 .
 12,99 (V₀) 13,46 . % (V₁).
 1,98 2,32 g/dm³,
 -
 -
 . -
 ()
 V₂ V₃. -
 -
 ,
 - 5,48
 (V₂) 6,23 g/dm³ (V₀). -
 .
 () 2,07 (V₂)
 2,50 g/dm³ (V₀),
 352,03 440,84
 mg/dm³, -
 -
 .
 -
 V₀ (78,75
 V₃
 .),
 (78,50 .). -
 ,
 ,
 .
 ,
 ,
 .
 ,
 , 2010 . -
 .
 12,22 (V₀)

Storgozia wines too (Table 3).

Wines of 2009 vintage had an alcohol content ranging from 12.99 (V₀) to 13.46 vol. % (V₁). The residual sugars quantity varied from 1.98 to 2.32 g/dm³ confirming the complete progress of the alcoholic fermentation. Wines from the variants V₂ and V₃ had lower rate of sugar-free extract (SFE). The titratable acidity of the experimental samples varied within the optimal rates for the variety – from 5.48 (V₂) to 6.23 g/dm³ (V₀). Volatile acids were within the normal rates. The concentration of total phenolic compounds (TFC) was within the range from 2.07 (V₂) to 2.50 g/dm³ (V₀), and of anthocyanins from 352.03 to 440.84 mg/dm³, as these values were in correlation with those of the wine color intensity. In the organoleptic analysis of the samples the best characteristics and therefore the highest score had the wine from the control V₀ (78.75 points), followed by variant V₃ (78.50 points). These samples had high content of SFE, TFC and anthocyanins. They were characterized by a ruby red color, pleasant fruity aroma, thick and harmonious taste.

The results of the chemical composition of the experimental wines, vintage 2010 did not show significant differences in the tested indicators between the four variants. The alcohol in the samples was in the range from

3.

2009-2011

Table 3. Chemical composition of wine of Storgozia variety for the period 2009-2011

Indicators Variants	/ Year	Alcohol, (vol. %)	Sugar (g/dm ³)	Sugar free xtract (g/dm ³)	Titratable acids (g/dm ³)	Volatile acids (g/dm ³)		/ TPC (g/dm ³)	Anthocyanins, (mg/dm ³)	Colour intensity (l)	Tasting score
V ₀	2009	12,99	2,32	21,28	6,23	0,59	3,35	2,50	440,84	10,10	78,75
	2010	12,22	1,71	21,29	6,15	0,60	3,30	1,68	314,50	9,55	78,38
	2011	13,78	1,95	21,65	5,70	0,62	3,36	1,94	385,95	9,09	78,14
	Average	13,00	1,99	21,41	6,03	0,60	3,34	2,04	380,43	9,58	78,42
V ₁	2009	13,46	1,98	21,22	5,68	0,66	3,33	2,21	367,86	9,57	76,88
	2010	12,45	1,94	22,16	5,40	0,60	3,35	1,82	344,00	9,85	75,76
	2011	13,86	2,39	21,61	5,40	0,68	3,35	1,96	384,14	9,07	76,57
	Average	13,26	2,10	21,66	5,49	0,65	3,34	2,00	365,33	9,50	76,40
V ₂	2009	13,16	2,18	20,52	5,48	0,60	3,30	2,07	352,03	9,28	77,63
	2010	12,39	1,23	21,57	5,93	0,66	3,32	1,74	324,64	9,75	76,77
	2011	14,68	2,29	21,51	5,18	0,68	3,36	2,00	394,99	9,26	76,71
	Average	13,41	1,90	21,20	5,53	0,65	3,33	1,94	357,22	9,43	77,04
V ₃	2009	13,41	2,24	20,45	5,53	0,66	3,30	2,10	355,99	9,46	78,50
	2010	12,28	1,59	20,71	4,88	0,64	3,34	1,77	327,97	9,70	75,30
	2011	13,83	3,73	22,07	5,85	0,64	3,35	1,86	368,02	8,92	77,85
	Average	13,17	2,52	21,08	5,42	0,65	3,33	1,91	350,66	9,36	77,22

2011 .
 (13,78 14,68 .%),
 V₃ (22,07 g/dm³),
 5,18 g/dm³ (V₂)
 5,85 g/dm³ (V₃),
 (78,14 .).

- That confirmed the impact of other components of wine composition on its organoleptic profile. The variant was characterized by fresh flavour with hints of forest berries.
 - The wine taste was well structured, harmonious and balanced, with lasting aftertaste.

- In 2011, the experimental wines were distinguished by higher alcohol content (from 13.78 to 14.68 vol. %), due to the higher sugar content in the grapes. SFE rate was the highest in V₃ (22.07 g/dm³), while in the rest of the variants it was almost similar. The titratable acids ranged from 5.18 g/dm³ (V₂) to 5.85 g/dm³ (V₃), as the higher rates did not affect adversely the organoleptic qualities of wines. There were no significant differences in TFC and anthocyanins content as well as in the colour intensity between the variants. In the organoleptic analysis the wine from the control variant (78.14 points) had the highest tasting score.

CONCLUSIONS

- On the basis of the carried out investigations and obtained results it could be summarized:
 - ❖ The applied treatments (shoot topping and retaining the number of clusters) determined the quantitative changes in the main

❖
 ()

2009 2011 . (V₃ V₁ 2010
 .),
 V₂
)
 (V₃)
 V₁

indicators characterizing the yield from the experimental vines.

- ❖ The grapes yield was higher in the variants of shoot topping (V₁ in 2009 and 2011 and V₃ in 2010), mainly at the account of the greater mass of the bunch.
- The lowest yield per vine was obtained from the variant V₂ because of the reduced number of clusters.

- ❖ The effect of the treatments was highly dependent on climatic factors. The weather conditions during the year modified the impact of the studied factor.

- ❖ Summer pruning treatments in the vineyards influenced primarily the sugar accumulation in grapes and less the titratable acids content. All experimental variants exceeded the control in sugar accumulation.
- The lowest rate of titratable acids had the variant with thinning out the clusters.

- ❖ The wines had a different chemical composition, as the variant with shoot topping in July (V₃) was characterized by the lowest average rates of SFE, titratable acids, TFC, anthocyanins and colour intensity. Wines from the experimental wines were not superior compared to the control concerning these indicators, except V₁ for SFE.

- ❖ The experimental samples differed in their tasting properties

as the control variant had the best organoleptic qualities.

/ REFERENCES

1. 1999.
2. 1985.
3. 1984.
- 84-87. XXI, 2,
4. 1979.
5. 2012.
- 4-5 (. . . . , 2012).
6. 127-132. 1972.
7. 2008.
8. 115-118. 2006.
- 888-901. Journal of Mountain Agriculture on the Balkans, Vol. 9, 5,
9. 2012.
10. 2001.
11. 1992.
12. **Amati A., B. Marangoni, R. Zironi, M. Castellari, G. Arfelli.** 1994. Prove di vendemmia differenziata. Effetti del diradamento dei grappoli sulla fisiologia della vite. (Nota III^a). Rivista di viticoltura e di enologia, 3, XLVII, 3-12.
13. **Amati A., G. Mazzavillani, R. Zironi, M. Castellari, G. Arfelli.** 1995. Prove di vendemmia differenziata. Effetti del diradamento dei grappoli sulla composizione dei mosti e dei vini. (Nota V^a). Rivista di viticoltura e di enologia, 1, XLVIII, 29-37.
14. **Dhillon A. S. and Singh L.** 1949. The influence of thinning and ringing on the cropping and quality of grapes and the vigor of grapevines. Proceedings of the American Society for horticultural science. East Lansing. Mich., Vol. 53.
15. **Pszczolkowski P., A. Morales, and S. Cava.** 1985. Composicion quimica y calidad de mostos y vino obtenidos de racimos diferentemente asoleados. Ciencia Investigation Agraria 12:181-8.

