

**CALLAPHIS**

**JUGLANDIS FRISCH. HROMAPHIS JUGLANDICOLA KALT.**  
**(HEMIPTERA: APHIDIDAE)**  
**(JUGLANS REGIA L.)**

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**NATURAL ENEMIES OF APHIDS CALLAPHIS JUGLANDIS**  
**FRISCH. AND HROMAPHIS JUGLANDICOLA KALT.**  
**(HEMIPTERA: APHIDIDAE) ON ENGLISH WALNUT**  
**(JUGLANS REGIA L.) IN PLOVDIV REGION**

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**SUMMARY**

(*Juglans regia* L.)  
 , *Callaphis juglandis* Frisch.  
*hromaphis juglandicola* Kalt.  
 (Hemiptera: Aphididae).  
*C. juglandis*

juglandicola, h.  
 juglandis h. juglandicola

Two species of aphids attack Persian (English) walnut (*Juglans regia* L.) in the region of Plovdiv, *Callaphis juglandis* Frisch. and *Chropmaphis juglandicola* Kalt. (Hemiptera: Aphididae). Walnut aphids *C. juglandis* develops on the upper side of leaves and formed numerous colonies, which suck juice around the main vein.

*C. juglandis* is much more common species, in comparison with the small walnut aphid, *Ch. juglandicola*, which settles and feeds on the underside of leaves. *C. juglandis* and *Ch. juglandicola* are potential pests of the Persian walnut in the region of Plovdiv. Present study was carried out to know the natural enemies of walnut aphids in the region of Plovdiv and their population dynamics and

possibilities for biological control of aphids.

The study was conducted in untreated walnut orchards of two different ecological regions – Plovdiv and Asenovgrad. Large number predators and parasitoids, belonging to the orders Coleoptera, Diptera, Neuroptera and Hymenoptera are found in the colonies of walnut aphids.

Although these predators are considered to play an important role in the regulation of aphid populations, they did not prevent walnut aphid damages. Some possible causes for this ineffectiveness are discussed.

**Key words:** aphids, walnut, predators, parasit ids

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**INTRODUCTION**

The common walnut (*Juglans regia* L.) is a walnut species of the family Juglandaceae which takes the third place in nuts production after cashews and almonds (FAOSTAT, 2011). In Bulgaria there are proper conditions for development of the common walnut and it is one of the most widely spread shell fruit crops species, grown for a long time. The walnut as an orchard species is attacked by a large number of pests among which the aphids *Callaphis juglandis* Frisch. and *hropmaphis juglandicola* Kalt.) are some of the most common phytophagous species.

(*Juglans regia* L.)  
 Juglandaceae,  
 (FAOSTAT, 2011).

*Callaphis juglandis* Frisch.  
*hropmaphis juglandicola* Kalt.)

*C. juglandis* Frisch.

- frequent and deleterious aphid species on walnuts in Bulgaria.

- The damages caused by this species are bigger than those caused by *h. juglandicola*. The latter is more rarely met in our country and usually occurs in lower numbers. The aphids of *C. juglandis* occupy the top of the leaves, grouped along the mid-vein where they suck plant juice, while those of *h. juglandicola* feed on the lower side of walnut leaves, mainly on the terminal leaflet of the compound leaf, usually along the veins.

The damages caused by two species of aphids are expressed in reduction of tree vigour as well as in nut size, yield, and quality. In addition to the direct damages, they cause indirect damages excreting abundantly amounts of honey-dew, which falls onto nut, leaves and shoots. On the contaminated with honey-dew organs subsequently develop the black sooty mould fungus. This fungus reduces light penetration to the leaf surface reduces its photosynthetic capacity. Furthermore, it form d coating which can cause death of the infected organs. At high temperatures the presence of such coating could lead to the occurrence of sunburn and become a reason for drying of the kernels.

(>15 | At high density (>15 pcs /leaf)

aphids can cause defoliation of the trees and contribute even more to the appearance of sun-scorching on the fruit. If the density of aphids keeps this level for more than 15 days, it can affect the quality of the fruit in the current year and reduce the yield in the coming year (Barnes & Sibbett, 1990).

(Barnes & Sibbett, 1990).

Natural enemies play an important role in regulating the aphid populations on walnuts. When the aphids feed on the organs of the plants; they are attacked by a large number of natural enemies (predators and parasitids).

In Bulgaria, focused studies to ascertain the species of predators and parasitoids on walnut aphids have not been held. The objective of this study is to investigate the natural enemies of *Callaphis juglandis* Frisch. and *hropmaphis juglandicola* Kalt and to estimate their effectiveness in populations control.

*Callaphis juglandis* Frisch.  
*hropmaphis juglandicola* Kalt

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## MATERIAL AND METHODS

The experiments took place in 2013 in two walnut orchards in Plovdiv and Asenovgrad. These orchards were observed once every 10 days through the whole year.