1-MCP

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IMPACT OF IMPLEMENTATION OF 1-MCP ON QUALITY OF FRUITS IN 'ADEL' AND 'GRANNY SMITH'

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SUMMARY

<p>1- (1-MCP)</p> <p>" " "</p> <p>2012/2013</p> <p>,</p> <p>,</p> <p>" " "</p> <p>K</p> <p>K/Ca,</p> <p>"</p> <p>,</p> <p>,</p>	<p>- The paper presents the impact made by implementation of 1-methylcyclopropene (1-MCP) on the firmness of fruits and appearance of scald in the 'adel' and 'Granny Smith' during 2012-2013, in the a ak agro-ecological conditions. In addition to the cultivar-related properties and the climatic conditions during the year, firmness of the fruit is influenced by the content of macro-elements and ethylene, as elements with a distinct impact on the quality of fruits during their storage in cold conditions.</p> <p>- While a high content of K and a high K/Ca ratio have been found in the 'adel', the same parameters were within optimum levels in 'Granny Smith'. The iodine-starch test has revealed significant differences between the two cultivars that were recorded at different measuring intervals and year of testing, as opposed to the soluble solids contents.</p>
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	<ul style="list-style-type: none"> - with highly susceptible cultivars the scald can appear even during storage (Folchi et al., 2005).
<p>(Folchi et al., 2005).</p> <p>-farnesene</p> <p>(Rowan et al., 2001).</p>	<ul style="list-style-type: none"> - Scald has been associated with oxidation of -farnesene and the consequent accumulation of conjugated trienes (Rowan et al., 2001).
<p>(Wang and Dilley, 2001).</p> <p>,</p> <p>,</p> <p>,</p> <p>,</p>	<ul style="list-style-type: none"> - The maturity stage at harvest may predispose fruits to some disorders (Wang and Dilley, 2001). If immature fruits are harvested, they will not reach their technological ripeness during the storage period, which typically results in weak colouring of the fruits, their accentuated acidity and aroma that is atypical for the cultivar.
<p>(Jemric et al., 2006)</p> <p>(Echeverría et al., 2004).</p> <p>,</p> <p>-</p> <p>,</p>	<ul style="list-style-type: none"> - Less mature fruits develop superficial scald more easily (Jemric et al., 2006) and do not develop typical full flavour (Echeverría et al., 2004). Fruits that are picked too late are much less prone to superficial scald, but soften and become mealy prior to marketing (Vielma et al., 2008).
<p>(Vielma et al., 2008).</p>	<ul style="list-style-type: none"> - Choice of optimum time for harvest can have a significant impact on reducing scald incidence and advancing the storage potential (Magazin et al., 2010).
<p>et al., 2010).</p> <p>(Magazin</p> <p>1-</p> <p>(1-MCP)</p>	<ul style="list-style-type: none"> - Application of the gaseous compound 1-methylcyclopropene (1-MCP) has been reported to

(Folchi et al., 2005; Watkins et al., 2000; Zanella 2003; Jung and Watkins, 2008).

1-MCP

have an inhibiting effect on superficial scald development (Folchi et al., 2005; Watkins et al., 2000; Zanella 2003; Jung and Watkins, 2008). 1-MCP postharvest treatment primarily prevents ethylene from binding to receptors and thus reducing ethylene sensitivity and respiration. Apart from this, there are many non-parasite diseases caused by disturbed balance in the plant's intake of certain nutrients, which is manifested in a variety of symptoms (Biškup et al., 2003).

(Biškup et al., 2003).

However, different correlations between the optimal time of harvest and the ethylene production rate can be found depending on cultivar (Watkins et al., 1989).

(Watkins et al., 1989).

Incorrect storage conditions can lead to storage disorders and loss of quality which can make entire batches unsuitable for consumption (Thompson, 1998). Ethylene receptors act as negative regulators of the ethylene response which are inactivated by binding of ethylene. For apple six ethylene receptor genes have been identified so far (Dal Cin et al., 2006; Wiersma et al., 2007; Tatsuki, 2010).

(Dal Cin et al., 2006; Wiersma et al., 2007; Tatsuki, 2010).

MATERIAL AND METHODS

Plant material: Fruits of the 'adel' and 'Granny Smith'

" " , M-9 ,
 , 2012 2013.
 : (16),
 (22)
 (SSC),
 , 30 :
 1- (SmartFresh™). (1-MCP)
)
 2 ,
 10 °C. ,

cultivars were picked from eight-year old fruit trees, set up on the M-9 rootstock from the experimental plantation of the Fruit Institute in a pak, during 2012 and 2013.
 - The fruits of the examined cultivars were collected at two intervals: one in mid-September (16th September) and the other one, in the third decade of October (22nd October) in both trial years.
 - The fruits collected in the first interval were used to determine the level of ripeness and soluble solids content (SSC), whereas the fruits from the second interval of picking were used to determine the content of macro-elements, as well as the level of ethylene and firmness of the fruit.

Treatments: For the testing purposes, 30 fruits in three replications were taken from each cultivar, making sure that these were of uniform shape and with no visible signs of mechanical damage. The selected fruits were treated with 1-Methylcyclopropen (1-MCP) (SmartFresh™). The control variant consisted of the same number of non-treated fruits. The selected fruits (treated and non-treated) were kept in a cold storage with standard atmosphere, where they were stored for 2 days at the temperature of 10 °C. The fruits that were selected for the treatment were arranged in crates and disposed in a plastic bag of 1

1 m³.
 20°C).
 MCP
 24
 0.625 µL L⁻¹.
 1- PC
 120
 [(2 ± 0.5) °C, (90 ± 5)% RH,
 (0.03% CO₂ + 21% O₂)]
 120
 (Code Amidon, Ctifl, 2002).
 (TSS)
 (0–32%).

m³ capacity. Treatment in order to treat the fruits with a medium that acts as ethylene blocker, 1g 1-MCP was dissolved in water (t 20 °C) using a small jar.

1- After dissolving 1-MCP in water, the plastic bag was air-tight sealed for the next 24 hours, until reaching the gas concentration of 0.625 µL·L⁻¹. Small battery-powered fan was attached to the jar so as to secure better distribution of the 1- PC gas inside the plastic bag.

After this, the bag was removed and the fruits were kept in the cold storage for the following 120 days.

The fruits undergoing the treatment, as well as the non-treated fruits were placed in wooden crates and kept in vertical rows until the moment of analysis in cold atmosphere [(2 ± 0.5) °C, (90 ± 5)% RH, (0.03% CO₂ + 21% O₂)] over a period of 120 days.

Determining degree of ripeness and soluble solids content:

The starch index was evaluated using the starch iodine test (Code Amidon, Ctifl, 2002). Total soluble solids (TSS) were determined using a hand refractometer (0–32%).

Determining content of macro-elements: The content of macro-elements was determined prior to the experiment set up, by

2

(21±1 °C and 60±5% RH).

30

FT 327
Forshchungsbedarf
Ahnbeck, Germany)
11 mm.

(Winopal
GmbH,

(ANOVA)

MSTAT-C (

).
(LSD)

$p = 0.05$.

(21±1 °C and 60±5% RH). Fruits intended for the third measuring interval were kept in the same conditions at room temperature for 30 days.

Measuring of fruit firmness was conducted using a penetrometer - fruit pressure tester, mod. FT 327 (Winopal Forshchungsbedarf GmbH, Ahnsbeck, Germany) fitted with a 11-mm probe. Two measurements were made on opposite sides of each fruit.

Part from measuring the firmness of the apple fruits, incidence of scald and rotting of fruits was also traced after each sampling for fruit firmness of fruits at room temperature.

Statistical analysis: The data was subjected to analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The Least Significance Difference (LSD) was used to compare treatment means and treatments declared different at $p = 0.05$ level of significance.

RESULTS AND DISCUSSION

Based on the information presented in Table 1, it can be seen that the air temperature values in the period between June and September, i.e. during the

2012 phase of growth and ripening of the fruits, were higher in 2012 than in the second trial year.

In addition to this, the air temperatures during the trial period had considerably exceeded their many-year average. As opposed to this, the average precipitation was higher in the second trial year, compared to the first year of study (Table 1).

1. 2012-2013
Table 1. The air temperatures (°C) and rainfall (mm) for the field of the Cacak in the year 2012-2013

Year	/Month							
	IV	V	VI	VII	VIII	IX	X	
2012	12.2	17.3	24.1	26.6	25.4	20.9	13.8	
2013	13.2	18.2	20.6	23.3	24.1	17.2	14.5	
Mean monthly temperature (°C)	Mean for 1991-2010	10,5	14,7	18,9	20,7	20,6	15,6	11,1
2012	47.0	68.0	38.0	22.0	0.00	7.2	30.0	
2013	37.0	78.5	61.5	10.0	62.5	87.0	16.7	
Rainfall (mm)	Mean for 1991-2010	71,6	86,9	107,1	91,1	81,8	98,1	72,2

In addition to this, the many-year average precipitation values are above the values recorded during the period of research. All of these factors ought to be taken into consideration in examining the effect of 1-MCP application on the characteristics and quality of the fruits.

The variance analysis showed the presence of significant difference in the degree of ripeness in both measuring intervals, for both factors under

et al. (2010), (Magazin et al. (2010),

- consideration. The interaction
 - effect of the examined factors was
 also significant. As opposed to
 this, significant differences in the
 - solid soluble content between the
 years of trial were established only
 in the second measuring interval.
 . In the same measuring interval,
 - the interaction effect of the
 examined factors made a
 significant impact on the contents
 - of soluble solids (Table 2).
 Magazin et al. (2010), also
 showed that the degree of maturity
 significantly affect the strength of
 the fruit.

2.