	20		In each garden 20 trees were examined and 5 leaves collected from each tree, a total of 100 leaves from a garden. The number of aphids and their natural enemies was registered after every examination in the respective garden. The eggs and larvae of predators, as well as the infested aphids, with the leaves, were taken to the laboratory to define their species.
	5		
100			
<i>juglandis</i>	<i>h. juglandicola</i>		The eggs and the preimaginal stages of the life cycle of the predator insects were grown individually in glass containers on <i>C. juglandis</i> and <i>h. juglandicola</i> at temperature 23-25 and 75% humidity. The identification of predatory species is done with the help of identification key for insect species in adult stage, Dorokhova et al. (1989).
	23-25	75%	
(1989).			Dorokhova et al.

## RESULTS AND DISCUSSION

### Aphids

<i>juglandicola</i>	<i>C. juglandis</i>	<i>h.</i>	The appearance of the two species of aphids, <i>C. juglandis</i> and <i>h. juglandicola</i> , in the investigated gardens was registered in the end of April. In the beginning of May the first colonies of the two species were observed; those of <i>C. juglandis</i> on the top of the leaves and of <i>h. juglandicola</i> —on the lower side. The peak of multiplication of aphids was observed in the middle of June in
<i>juglandis</i>	<i>h. juglandicola</i>	<i>C.</i>	

( .1)

the region of Plovdiv, and in the end of June – in the region of Asenovgrad. From the beginning of July the density of aphids started to decrease and with small fluctuations this tendency continued until the end of the vegetation period (Fig. 1).

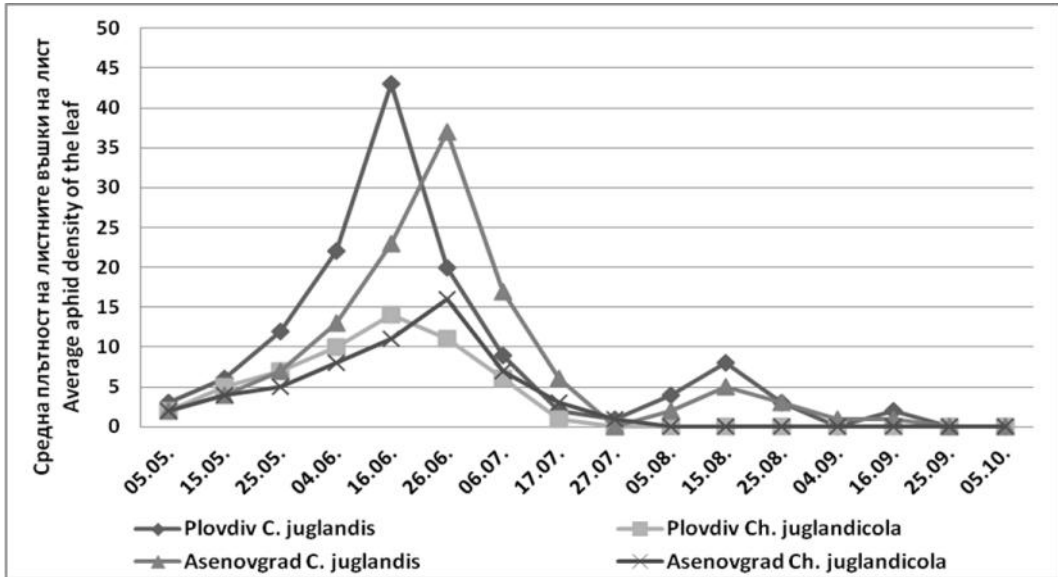


Fig. 1. Population dynamics of walnut aphid *C. juglandis* *h. juglandicola* in the region of Plovdiv and Asenovgrad in 2013

*C. juglandis* *h. juglandicola*  
 15  
 Hemiptera, Coleoptera,  
 Neuroptera Diptera, 1  
 Hymenoptera,  
 Braconidae.

### Natural enemies

In the course of the research in the colonies of *C. juglandis* and *h. juglandicola* were identified 15 species of predatory insects, belonging to four orders Hemiptera, Coleoptera, Neuroptera and Diptera, and 1 parasitoid species, representative of order Hymenoptera, Family Braconidae. The submitted data about the species and frequency of occurrence of aphidophagous

( 1)

Coccinellidae),  
 (Diptera: Syrphidae)  
 (Neuroptera: Chrysopidae).  
 Anthocoridae Miridae

Haliday  
 Braconidae),  
*Trioxis pallidus*  
 (Hymenoptera:  
*h. juglandicola*.

*h.*  
*juglandicola*

*C. juglandis*,

( 1).

show (Table 1), that aphid's populations on walnuts are controlled by a large number of natural enemies, including predators and parasitoids. The most frequent in occurrence and number of the predatory insects are the predatory ladybetles, (Coleoptera: Coccinellidae), followed by the larvae of Syrphid flies (Diptera: Syrphidae) and lacewings (Neuroptera: Chrysopidae). The predatory pirate bugs of family Anthocoridae and Miridae and the larvae of the brown-winged lacewings were observed to feed with aphids too, but their occurrence on the leaves was most probably connected not only with these food hosts.

Only one of the parasite species was found in this study *Trioxis pallidus* Haliday (Hymenoptera: Braconidae), which parasitized only in the colonies of *h. juglandicola*. Although they different species of predators feed on wide range of plants, they do not show the same preference to the different types of aphids. The colonies of *h. juglandicola* are attacked by more predators but in lower numbers unlike the colonies of *C. juglandis*, in which smaller number of predators occurs but in higher numbers (Table. 1).

**Table 1. Species composition and frequency of natural enemies of walnut aphids in the region of Plovdiv and Asenovgrad in 2013**

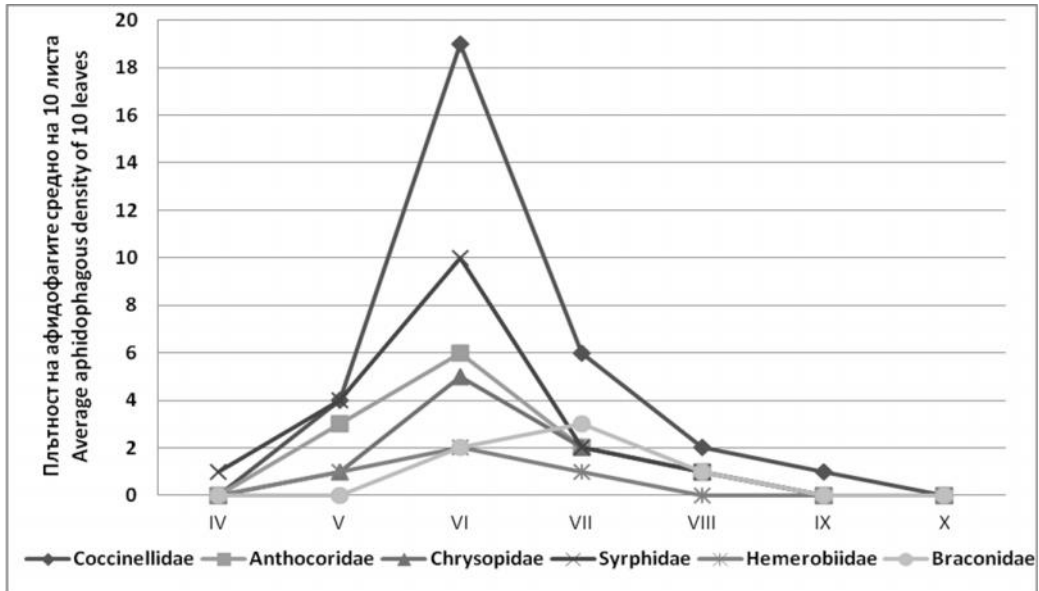
Order Suborder	Family	Species	Region			
			Plovdiv		Asenovgrad	
			C. juglandis	h. juglandicola	C. juglandis	h. juglandicola
Coleoptera	Coccinellidae	<i>Adalia bipunctata</i> L.	**		***	
		<i>Adalia decempunctata</i> L.	***	*	***	**
		<i>Adonia variegata</i> Gz.	*		*	**
		<i>Coccinella septempunctata</i> L.	*		*	*
		<i>Syncharmonia conglobata</i> L.		*		
		<i>Scymnus subvilosus</i> Gz.	**	*	*	*
Hemiptera / Heteroptera	Anthocoridae	<i>Anthocoris nemoralis</i> F.		*		*
		<i>Orius niger</i> Wolff.				*
	Miridae	<i>Deraeoeoris luteseens</i> Schill.		*		*
Neuroptera	Chrysopidae	<i>Chrysopa carnea</i> Stef.	**	**	**	**
		<i>Chrysopa formosa</i> Br.	*	*	*	*
		<i>Chrysopa perla</i> L.	*		**	*
	Hemerobiidae	<i>Hemerobius humulinus</i> L.		*		*
Diptera	Syrphidae	<i>Episyrphus balteatus</i> DeGeer	**	**	**	**
		<i>Metasyrphus corollae</i> F.	*	*	*	*
Hymenoptera	Braconidae	<i>Trioxis pallidus</i> Haliday		**		**

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-  
*Episyrphus balteatus*  
DeGeer  
*Metasyrphus corollae* F.  
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.  
*A. bipunctata* L.  
*decempunctata* L.

( .2 3).