Table 2. Degree of ripeness and soluble solids content in apple fruits

Factor	Parameters	Starch index (1–10)		TSS	
		16.9.2012	22.10.2012	16.9.2013	22.10.2013
A	'adel'	5.55±0.37a	7.05±0.25a	12.86±0.14a	14.10±0.21a
	'Granny Smith'	2.48±0.22b	5.15±0.43b	12.18±0.36a	14.53±0.12a
B	2012	2.85±0.30b	4.70±0.35b	12.33±0.19a	14.63±0.14a
	2013	5.18±0.45a	7.50±0.11a	12.71±0.35a	13.99±0.0.18b
AXB	'adel' 2012	4.00±0.21b	6.10±0.23c	12.68±0.20a	14.63±0.22a
	'adel' 2013	7.10±0.10a	8.00±0.00a	13.04±0.18a	13.56±0.26b
	'Granny Smith' 2012	1.70±0.21d	3.30±0.15d	11.97±0.29a	14.62±0.19a
	'Granny Smith' 2013	3.25±0.13c	7.00±0.00b	12.38±0.68a	14.43±0.16a
ANOVA					
A		*	*	ns	ns
B		*	*	ns	*
AXB		*	*	ns	*

p 0.05

(LSD)

Values within each column followed by the same small letter are insignificantly different at the *p* 0.05 by LSD test

(Drazeta et al., 2004).

- The chemical composition of
 fruits is determined by a multitude
 - of factors, including ecological and
 genetic ones (Drazeta et al.,
 2004).
 - The K content and the K/Ca

/
 " " ,
 Ca " "
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 K.
 , / .
 Krysiak Łata (2010)
 , /C ,
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 2013.
 ,
 'SmartFresh',

- ratio were statistically significantly higher in the 'adel' cultivar, whereas the Ca content recorded a statistically significant difference in 'Granny Smith'. The analysis
- into the impact of the trial year on the content of the analysed macro-
- elements revealed a significant
- influence on K contents.
-
- The interaction effect of the analysed factors made a
- significant impact on K contents, as well as on the K/Ca ratio.
-
- According to Wi ska-Krysiak and Łata (2010) fruits of low Ca content and high K content, and consequently, a high K/Ca ratio, are susceptible to bitter pit occurrence. Moreover, the development of this disorder is
- also influenced by storage
- conditions and the activity of the enzymes involved in fruit respiration.
-
- The ethylene contents showed significant differences
- , between the cultivars and years
- of trial, before and after the treatment. A considerably higher ethylene contents were observed in 'Granny Smith', as opposed to the 'adel' cultivar. A significantly
- higher ethylene content was recorded in 2013. As opposed to
- the ethylene contents values
- established in the control group,
- owing the implementation of 'SmartFresh' it was possible to reduce the ethylene levels to

approximate values, in both years in the 'adel' cultivar, i.e. in the 'Granny Smith' cultivar in the first trial year; at the same time, the only significant difference was established only in the 'Granny Smith' cultivar, in 2013. However, the content of ethylene before the experiment and after the application of 1-MPC is significantly reduced, which is later reflected in the firmness of apple fruit during storage chamber (Table 3).

Similar results were noted by Calhan et al. (2013).

3. K, Ca, K/Ca

Table 3. Contents of K, Ca, K/Ca ratio and ethylene contents in apple fruits

Factor	Parameters	Content of K mg kg ⁻¹	Content of Ca	K/Ca	Ethylene content $\mu\text{L kg}^{-1}\text{h}^{-1}$	
					Control	SmartFresh TM
A	'Cadel'	1800.8±31.06a	39.3±4.38b	48.7±5.47a	14.2±0.98b	2.3±0.38b
	'Granny Smith'	1390.5±41.80b	59.5±3.89a	24.0±2.02b	33.6±3.41a	8.90±3.66a
B	2012	1655.0±94.86a	52.50±3.77a	32.58±3.47a	20.80±2.86b	1.45±0.39b
	2013	1536.3±95.61b	46.3±7.54a	40.1±8.79a	27.0±6.18a	9.75±3.30a
AXB	'adel' 2012	1860.0±14.29a	48.0±3.46a	39.2±2.96bc	14.8±1.48c	2.0±0.67b
	'adel' 2013	1741.7±33.46a	30.7±2.96b	58.2±7.09a	13.6±1.51c	2.6±0.44b
	'Granny Smith' 2012	1450.0±52.54b	57.0±6.24a	26.0±2.85cd	26.8±1.66b	0.9±0.15b
	'Granny Smith' 2013	1331.0±49.34b	62.0±5.51a	22.0±2.86d	40.4±3.02a	16.9±1.76a
ANOVA						
A		*	*	*	*	*
B		*	ns	ns	*	*
AXB		ns	*	*	*	*

p 0.05

(LSD)

Values within each column followed by the same small letter are insignificantly different at the *p* 0.05 by LSD test

The analysis of variance established that there was a statistically significant difference among all of the intervals of measuring the fruit firmness in

both cultivars under consideration, as well as in both trial years. A significant reduction in firmness occurred upon taking the fruits out of the cold storage and leaving to rest at room temperature.

Factor B (control group and treatment with SmartFresh™) produced a significant effect in the ‘adel’ cultivar in 2012, i.e. in both trial years in the ‘Granny Smith’ cultivar.

The interaction effect of the analysed factors was statistically important in the ‘adel’ cultivar in 2012, as well as in the ‘Granny Smith’ cultivar, in both trial years. (Table 4).

Table 4. Firmness of apple fruits (kg cm⁻²)

Factor	adel		Granny Smith		
	2012	2013	2012	2013	
A	Control	8.71±0.15a	5.59±0.15a	10.39±0.13a	9.63±0.19a
	Measurement 1	6.71±0.20b	5.62±0.09a	10.11±0.18a	8.91±0.11ab
	Measurement 2	5.87±0.14c	5.20±0.09b	8.87±0.31b	9.08±0.16a
B	Control	6.38±0.25b	5.35±0.10a	8.64±0.26b	8.95±0.11a
	SmartFresh™	6.93±0.21a	5.38±0.07a	10.04±0.13a	8.64±0.10b
AXB	Control	8.71±0.22a	5.59±0.21a	10.39±0.18a	9.63±0.19a
	Measurement 1xControl	6.14±0.25cd	5.81±0.14a	9.84±0.20ab	8.93±0.18b
	Measurement 1x SmartFresh™	7.36±0.15b	5.42±0.11ab	10.41±0.28a	8.88±0.14b
	Measurement 2xControl	5.55±0.20e	4.96±0.14c	7.58±0.18c	8.46±0.15b
	Measurement 2x SmartFresh™	6.16±0.17c	5.42±0.07ab	10.05±0.22a	8.59±0.12b
	Measurement 3x Control	5.11±0.19e	5.05±0.17bc	6.76±0.14d	8.79±0.21b
	Measurement 3x SmartFresh™	5.62±0.16dc	5.07±0.11bc	9.34±0.27b	8.67±0.32b
ANOVA					
A		*	*	*	*
B		*	ns	*	*
AXB		*	ns	*	*

p 0.05

(LSD)

Values within each column followed by the same small letter are insignificantly different at the *p* 0.05 by LSD test

" "

100%

1-MPC

2012,

2013

50%

1-MPC.

1-MCP

(Rupasinghe et al., 2000; Watkins et al., 2000; Fan et al., 1999).

- No incidence of scald was observed in the 'adel' cultivar. Following the second measuring interval at the control group in both trial years, there was 100% incidence of scald in the 'Granny Smith' cultivar.

- As opposed to this, the fruits treated with 1-MPC recorded no scald in 2012, whereas scald incidence in 2013 was determined in 50% of the fruits treated with 1-MPC. The effectiveness of 1-MCP in the inhibition of the ethylene has been published in numerous studies (Rupasinghe et al., 2000; Watkins et al., 2000; Fan et al., 1999).

CONCLUSIONS

Based on the results obtained during 2012-2013 in the agro-ecological conditions of a ak, concerning the application of 1-methylcyclopropene (1-MCP) and its impact on fruit firmness and incidence of scald in the 'adel' and 'Granny Smith' cultivars, it is possible to make the following conclusions:

In addition to the typical cultivar characteristics and the climatic conditions of the year of the study, the firmness of the fruit is also dependent on the content of macro-elements and ethylene, making an impact on the quality of fruits during the cold storage.

Ca, K/Ca, 1-MCP 2012, 2013 1-MPC

While the 'adel' cultivar recorded high K contents and a high K/Ca ratio, coupled with a low Ca content, the same parameters were at their optimum levels in the 'Granny Smith'.

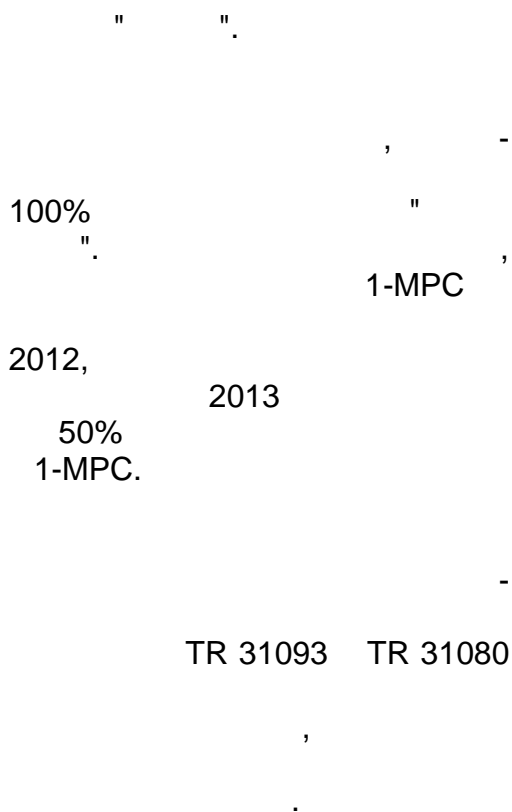
In both trial years, different measuring intervals revealed significant differences in relation to the degree of fruit ripeness of both 'adel' and 'Granny Smith' cultivars; however, this tendency was not present in the soluble solids contents.