In May the most frequent and numerous were the larvae of the syrphid flies *Episyrphus balteatus* DeGeer and *Metasyrphus corollae* F., although in the aphid colonies were found other predatory species as well, mostly old larvae of predatory pirate bugs, ladybirds, lacewing larvae but in lower numbers. The predatory ladybetles *A. bipunctata* L. and *A. decempunctata* L. were widely spread too (Fig. 2 and 3).

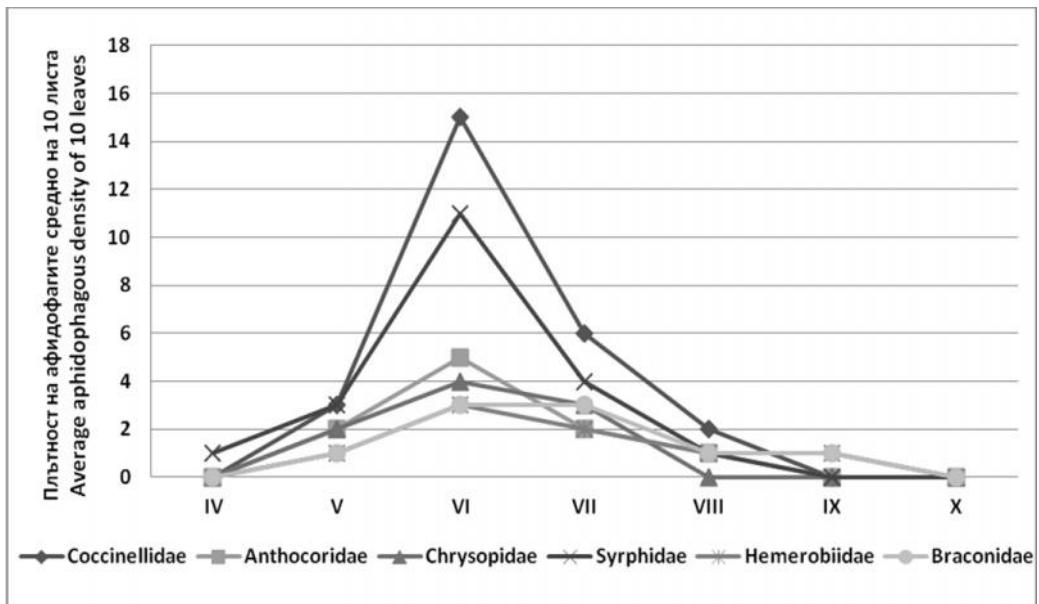




. 2.

2013

Fig. 2. Population dynamics of natural enemies of walnut aphids in the region of Plovdiv in 2013



. 3.

2013

Fig. 3. Population dynamics of natural enemies of walnut aphids in the region of Plovdiv in 2013

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*Adalia bipunctata* L. A.  
*decempunctata* L,

- ( .2 3).

*pallidus*,

( . 1).

*h. juglandicola*,

*Trioxis*

*h. juglandicola*

At the moment of mass multiplication of aphids (the second half of July), *Adalia bipunctata* L. and *A. decempunctata* L occurred most frequently and were most numerous. In the aphid colonies were found other species of ladybetles, larvae of syrphid flies, pirate bugs and lacewings as well but in lower numbers (Fig. 2 3).

In July, due to the decrease in the number of the aphids, the density of aphidophagous fell down sharply and this tendency continued in the following months. Under the conditions of the gradually decreasing number of aphidophagous, the predatory ladybetles kept their dominance over the rest of the predatory species.

In the peak of the mass multiplication, the aphids in the colonies of *h. juglandicola*, except by predators, began to notice more and more individuals, parasitized from *Trioxis pallidus*, whose rate gradually increased. This led to a sharp decline in the population size of *h. juglandicola* (Fig. 1).

Summarizing the results of the research, we can conclude that the predators and parasitoids are capable of keeping populations only of *h. juglandicola* below the economic injury level but not those of C.

*h. juglandicola*,  
*C. juglandis*.

*h. juglandicola*

*juglandicola*

(Magnussen & Hansen, 2014),  
Heie (1982)

*pallidus*,

*C. juglandis*

*juglandis*. The populations of *Ch. juglandicola* have a better controlled by their natural enemies. Our explanation of this is, on the one hand, the lower population numbers of *Ch. juglandicola* in this study and on the other hand, the fact that colonies of this species are not infested by ants to protect them from their natural enemies.

The lack of ants in the colonies of *h. juglandicola* was confirmed in other similar studies (Magnussen & Hansen, 2014), however Heie (1982) reports that ants could be found in the colonies of the two species of walnut aphids.

The larvae of syrphid flies, predatory ladybirds, lacewings and the rest of the predatory species, together with the parasitoid *T. pallidus* can regulate the populations of *h. juglandicola* at an innocuous economic injury level without the use of insecticides.

At the same time, the same aphidophagous except *T. pallidus*, which does not parasitize on this species of aphids, can not keep the populations of *C. juglandis* below the injury level and prevent damages.

The reason for this is the significant difference in the rate of development of the predator and the victim which is most indicative

(Dixon *et al.*, 1997).

( )

*h. juglandicola*,

*C. juglandis*.

with the predatory ladybetles (Dixon *et al.*, 1997). Predators reproduce sexually and their victims – asexually (parthenogenetically).

Thus they can not respond either functionally or numerically the growing numbers of its victims. Another reason to consider explaining their inefficiency could be the fact that a large part of their larvae prefer to feed initially in the colonies of *h. juglandicola*, which are not protected from the influence of the ants and only when they destroy them to move into colonies of *C. juglandis*.

### CONCLUSIONS

1. *Callaphis juglandis* Frisch. and *hropmaphis juglandicola* Kalt. (Hemiptera: Aphididae)

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Hemiptera, Coleoptera, Neuroptera  
Diptera, 1  
Hymenoptera,  
Braconidae.

2. *hropmaphis juglandicola*

3.

1. The colonies of walnut aphids *Callaphis juglandis* Frisch. and *hropmaphis juglandicola* Kalt. (Hemiptera: Aphididae) are attacked by a large number of natural enemies, including 15 species predatory insects, belonging to the order Hemiptera, Coleoptera, Neuroptera and Diptera, and 1 parasitoid species of order Hymenoptera, family Braconidae.

2. Colonies of walnut aphid *Chromaphis juglandicola* are few in number and do not attend ants, which contributes to their better biological control.

3. Natural enemies can regulate populations of

	<p><i>h. juglandicola</i>   <i>hropmaphis juglandicola</i> of economic harmless level without the use of insecticides.</p>
<p>4. <i>Callaphis juglandis</i></p>	<p>4. The natural enemies of <i>Callaphis juglandis</i> play an important role in regulating the populations of the species but they are not able to keep them below the injury level and prevent from causing damages.</p>

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**(GUIGNARDIA BIDWELLII (ELLIS))**

**VIALA & RAVAZ)**

**2014**

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**MANIFESTATIONS OF BLACK ROT (*GUIGNARDIA BIDWELLII* (ELLIS) VIALA & RAVAZ) IN CONVENTIONAL AND ORGANIC CULTIVATION OF VINES IN THE CONDITIONS OF YEAR 2014**

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**SUMMARY**

2014 . -

- Routing investigation was carried out during the vegetation period of 2014 at IVE-Pleven for black rot spread in vineyards for conventional and organic grapes production.

, The agro-climatic conditions of the year were analyzed as a precondition for the development of vine fungal diseases. The results showed almost tenfold stronger attack of black rot in organic cultivation compared to conventionally grown varieties.

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1.9% 18.5%.

- The lowest rate of the disease spread was recorded for Muscat Kaylashki variety for both systems of cultivation, 1.9% and 18.5%, respectively. High sensitivity was found for Muscat Ottonel variety, which had almost 100% infested vines in the organic cultivation. It was accounted a relatively weak attack on berries and clusters that was probably due to the more frequent use of fungicides against

, 100%

- , : , , ( black rot) . 1885 . Viala Ravaz, - , ( , 1956 1963). - 2002/2003 . (Ullrich et al, 2009)

downy mildew and powdery mildew, some of which were effective against black rot too.

**Key words:** black rot, varietal susceptibility, organic cultivation, vine

**INTRODUCTION**

Black rot until recently was considered almost unknown vine disease in Bulgaria. It was brought to Europe from North America. In 1885 it was observed in France by Viala and Ravaz, who reported it for the first time in phytopathological literature.

This was the most spread and harmful disease in the humid regions of the southern states and along the Gulf of Mexico (Brown, 1956 acc. to Vanev 1963). Later, the disease was found in Italy, Russia, Georgia, Azerbaijan, Ukraine and others. Since 2002/2003, black rot occurred regularly in the organic vineyards in the regions around Moselle and the Middle Rhine valley in Germany (Ullrich et al., 2009).

In Bulgaria black rot was thought to be not so widely spread however in certain years of wet weather and very frequent rainfall it may cause significant losses.

It attacked most often the vineyards located along the Black Sea coast and those in lowlands,

., 1963; (, 2003).

1904 . ( (1960), , 1963).

1963 .). 1963 . (*Phoma uvicola* (Viala et Rawaz), 100 198 µm

µm 3,1 5 11,5 5 µm

*Guignardia bidwellii* (, 1963;

not well aired (Raykov et al., 1963; Malenin, 2003).

In our country the disease was detected for the first time by Kozarev (1904) in Vidin (Raykov et al., 1963). Margaritov (1960) found black rot on vine leaves, shoots and berries in Pomorie region.

At the same time the disease was found on ripened berries of Bolgar variety in the experimental field of IVE-Pleven.