Whereas the level of ethylene showed significant differences between the cultivars, this parameter also varied between the years of trial in the 'Granny Smith' cultivar.

Firmness of fruits showed significant differences between different measuring intervals in both cultivars. While the implementation of 1-MCP had a highly significant effect on the preservation of fruit firmness in 'Granny Smith' in 2012, in the following 2013 a difference was established only in relation to the initial measuring, but not between the measuring before and after the cold storage.

Similar results in fruit firmness were achieved in applying 1-MCP on the 'adel' cultivar.

No occurrence of scald was



established in the 'adel' cultivar. Following the second measuring interval at the control group in both trial years, there was 100% incidence of scald in the 'Granny Smith' cultivar.

As opposed to this, the fruits treated with 1-MPC recorded no scald in 2012, whereas scald incidence in 2013 was determined in 50% of the fruits treated with 1-MPC.

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/ REFERENCES

1. **Biškup S., osi T., Pecina M., Miljkovi I.** 2003. Relations of nutrient in apple leaves and fruits grown on acid soil and their influence on bitter pit occurrence. *Pomologia Croatica*, 9, 1-4.
2. **Çalhan Ö., EREN ., Onursal C. E., Güneyli A., Emre M., Öztürk F. P.** 2013. Antalya (Korkuteli-Elmalı) Bölgesinde Elmalara 1-MCP (SmartFresh™) Uygulamaları ve Etkileri. *Tarım Bilimleri Ara tırma Dergisi*, 6, 1, 21-25.
3. **Dal Cin V., Rizzini F.M., Botton A., Tonutti P.** 2006. The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. *Postharvest Biology and Technology*, 42, 125–133.
4. **Drazeta L., Lang A., Hall A.J., Volz R.K., Jameson P.E.** 2004. Causes and effects of changes in xylem functionality in apple fruit. *Ann. Bot. (Lond)*. 93, 3, 275-82.
5. **Echeverría G., Fuentes T., Graell J., Lara I., López M.L.** 2004. Aroma volatile compounds of 'Fuji' apples in relation to harvest date and cold storage technology. A comparison of two seasons, *Postharvest Biology and Technology*, 32, 29-44.
6. **Fan X., S. M. Blankenship, J.P. Mattheis.** 1999. 1-Methylcyclopropene inhibits apple ripening. *Journal of the American Society for Horticultural Science*, 124, 690-695.
7. **Folchi A., Pratella G.C., Gregori R.** 2005. Relationship between O₂ levels, 1-methylcyclopropene (1-MCP), conjugated trienes (CTs) and superficial scald in 'Granny Smith' apples. *Acta Horticulturae*, 682, 2063-2068.

8. **Jemric T., Lurie S., Dumija Lj., Pavicic N., Hribar J.** 2006. Heat treatment and harvest date interact in their effect on superficial scald of 'Granny Smith' apple. *Scientia Horticulturae*, 107, 155-163.
9. **Jung S.K., Watkins C.** 2008. Superficial scald control after delayed treatment of apple fruit with diphenylamine (DPA) and 1-methylcyclopropene (1-MCP). *Postharvest Biology and Technology*, 50, 45-52.
10. **Magazin N., Gvozdenovi D., Keserovi Z., Mili B.** 2010. Fruit quality of Granny Smith apples picked at different harvest times and treated with 1-MCP. *Fruits*, 65, 3, 191-197.
11. **Rowan D.D., Hunt M.B., Fielder S., Norris J., Sherburn M.S.** 2001. Conjugated triene oxidation products of β -farnesene induce symptoms of superficial scald on stored apples. *Journal of Agricultural and Food Chemistry*, 49, 2780-2787.
12. **Rupasinghe H. P. V., D. P. Murr G. Paliyath L. Skog.** 2000. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. *The Journal of Horticultural Science and Biotechnology*, 75, 271-276.
13. **Tatsuki M.** 2010. Ethylene biosynthesis and perception in fruit. *Journal of the Japanese Society for Horticultural Science*, 79, 315-326.
14. **Thompson A.K.** 1998. *Controlled Atmosphere Storage of Fruit and Vegetables*. CAB International, Wallingford, UK.
15. **Vaysse P.** 2002. Code Amidon Pomme // CTIFL.
16. **Vielma M., Matta F., Silva J.** 2008. Optimal harvest time of various apple cultivars grown in Northern Mississippi. *Journal- American Pomological Society*, 62, 13-21.
17. **Wang Z., Dilley D.R.** 2001. Initial low oxygen stress (ILOS) controls scald of apples without using postharvest chemical treatments. *Acta Horticulturae*, 553, 261-266.
18. **Watkins C., J. Nock, B. Whitaker.** 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology*, 19, 17-32.
19. **Watkins C., Nock J., Whitaker B.** 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology*, 19, 17-32.
20. **Wiersma P.A., Zhang H.Y., Lu C.W., Quail A., Toivonen P.M.A.** 2007. Survey of the expression of genes for ethylene synthesis and perception during maturation and ripening of 'Sunrise' and 'Golden Delicious' apple fruit. *Postharvest Biology and Technology*, 44, 204-211.
21. **Wi ska-Krysiak M., B. Łata.** 2010. Influence of lipoxygenase activity and calcium and potassium contents on bitter pit occurrence in commercial apple cultivars. *Folia Horticulturae*, 22, 13-17.
22. **Zanella A.** 2003. Control of apple superficial scald and ripening – a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. *Postharvest Biology and Technology*, 27, 69-78.

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CHANGES IN PHYSICAL PROPERTIES, SOLUBLE SOLIDS AND VITAMIN C IN FRUIT OF 'CLERY' STRAWBERRY CULTIVAR DURING FIVE HARVEST TIMES

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SUMMARY

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The paper presents the results of the research into the impact made by harvesting time and fertilizer on the dry soluble solids contents (SSC) and vitamin C in the fruit of the 'Clery' strawberry cultivar, in 2014. Fruit samples for analysis were collected in the full maturity phase, at five rounds. Two types of fertiliser were applied – mineral NPK fertiliser and microbiological fertiliser. The obtained results have shown that the mass, length and width of the fruit were under a significant impact of the harvesting times, whereas the fruit shape index was significantly influenced by both factors under consideration (period of harvest and fertiliser).

The fruit mass and length were considerably larger in the first three consecutive times of harvest, whereas the width of the fruit was larger in the first two harvest times.

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 (Fragaria x ananassa Duch.),
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20-25 t/ha

- Significantly higher values of the fruit shape index were recorded in the first four times of harvest, when compared to the final harvest time, which was characterised by the lowest fruit shape index. The mineral fertiliser made a positive impact on the fruit shape index, compared to the effect made by the applied micro-biological fertiliser.

- While the highest values of the soluble solids content were recorded in the second time of harvest, the highest content of vitamin C was determined in the fourth harvest time.

- The interaction effect between the third harvest time and the micro-biological fertiliser had a relevant effect on the content of vitamin C, compared to other interaction effects. In the course of the contemplated harvest times, no regular patterns concerning the quantity of SSC and vitamin C were observed in strawberry fruits.

- In the overall conclusion, the earlier times of harvest have demonstrated more favourable physical properties, as well as a higher content of SSC and vitamin C in the strawberry fruits.

- **Key words:** garden strawberry (*Fragaria x ananassa* Duch.), harvest times, fertilisers, physical properties, soluble solids, vitamin C

INTRODUCTION

- Strawberry is a type of fruit which, when supported by a full-scale agro-technical and pomotechnical measures, produces average yields in the range of 20-25 t/ha of high quality fruits – a fact that indicates a very high economic potential of the production.

- Contrary to the wishes of

producers, who seek highly-productive cultivars with large-size fruits and a long harvesting season, the consumers typically prefer cultivars with a wide range of sensory qualities. By improving the structure of the strawberry assortment in plantations, i.e. by introducing new cultivars which apart from good physical and chemical characteristics of the fruits also possess high nutritive and anti-oxidant values, it is possible to make a positive impact on increased consumption of the fruits known for their beneficial healing effect, without reducing the commercial benefits of the production (Milivojević et al., 2009).