In 1961, limited manifestations of black rot were observed in Pleven, Ruse, Plovdiv and Vidin districts (Vanev, 1963).

In 1963 Vanev isolated the fungus *Phoma uvicola* (Viala et Rawaz), which had black, spherical or slightly flattened pycnidia, 100 to 198 µm in diameter, and a circular ostiole from which an exudate of pycnidia spores leaked out.

Exposed to air the exudate dried out in the form of yellowish ribbons releasing the pycnidia in water. The size of the spores varied between 5 and 11.5 µm in length and 3.1 to 5 µm in width.

There is morphological diversity of spores – with rounded corners and sharp at both ends. In our country the pathogen overwinters in the infested parts as pycnidia while the teleomorph *Guignardia bidwellii* has not been detected (Vanev, 1963; Vanev, 1995).



, 1995).  
*Phyllosticta ampellicida* (Englem.) Van Der Aa = *Phoma uvicola* (Viala et Rawaz)  
*Guignardia bidwellii* (Ellis) Viala & Ravaz (Wikee et al., 2011). *G. bidwellii*

8  
 1963; Wilcox (2003),  
 15,5° 29° ,  
 6 9  
 Molitor et al. (2012),  
*G. bidwellii*

6 24°

Causative agent of black rot on grapevine is *Phyllosticta ampellicida* (Englem.) Van Der Aa = *Phoma uvicola* (Viala et Rawaz) with telemorph *Guignardia bidwellii* (Ellis) Viala & Ravaz (Wikee et al., 2011). *G. bidwellii* is ascomycetous fungus, forming pseudothecia with cylindrical or club-shaped ascus containing 8 monocellular hyaline ascospores (Vanev, 1963; Harizanov et al., 2009).

Wilcox (2003) described the infection of black rot at different temperatures and moisture. The shortest period of spreading the infection was observed at temperatures within the range of 15.5° to 29° , for which only 6 to 9 hours were needed and high humidity. According to Molitor et al. (2012), the incubation period of *G. bidwellii* was strongly influenced by temperature. In this regard there has been developed a pattern according to which, the first symptoms of the disease on the leaves appear after reaching the threshold of 175 cumulative degree-day, calculated on the base of the sum of average daily temperatures between 6 and 24°C from the day after infection.

Typical symptoms are the occurrence of spots on the vine green parts, leaf petioles, leaf stems and shoots. Leaves are susceptible to infection about a week after sprouting.

2-10mm

Yellow-brownish rounded spots of different sizes from 2-10mm appear on leaves one to two weeks after infection. Small, black pycnidia in the form of a ring appear soon in wet weather. Black oval lesions occur on shoots.

The flower clusters and berries are sensitive from the period of the caps falling to interspersing. Small whitish spots surrounded by a reddish ring are noticed at first on the berries.

The infected berries become whitish or brown and quickly darken, covered with pycnidia, wither and mummify. The early researchers thought that only young above ground parts were susceptible to infection by *G. bidwellii*, i. e., there was age-related resistance, but according to Hoffman et al. (2004), conidia could infect the leaves of all ages and the occurrence of the fruit ontogenetic resistance was not well documented.

Hoffman et al. (2004),

In Bulgaria until recently there were no registered licensed plant protection products against black rot.

The main practices used were aimed at reducing the size of infection, namely: collection and destroying of all infected parts, spring plowing of attacked vineyards, pruning of infested shoots at rest and their destruction

(Kostadinova 2011).

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DMI

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2003).

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(DMI)

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(Hoffman and Wilcox, 2002;  
Hoffman and Wilcox, 2003;  
Hoffman et al, 2004).

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85%  
(Molitor et

al., 2012).

2014

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(Kostadinova 2011).

In France and Switzerland plant protection products (PPP) with active substances from different groups are applied for the disease control: Strobilurin – trifloxystrobin + tebuconazole, DMI fungicides – tebuconazole, myclobutanil, flusilazole, penconazole; Dithiocarbamates – metiram, mancozeb (Kostadinova, 2011; Malenin 2003).

In the USA the most effective preparations for black rot control are (DMI) fungicides with active substance myclobutanil and tebuconazole (Hoffman and Wilcox, 2002; Hoffman and Wilcox, 2003; Hoffman et al., 2004). Full control of the infection development on leaves and clusters may be achieved by the use of strobilurin and pyraclostrobin, even after the expiry of more than 85% of the incubation period (Molitor et al., 2012).

The aim of this study was to assess the development of black rot in organic and conventionally grown vineyards in terms of 2014 as a basis for future analysis and assessment of the risk of diseases not covered by the approved fungicide products for organic production.

## MATERIAL AND METHODS

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Data  
Analysis MS Excell (1997-2003)  
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iMETOS 000003CA (Pessl  
Instruments GmbH).