In the past decade, the strawberry assortment in Serbia has undergone dynamic changes, starting with the introduction of cultivars such as 'Elsanta', 'Queen Elisa', 'Miss', 'Dora' and 'Patty' - all of which have now gone out of use, but also 'Clery', a cultivar that has retained its position as the leader in production up to the present time (Milivojević and Nikolić, 2015). Fruits of the 'Clery' cultivar have an exceptional commercial value which is based on the specific combination of the early ripening and attractiveness of the fruit. In addition to this, the cultivar shows the fall-bearing tendency which, coupled with the marked ability to adapt to different

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- In the past decade, the strawberry assortment in Serbia has undergone dynamic changes, starting with the introduction of cultivars such as 'Elsanta', 'Queen Elisa', 'Miss', 'Dora' and 'Patty' - all of which have now gone out of use, but also 'Clery', a cultivar that has retained its position as the leader in production up to the present time (Milivojević and Nikolić, 2015). Fruits of the 'Clery' cultivar have an exceptional commercial value which is based on the specific combination of the early ripening and attractiveness of the fruit. In addition to this, the cultivar shows the fall-bearing tendency which, coupled with the marked ability to adapt to different

cultivation systems, makes it conducive to successful growing both within and out of the standard seasonal production.

In terms of their optimum ripeness for harvesting, the maturity of strawberry fruits is determined based on the colour of epidermis, as well as the fruit firmness and taste. In order to achieve the maximum fruit quality in terms of its flavour and colour, the harvest of strawberry fruits ought to take place in the phase of full ripeness.

The main changes in the composition of the strawberry fruit occur prior to the harvest, while the fruit is attached to the mother plant. It is therefore crucial that the freshly consumed fruits be picked at the time when they are ready for consumption. Consequently, the time period within which the fruits are marked by the best quality is a very short one (Cordenunsi et al., 2003).

There are numerous studies that point to the fact that berry fruits possess high concentrations of bio-active substances (Manach et al., 2004; Seerem, 2009) and that there is a clear correlation between the daily consumption of fruit and human health. Vitamin C which is present in strawberry fruits acts as a potent anti-oxidant, and makes a significant positive contribution to the manifested anti-oxidant activity of the fruit, either

al., 2003).

(Manach et al., 2004; Seerem, 2009)

(V et al., 2006).
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 NPK

- by itself or in a combination with different phenol components (V et al., 2006).

- The aim of this paper is to examine the changes of physical properties, contents of soluble solids and vitamin C in the fruits of the 'Clery' strawberry cultivar, during five harvest times and under conditions of applying mineral and micro-biological fertilisers.

MATERIAL AND METHODS

- The research was conducted in the experimental plantation of the 'Clery' strawberry cultivar, at the Fruit Research Institute in a ak (43° 53' Northern Latitude and 20° 20' Eastern Longitude, 225 m altitude) during 2014. The planting was performed in summer 2011 (18th July), using the form of the double ribbon-row beds, covered in black PE foil, applying the 30 x 30 cm planting distance.

- During the experiment, the plantation was treated with standard agro-technical and pomological measures, supported with drip-irrigation. The fruits for analysis were picked at five times, at every five days during the harvesting season (period I – 26th May, period II – 31st May, period III – 5th June, period IV – 11th June, period V – 16th June).

- The mineral fertiliser treatment involved application of Poly-Feed water-soluble NPK fertilisers of

Poly-Feed, : Poly-Feed 11:44:11+Me (,); Poly-Feed 20:20:20+Me (); Poly-Feed 16:8:32+2MgO+Me (); Multi-Cal 15,5:0:0+26,5 CaO (); Multi-K Mg 12:0:43 + 2MgO (Baktofil B-10 , : *Azotobacter*, *Azospirillum*, *Bacillus* *Pseudomonas*. (,), () " "

formulations, which were determined taking into consideration the phenological phases of the plants' development: Poly-Feed 11:44:11+Me (after the planting and at the start of vegetation, to stimulate the root development and restore the activity of the root system); Poly-Feed 20:20:20+Me (in the phase of intensive vegetative growth); Poly-Feed 16:8:32+2MgO+Me (in the phenophase of flowering, intensive growth and ripening of fruits); Multi-Cal 15,5:0:0+26,5 CaO (in the phase of the fruits growth and development, aimed at eliminating the physiological calcium insufficiency and increasing the firmness of the fruits); Multi-K Mg 12:0:43 + 2MgO (in the phenophase of the fruit growth and ripening). Baktofil B-10 was applied in the treatment with microbiological fertilisers, as a microbe bio-fertiliser containing combinations of different bacteria genera: *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas*. The research included testing the physical characteristics (mass, length, width, fruit shape index), soluble solids contents (SSC) and contents of vitamin C in the fruit of the 'Clery' strawberry cultivar. Picking of fruits for the analysis of physical properties (mass, length, width and fruit shape index), as well as the SSC and contents of vitamin C was performed in the phase of full ripeness (when 20 fruits were

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Metler
± 0,01 g.
"Inox",
± 0,05 mm.

(Krüss, Germany),

(Rikovski et al., 1989),
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100 g
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(ANOVA).

(LSD)
0.05

- picked in each of the 3 repetitions, resulting in a total of 60 fruits per time).
- The fruit mass was determined by measuring, using the Metler analytical scales of ± 0,01 g precision. The length and width of the fruit were measured using the digital Inox calliper, with the precision of ± 0,05 mm.
- Values of the fruit shape index were calculated as a ration of the fruit length and width.
- The soluble solids content was determined using a digital refractometer (Krüss, Germany), and the corresponding values are expressed in %.
- The content of vitamin C in the strawberry fruit was determined using the method iodometric titration (Rikovski et al., 1989), and expressed as mg of vitamin C per 100 g of fresh fruit mass (mg 100 g⁻¹ dry mat. of fruit).
- The data obtained in the research have been processed using the SPSS statistical programme, based on the two-way factor analysis of variance (ANOVA). The results shown in the table represent the mean value from three replications. The LSD test at the significance level of 0.05 was used to determine the significance of differences among the mean values.

RESULTS AND DISCUSSION

Table 1 shows the results pertaining to the physical properties of the fruits, contents of soluble solids and vitamin C in the fruits of the 'Clery' strawberry cultivar, in relation to the time of harvesting and applied fertilisers.

Table 1. Impact of harvesting time and fertiliser on the physical properties of 'Clery' strawberry cultivar

Factor		Fruit mass (g)	Fruit length (mm)	Fruit width (mm)	Fruit shape index
Harvesting time (A)	I	18,9 ± 1,6 a	38,7 ± 1,6 a	33,9 ± 0,8 a	1,13 ± 0,02 a
	II	17,6 ± 2,2 a	40,3 ± 2,7 a	33,2 ± 1,5 a	1,21 ± 0,03 a
	III	16,5 ± 0,8 a	36,8 ± 0,7 a	31,8 ± 0,6 ab	1,16 ± 0,03 a
	IV	9,0 ± 1,2 b	29,4 ± 0,6 b	24,9 ± 1,4 c	1,19 ± 0,06 a
	V	10,8 ± 0,6 b	29,4 ± 0,6 b	28,9 ± 0,8 b	1,02 ± 0,04 b
Fertiliser (B)	Mineral fertiliser	14,9 ± 1,5 a	35,9 ± 1,7 a	30,2 ± 1,3 a	1,19 ± 0,03 a
	Baktofil B-10	14,2 ± 1,1 a	33,9 ± 1,3 a	30,9 ± 0,9 a	1,09 ± 0,02 b
ANOVA					
A		*	*	*	*
B		ns	ns	ns	*
AxB		ns	ns	ns	ns

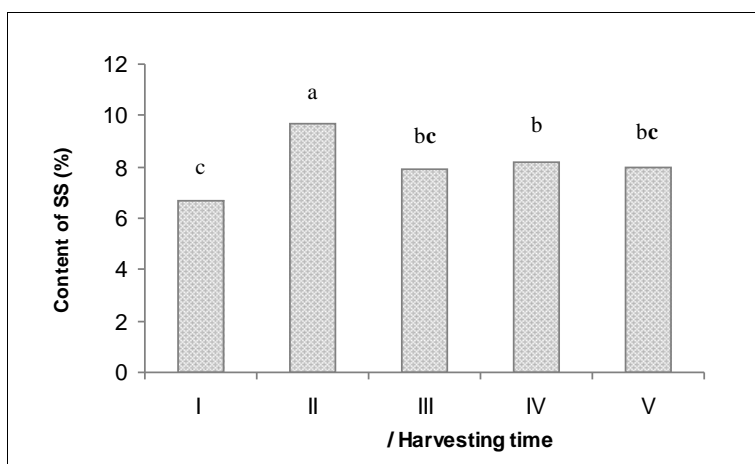
Mean values in three replications ± standard error; values in columns marked with different letters represent significant differences at the level of $p ,05$ (*LSD test*); ns – nonsignificant differences.

Based on the analysis of the data shown in the table, it is possible to conclude that the mass, length and width of the fruit were under a significant impact of the harvesting time, whereas the fruit shape index was influenced by both factors under consideration (harvesting time and fertiliser). In assessing new strawberry selections, large fruits are given particular preference,

since this property is important from the aspect of increasing the efficiency of manual picking and enhancing sales of fruits for fresh consumption.

Our research has revealed significantly higher values of fruit mass and length in the fruits picked during the first three times, compared to the fourth and the fifth time. The width of the strawberry fruit was significantly larger in the first two harvesting times. Significantly larger values of the fruit shape index were recorded in the first four harvesting times, compared to the final harvesting time, when the fruit shape index was at its lowest.

Mineral fertiliser made a positive impact on the fruit shape index, compared to the applied micro-biological fertiliser.



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Fig. 1. Impact made by harvesting time on SSC in the fruit of 'Clery' strawberry cultivar

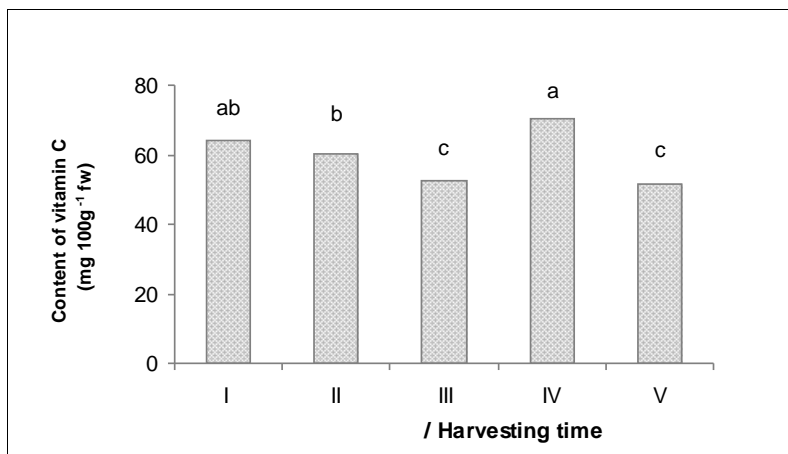
(Tulipani et al., 2008).

(Jouquand et al., 2008).

The appeal of strawberry fruits on consumers is largely dependant on the fruit taste, a property that stands in a close correlation with the content of soluble solids (Tulipani et al., 2008).

During sensory testing, the best marks are awarded to fruits the highest soluble solids content, or those with the highest ratio SSC – total acidity (Jouquand et al., 2008).

Our research revealed that a significant impact on the soluble solids contents was made only by the harvesting time (Figure 1). A significantly higher value of this important parameter of fruit quality was recorded in the second harvesting time, compared to the other times.



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, B-Baktofil B-10)

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Fig. 2. Impact of interaction between harvesting time and fertiliser on the content of vitamin C in the fruit of 'Clery' strawberry cultivar (M-mineral fertiliser, B-Baktofil B-10)

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 et al., 2008).
 (Jacob and Sotoudeh, 2002). Nile
 and Park (2014)
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 Erturk
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 PGPR (

Strawberry fruits contain high concentrations of useful antioxidants, including the highly important ascorbic acid (vitamin C). In certain cultivars, vitamin C participates in the overall antioxidant capacity of the fruit with a share of 30-35% (Tulipani et al., 2008). There are numerous studies that indicate that individuals whose nutrition provides them with a large intake of vitamin C significantly reduce the risk of a large number of chronic diseases (Jacob and Sotoudeh, 2002). Nile and Park (2014) point out that the contents of vitamin C in the fruits of berries are dependent on a large number of factors; variety, cultivar, cultivation technology, ecological conditions, fruit ripeness, region of cultivation, length and conditions of storage. Figure 2 shows the dynamics of vitamin C contents in the fruit of the 'Clery' strawberry cultivar in relation to the harvesting time and the type of fertiliser applied. The interaction effect between the third harvesting time and the microbiological fertiliser made a significant impact on the vitamin C contents, in relation to other examined interaction effects.
 Similar results were reported by Erturk et al. (2012), reporting that the application of PGPR (plant growth-promotion rhizobacteria)

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, Umar et al. (2009)

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Azotobacter.

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(Mozafar, 1993).

- made a significant effect on the
 - content of vitamin C in the fruit of
 - the 'Fern' strawberry cultivar.

- On the other hand, Umar et al.
 - (2009) report that the maximum
 - content of ascorbic acid in
 - strawberry fruits was recorded in a
 - treatment involving application of
 - organic and mineral nitrogen
 - fertiliser, combined with
 - *Azotobacter*.

- However, there is a number of
 - data sources in the literature
 - pointing to the act that excessive
 - use of nitrogen mineral fertilisers
 - has a negative impact on the
 - vitamin C contents in different
 - types of fruits and vegetables,
 - which represent the major source
 - of this vitamin in human nutrition
 - (Mozafar, 1993).

CONCLUSIONS

- In the overall conclusion, it
 - can be said that more favourable
 - physical properties and a higher
 - content of the soluble solids and
 - vitamin C in the fruit of the 'Clery'
 - strawberry cultivar were
 - established in the early-season
 - harvesting. Taking into
 - consideration that the largest part
 - of strawberry fruits are picked in
 - the second and third harvests, and
 - also having in mind the results of
 - this study, it can be concluded that
 - the fruits of the 'Clery' cultivar
 - harvested in the early season can

satisfy the consumers' demands related to fresh consumption.

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/ REFERENCES

1. **Cordenunsi B.R., Genovese M.I., João R.O., Hassimotto N.A, Santos R.J., Lajolo F.M.** 2005. Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chemistry*, 91, 1, 113-121.
2. **Erturk Y., Ercisli S., Cakmakci R.** 2012. Yield and growth response of strawberry to plant growth-promoting rhizobacteria inoculation. *Journal of Plant Nutrition*, 35, 6, 817-826.
3. **Jacob R.A., Sotoudeh G.** 2002. Vitamin C function and status in chronic disease. *Nutrition Clinical Care*, 5, 66–74.
4. **Jouquand C., Chandler C., Plotto A., Goodner K.** 2008. A sensory and chemical analysis of fresh strawberries over harvest dates and seasons reveals factors that affect eating quality. *Journal of the American Society for Horticultural Science*, 133, 859-867.
5. **Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L.** 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, 79, 727-747.
6. **Milivojevi J., Nikoli M., urovi D.** 2009. The influence of growing system on cropping potential of strawberry cultivar 'Clery' grown in plastic tunnel. *Acta Horticulturae*, 842, 115-118.
7. **Milivojevi J., Nikoli M.** 2015. Dostignu a i trendovi u proizvodnji jagode u Srbiji. Zbornik radova V savetovanja Inovacije u vo arstvu "Savremena proizvodnja jagode", Beograd (Republika Srbija).
8. **Mozafar A.** 1993. Nitrogen fertilizers and the amount of vitamins in plants: A review. *Journal of Plant Nutrition*, 16, 12, 2479-2506.
9. **Nile S.H., Park S.W.** 2014. Edible berries: Bioactive components and their effect on human health. *Nutrition*, 30, 2, 134-144.
10. **Rikovski I., Džami M., Rajkovi M.** 1989. Praktikum iz analiti ke hemije. Gra evinska knjiga, Beograd.
11. **Seerem N.P.** 2009. Bioactive polyphenols from foods and dietary supplements: challenges and opportunity, in: Ho C.T., Wang M., Sang S. (Eds.) *Herbs: Challenges in Chemistry and Biology*, (Chapter 3), 5308-5312.

12. **Tulipani S, Mezzetti B, Capocasa F, Bompadre S, Beekwilder J, de Vos C.H.R., Capanoglu E, Bovy A, Battino M.** 2008. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry*, 56, 3, 696-704.
13. **Umar I., Wali V.K., Kher R., Jamwal M.** 2009. Effect of fym, urea and azotobacter on growth, yield and quality of strawberry cv. Chandler. *Notule Botanicae Horti Agrobotanici Cluj*, 37, 139–143.
14. **Voca S., Duralija B., Druzic J., Babojelic M.S., Dobricevic N., Cmelik Z.** 2006. Influence of cultivation systems on physical and chemical composition of strawberry fruits cv. Elsanta. *Agriculturae Conspectus Scientificus*, 71, 4, 171–174.

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POTYVIRUSES IN HUNGARY

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SUMMARY

- We summarize the results obtained
 - from survey of sharka disease, characterization of PPV isolates (by biological, serological and RT-PCR assays) in Northern and Central Hungary.

- Molecular characterization of Maize dwarf mosaic virus strains was conducted and coat protein genes of Hungarian (and also some Bulgarian) strains were compared as well.
 - The coat protein genes were compared to other members of Sugarcane mosaic virus subgroup and phylogenetic tree was constructed.

- Aphid transmission of potyviruses is governed by interaction of HC-Pro and CP proteins, namely highly conserved HC-Pro (KITC and PTK) and CP (DAG) motifs.

- Y, Two Potato virus Y strains isolated in Hungary, differing in aphid transmissibility were studied and HC-Pro and CP genes were sequenced. Our studies have indicated that besides the highly conserved motifs of HC-Pro and CP, other

Pro CP,

PVY.

1995.

ZYMV

: MDMV, PPV, PVY

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Potyvirus,

Potyviridae.

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11-13 nm.

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protein regions may influence the aphid transmissibility in case of PVY.

Zucchini yellow mosaic virus was first detected in Hungary in 1995. The results of molecular characterization and seed transmission experiments will be discussed.