Routing investigation was carried out during the vegetation period of 2014 at the Experimental Base of IVE-Pleven for black rot spread in vineyards for conventional and organic grapes production.

The investigation included the wine varieties: Muscat Ottonel, Muscat Kaylashki and Rubin – conventional cultivation as well as Muscat Ottonel, Muscat Kaylashki, Cabernet Sauvignon and Drujba – organic cultivation.

Account is taken of number of vines with symptoms of the disease by variety and repetition in the two variants of cultivation. The results for the disease spread as a rate of attacked vines per varieties, were processed by the methods of descriptive statistics from Data Analysis package of MS Excell (1997-2003).

The climatic factors temperature, rainfall and relative humidity were recorded by an electronic weather station iMETOS 000003CA (Pessl Instruments GmbH).

## RESULTS AND DISCUSSION

2014

The weather factors influencing the development of the main vine diseases in 2014 were distinguished from the previous two years in several aspects.

( . 1).

For the region of Pleven the monthly rainfall in April was almost three times higher compared to 2012 and 2013 (Fig. 1).

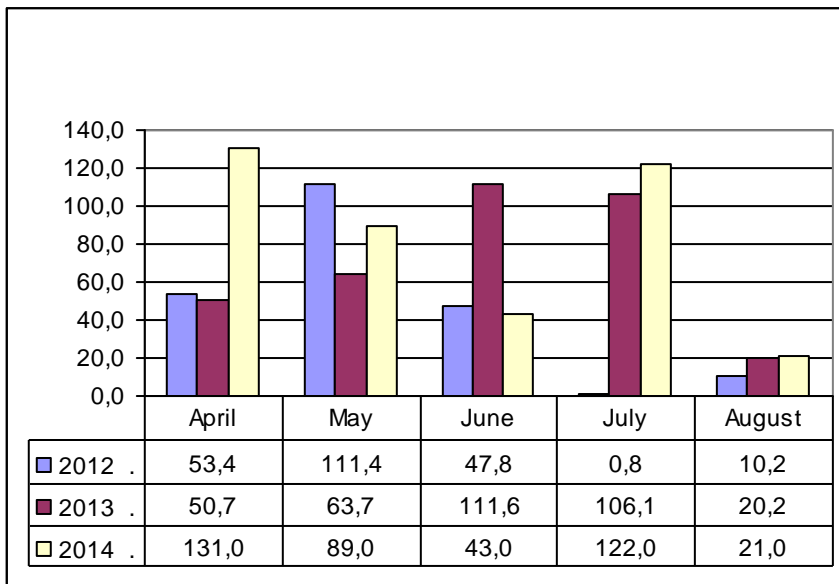


Fig.1. Month sum of the rainfall (mm)

2013

70% ( . 2).

That was a favourable prerequisite concerning soil moisture and air humidity for fungal diseases development already in early May. The rainfalls continued in May and June, but unlike in 2013 they remained unchanged in July too.

Consequently, the average relative humidity during the whole vegetation period remained above 70% (Fig. 2).

The maximum air temperature in July with values that typically have an inhibiting effect on fungal

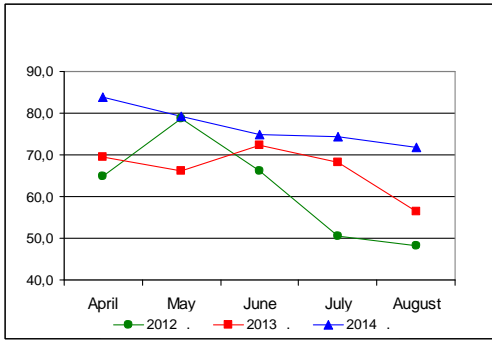
2014

35°

( . 3).

diseases development in 2014 did not exceed 35°C unlike in the previous two years (Fig. 3).

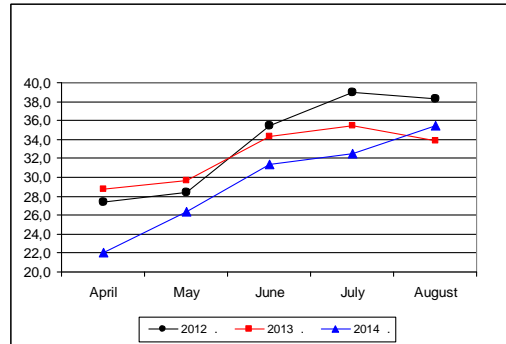
In aggregate all these factors created an extreme situation regarding the disease control.



. 2.

[%]

Fig. 2. Relative humidity [%]



. 3.

[°C]

Fig. 3. Max temperature of the air [°C]

2014

( . 4

. 5).