Key words: Characteristics of Potyviruses, MDMV, PPV, PVY and ZYMV

Potyviruses represent one of the largest and economically most important group of plant viruses. More than 100 known species belong to the genus *Potyvirus*, which is one of the six genera of the family *Potyviridae*. They infect a wide range of mono- and dicotyledon plants and spread all over the world.

Potyviruses are transmitted by aphids in a non-persistent manner; namely the virus is acquired in a short time (seconds to minute) and without a latent period it can be transmitted in a similarly short time. They can also be easily transmitted by mechanical means. These viruses have non-enveloped rod-shaped flexuous particles 700-850 nm long and 11-13 nm in diameter, encapsidating a genome that is a single-stranded positive sense RNA molecule of approximately 10000 nucleotides. Genome expression strategy based on the synthesis of a single polyprotein, which is then proteolytically processed to yield 10 individual

10	-	-	proteins. Recently the 11th potyviral protein PIPO (pretty interesting protein) was also detected
11- PIPO	.	.	The biochemical and biological features of the processed proteins are:
P1:	:	-	P1: C-terminal autocleavage and trypsin-like serine proteinase, symptomatology, genome amplification, binding RNA;
()	,	-	
HC-Pro:	,	-	HC-Pro: proteolytic activity, C terminal autocleavage, aphid transmission, cell-to-cell and long-distance movement, genome amplification, self interaction, enhancement of pathogenicity, longdistance movement of other viruses in mixed infections, suppression of gene silencing, synergism and symptom expression, papain-like cysteine proteinase, binding RNA;
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P3:	.	-	P3: plant pathogenicity, genome replication;
6K1:	;	-	6K1: plant pathogenicity together with the C-terminal region of P3, genome amplification;
C-	;	-	
CI:	;	-	CI: cell-to-cell movement, ATP-ase/RNA helicase, genome amplification, binding RNA;
	,	-	
	,	-	
6K2:	;	-	6K2: anchoring the viral replication complex to membranes, genome amplification;
	,	-	
VPg:	;	-	VPg: genome replication, binding RNA;
Nla:	;	-	Nla: cellular localization, trypsin-like serine protease (acts in
-	-	-	

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 Nlb:
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 (RdRp),
 CP:
 ,
 ,
 ,
 (Oana et al., 2009).

2000
 (Riechmann et al., 1992)

(Shukla, 1991).

- *cis* and in *trans*), binding RNA,
 , genome amplification, protein-
 protein interaction;

, Nlb: genome replication,
 RNA-dependent RNA polymerase
 (RdRp), binding RNA;

- CP: aphid transmission, cell-
 to-cell and systemic movement
 and virus assembly, binding RNA,
 , genome amplification.(Oana et al.,
 - 2009).

- The structural and replication
 . proteins are produced in an
 . equimolar ratio. Since the virion is
 composed of about 2000 copies of
 capsid protein (Riechmann et al.,
 - 1992) huge amount of processed
 - viral proteins form cylindrical and
 - amorphous inclusion bodies in
 infected cells.

. These inclusion bodies can be
 - considered as the most important
 phenotypic criterion for assigning
 viruses to potyvirus group (Shukla,
 1991).

- In Hungary, the following
 - potyviruses were detected: *Bean*
 - *common mosaic virus*, *Bean yellow*
 - *mosaic virus*, *Celery mosaic virus*,
 - *Columbian datura virus*, *Henbane*
mosaic virus, *Lettuce mosaic virus*,
Lily mottle virus, *Maize dwarf*
mosaic virus, *Onion yellow mosaic*
 - *virus*, *Pea mosaic virus*, *Plum pox*
 - *virus*, *Potato virus A*, *Potato virus*
 - *Y*, *Sugarcane mosaic virus*, *Tulip*
 , *breaking virus*, *Turnip mosaic*

(
)
,
,
(Tóbiás et al., 2007).
,
MDMV
MDMV
95.1 % 99.6 %.
MDMV
SCMV (1).

- According to biological (symptoms on test plants) and serological properties the samples were identified as MDMV (Tóbiás et al., 2007). The coat protein sequence analysis has the primary importance in potyvirus taxonomy, hence the coat protein genes were determined of selected MDMV isolates. The coat protein sequence identity varied from 95.1 % to 99.6 %. The Bulgarian and Hungarian MDMV isolates are very similar to each other and can be differentiated from other members of SCMV potyvirus subgroup (Figure 1).

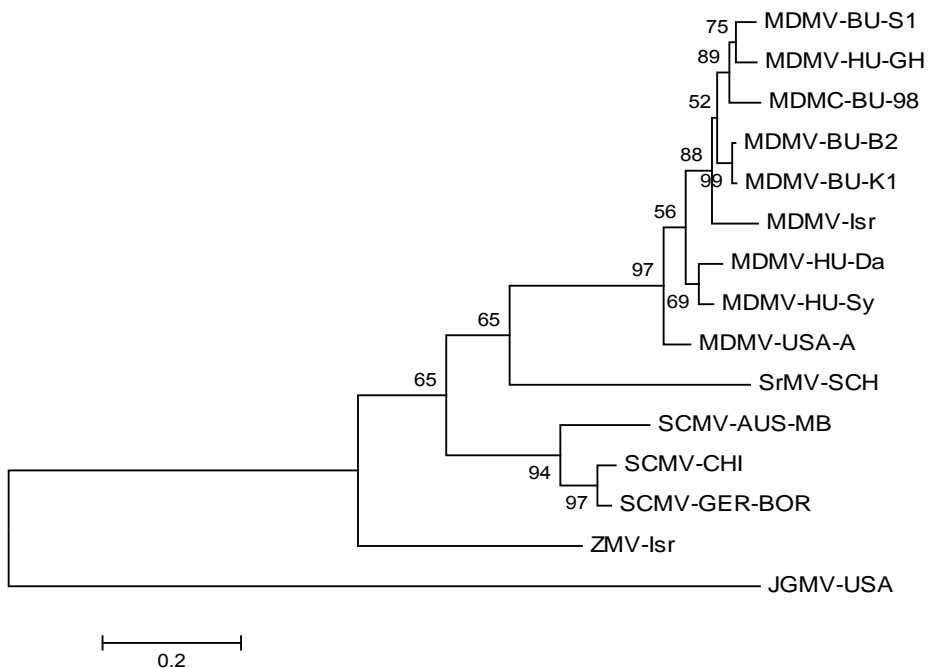


Fig. 1.

SCMV
„bootstrap analysis“.

Fig. 1. Phylogenetic tree obtained from the coat protein sequences of some members of SCMV potyvirus subgroup using maximum likelihood model and bootstrap analysis.

SCMV

MDMV

(PPV)

1948

1950

1963

PPV

(*Prunus domestica* L.),
(*Prunus armeniaca* L.),
(*Prunus persica* (L.) Batsch.),
(*Prunus persica* var. *nucipersica* (Borkh.) C.K. Schneid., hybrids),
(*Amygdalus communis* L.,
syn. *P. amygdalus* (L.) Batsch.,
Amygdalus dulcis Mill.),
(*Prunus avium* (L.))
(*Prunus cerasus* L.),

(*P. spinosa* L.)

(Gáborjányi et al., 2001; Pribék et al., 2001; Salamon and Palkovics, 2002).

- The coat protein amino acid sequences of strains or isolates of different members of the SCMV subgroup are separated from each other and form distinct clusters in the phylogenetic tree. The MDMV isolates from Hungary and Bulgaria were clustered together.

Plum pox virus

- Sharka or pox or plum pox disease is one of the most devastating viral diseases of stone fruits in Hungary. The *Plum pox virus* (PPV) was first described in Hungary in 1948 from apricot, in 1950 from plum and in 1963 from peach, but by late seventies the virus was spread all over the country. The PPV damages plums (especially *Prunus domestica* L.), apricots (*Prunus armeniaca* L.), peaches (*Prunus persica* (L.) Batsch.), nectarines (*Prunus persica* var. *nucipersica* (Borkh.) C. K. Schneid., hybrids), almonds (*Amygdalus communis* L., syn. *P. amygdalus* (L.) Batsch., *Amygdalus dulcis* Mill.), sweet cherries (*Prunus avium* (L.)) and tart cherries (*Prunus cerasus* L.), but was detected from symptomless blackthorn (*P. spinosa* L.) as well (Gáborjányi et al., 2001; Pribék et al., 2001; Salamon and Palkovics, 2002).

- A survey of Sharka disease was conducted in the Central- and

PPV-D

,
PPV-M.

region, but PPV-M was also identified. The apricot and peach orchards were less infected than plum orchards in the northern counties of Hungary.

(M, D Rec).

A triple-mixed infection (M, D and Rec) was identified during the studies. After the survey a map was prepared, the dominant isolates, the infection rate, the host plants and the type of the sample collection places were marked, and a bilingual (HUN-SK) booklet was published containing the results of the survey.

Y

Potato virus Y

, PVY^N (, PVY^O,), PVY^C

The main potato-infecting strains are the common, PVY^O, the necrotic, PVY^N (causing veinal necrosis on tobacco leaves), the stipple-streak strain, PVY^C that lost its aphid transmission ability and PVY^{NTN}, causing severe tuber necrotic symptoms in potato.

PVY^{NTN},

In Hungary several different strains of *Potato virus Y* (PVY) were isolated from potato. PVY-5 belongs to the common "O" strain (PVY^O), PVY-98 and PVY-111 belong to the "N" (PVY^N) and the NTN (PVY^{NTN}) strains, respectively. The PVY-5 differed significantly from the PVY-98 in efficiency of aphid transmission (Basky and Almási, 2005).

Y (PVY)
PVY-5 " " (PVY^O),
PVY-98 PVY-111
"N" (PVY^N) NTN (PVY^{NTN})
PVY-5
PVY-98
(Basky and Almási, 2005).

Aphid transmission of

(90,1-97,6% 92,9-98,9%).

PVY-98 PVY-5

CP HC-Pro

PVY-5

HC-Pro

PVY-98. 23

(-) CP (-)

(-) PVY

(HC-Pro CP).

(ZYMV)

CP case of the CP (90,1-97,6% and 92,9-98,9%, respectively).

- In summary, we conclude that the differences in the vectoring aphid species and the efficiency of aphid transmission between PVY-98 and PVY-5 do not correlate with any amino acid changes in the highly conserved HC-Pro and CP motifs. This may indicate the role of amino acid substitutions at other positions of these proteins. The PVY-5 isolate differed in two amino acid residues in the HC-Pro sequences from all other isolates and in nine residues from PVY-98. Within the CP region there were 23 amino acid variations between the two isolates.

- Some of these changes may affect virus-vector interactions (e.g. binding to the insect cuticle protein(s) of the stylet) or in the case of CP may abolish or alter reactions to certain monoclonal antibodies.

Determination of the exact factors and the details of the mechanism(s) affecting aphid transmissibility of PVY needs further investigation (e.g. with HC-Pro or CP mutant viruses).

Zucchini yellow mosaic virus

- To assess the diversity within Hungarian ZYMV isolates the

ZYMV

12

ZYMV

2008.

ZYMV

99,2%.

97.8 %

ZYMV

ZYMV

ZYMV. N-

(Shukla et al., 1991).

N-

nucleotide sequences of coat protein genes were determined and compared. The selected 12 ZYMV isolates originated from different parts of Hungary, from different hosts (oilseed pumpkin, cucumber, melon, zucchini and winter squash) and were isolated between 1995 and 2008. Six ZYMV isolates originated from oilseed pumpkin seeds transmitting the virus.

The coat protein of the ZYMV isolates showed very high similarity to each other. The nucleotide and amino acid sequence identities were above 97.8 % and 99,2 %, respectively. A phylogenetic tree was constructed from the amino acid sequences of the coat protein of ZYMV isolates from different parts of the world. Isolates from Austria, Slovenia and Hungary clustered together and form a Central European branch. Isolates from China, Taiwan, Japan, Italia and USA are forming other clusters, but isolates from Singapore, China-Shanzi and Florida ZYMV were highly divergent from all other ZYMV strains. The N-terminus is the only region within the entire coat protein that is unique to potyviruses and contains virus-specific and strain specific epitopes (Shukla et al., 1991).

The amino acid sequence identities of the N-terminal region of ZYMV isolates are shown in

ZYMV
 ZYMV
 ()
 (N)
 (D) (K)
 16 17,
 () 27,
 37
 ZYMV .
 .
 ZYMV
 (98-99,8%
 N-
 (Tóbiás and
 Palkovics, 2003).
 62
 ,
 . 19997
 , 16039
 225
 . 25
 ,
 15.34%
 .

2. Figure 2. The ZYMV isolates from
 - Central Europe (Austria, Slovenia,
 - , Hungary) have uniformly
 - asparagine (N) instead of aspartic
 acid (D) and lysine (K) at position
 16 and 17, respectively, alanin (A)
 - substitutes for valine (V) at
 position 27, and valine (V)
 (V) changes to methionine (M) at
 position 37 compared to other
 ZYMV isolates. These sequences
 are stable over a period of 10
 years and are specific only to
 these isolates suggesting a
 common ancestor.

- These results confirm our
 - previous data that Central
 - European ZYMV isolates show
 - high similarity (98-99,8% identity)
 and possess unique motifs in the
 N-terminal region of coat protein
 (Tóbiás and Palkovics, 2003).

- In seedtransmission
 experiments 62 seedlots
 originating from hulles pumpkin
 fruits exhibiting typical symptoms
 of ZYMV infection were tested. In
 total 19997 seeds were sowed,
 16039 plants were monitored and
 225 plants were seedbourne virus
 infected. 25 seedlots showed
 seedborne virus transmission that
 ranged from 0.29 % to 15.34 %
 of the seeds.

ZYMV-Austria-10	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Austria-11	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Austria-12	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Austria-2	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Austria-5	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Austria-6	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Austria-Slovenia-1	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-2	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-fl1	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-sz6	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
zymv-15/1	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
zymv-59/2	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
zymv-80/1	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-5	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-10	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-8	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Hungary-sz3	1	SGTQPTVADAGATKKNNE	DDKGKNKDATGSGSGEKT	MAAVTKD
ZYMV-Taiwan-CY2	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Taiwan-PT5	1	SGTQPTVADAGATKKDKED	DKGKNKDVAS	SGSGEKTVA
ZYMV-Korea-A	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Taiwan-TN3	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Austria-Berlin-1	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Austria-Italy	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-China-Hangzhou	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Japan-M39	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-USA-California	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-USA-Connecticut	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Japan-M	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Taiwan-TNML1	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-China-Beijing	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Taiwan-TC1	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Israel	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Taiwan-NT1	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-China-Hainan	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-China-Ningbo	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Japan-169	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-USA-Florida	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Korea-cu	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-China-Shanxi	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA
ZYMV-Singapore	1	SGTQPTVADAGATKKDKED	DKGKNKDVTS	SGSGEKTVA

. 2.

ZYMV

N-

Fig. 2. Amino acid sequence alignment for the N-terminal of the coat protein of ZYMV isolates.

/ REFERENCES

1. **Basky Zs. and Almási A.** 2005. Differences in aphid transmissibility and translocation between PVY^N and PVY^O isolates. *Journal of Pest Science*, 78, 67-78.
2. **Blanc S., LopezMoya J. J., Wang R. Y., Garcia Lampasona S., Thornbury D. W. and Pirone T. P.** 1997. A specific interaction between coat protein and helper component correlates with aphid transmission of a potyvirus. *Virology*, 231, 141-147.

3. **Danci Oana, Angelika Ziegler, Lesley Torrance, Gasemi S., Danci M.** 2009. Potyviridae Family – short review. *Journal of Horticulture, Forestry and Biotechnology*, Vol. 13 pp. 410-420.
4. **Gáborjányi R., Palkovics L. and Pribék D.** 2001. Plum pox virus in Hungary: Attempts to solve the problem. *Georgikon Agriculture*, 12:1, 71-85.
5. **Harrison B. D. and Robinson D. J.** 1988. Molecular variation in vector-borne plant viruses: Epidemiological significance. *Philos. Trans. R. Soc. London Biol.* 321, 447-462.
6. **Janson B.F. and Ellett C.W.** 1963. A new corn disease in Ohio. *Plant Disease Reporter*, 47: 1107-1108.
7. **Pirone T. P.** 1991. Viral genes and gene products that determine insect transmissibility. *Seminars in Virology*, 2, 81-87.
8. **Pirone T. P. and Blanc S.** 1996. Helper-dependent vector transmission of plant viruses. *Ann. Rev. Phytopathol.* 34, 227-247.
9. **Pribék D., Palkovics L. and Gáborjányi R.** 2001. Molecular characterization of *plum pox virus* almond isolate. *Acta Horturae*, 550, 91-95.
10. **Riechmann J. L., Lain S., Garcia J. A.** 1992. Highlights and prospects of potyvirus molecular biology. *J. Gen. Virol.* 73, 1-16.
11. **Salamon P. and Palkovics L.** 2002. Characterization of *Plum pox virus* PPV-BT-H isolated from naturally infected blackthorn (*Prunus spinosa* L.) in Hungary. *European Journal of Plant Pathology*, 108, 903-907.
12. **Szirmai J., Paizs L-né** 1963. Maize mosaic or stripe. *Növénytermelés* 12: 43-60. (in Hungarian with English summary)
13. **Tóbiás I. and Palkovics L.** 2003. Characterization of Hungarian isolates of zucchini yellowmosaic virus (ZYMV, potyvirus) transmitted by seeds of *Cucurbita pepo* var. *Styriaca*. *Pest Management Science* 59, 4: 493-499.
14. **Tóbiás I., Bakardjieva N. and Palkovics L.** 2007. Comparison of Hungarian and Bulgarian Isolates of Maize dwarf mosaic virus. *Cereal Research Communications* 35: 1643-1651.
15. **Wang R. Y., Powell G., Hardie J. and Pirone T. P.** 1998. Role of the helper component in vector-specific transmission of potyviruses. *Journal of General Virology*, 79, 1519-1524.