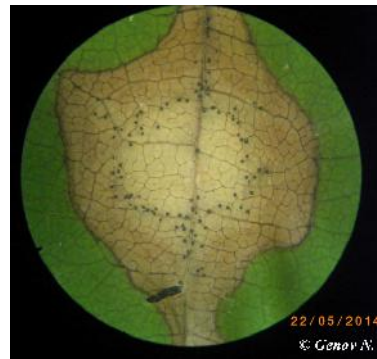
The first symptoms of black rot on leaves in 2014 appeared in the middle of May (Fig. 4 and Fig. 5). The continued frequent precipitation and the temperature in the range between 20 and 30°C degrees in June and July were favourable for the disease development.

30°

20



. 4.



. 5.

Of the examined conventionally grown varieties (Table 1), the highest rate of black rot infested leaves on vines was reported for Rubin variety (8.0%). The smallest rate of attack was accounted in Muscat Kaylashki variety (1.9%). From the organic cultivated varieties (Tabl. 1), the varieties Muscat Ottonel (97.8%) and Drujba (88.3%) were the most affected. The lowest rate of infested vines was reported again for Muscat Kaylashki variety (18.5%).

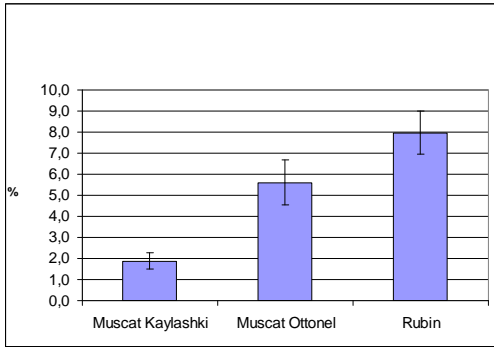
1.

**Table 1. Average values for spread of black rot on conventional and organically grown varieties**

/ Variety	/ Mean [% ± SE]	
	Conventional	Organic
Muscat Kaylashki	1.9 ± 0.4	18.5 ± 4.1
Muscat Ottonel	5.6 ± 1.1	97.8 ± 1.6
Rubin	8.0 ± 1.0	n. d.
Drujba	n. d.	88.3 ± 5.1
Cabernet Sauvignon	n. d.	53.3 ± 4.7

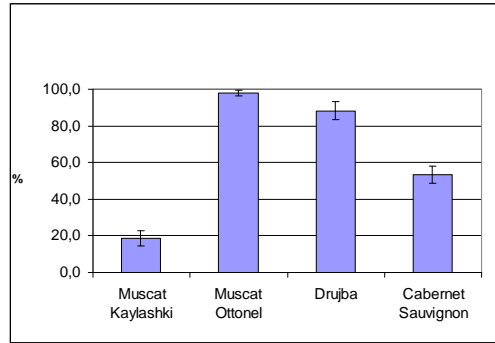
SE – standard error of mean; n. d. not determined

The results showed almost tenfold stronger attack of black rot in organic cultivation compared to conventionally grown varieties (Fig. 6 and Fig. 7).



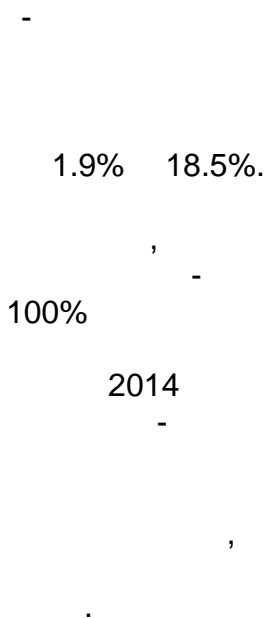
6.

Fig. 6. Black rot on conventionally grown varieties



7.

Fig. 7. Black rot on organically grown varieties



The lowest rate of the disease spread was recorded for Muscat Kaylashki variety for both systems of cultivation, 1.9% and 18.5%, respectively. High susceptibility was found for Muscat Ottonel variety, which had almost 100% infested vines in the organic cultivation. In 2014 it was accounted a relatively weak attack on berries and clusters that was probably due to the more frequent use of fungicides against downy mildew and powdery mildew, some of which were effective against black rot too.

### CONCLUSIONS

The organic grapes production has much higher risk of emergence and spread of black rot in comparison with conventionally grown vineyards.

For overcoming this problem it is of great significance the



implementation of all agricultural and other measures limiting the spread of the disease in vineyards including the proper choice of a variety and location for planting.

Muscat Kaylashki variety is suitable for organic farming concerning black rot disease. The susceptible varieties Rubin and Muscat Ottonel are suitable for control varieties in testing new active substances against black rot on vine.

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## COMPARATIVE ECONOMIC ASSESSMENT OF THE RESULTS OF TESTED IRRIGATION METHODS IN VINE NURSERY

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### SUMMARY

The study was based on the results derived from an experiment carried out in the nursery of the Institute of Viticulture and Enology-Pleven in 2006-2007. The rooted cuttings were from Misket Kaylashki variety grafted to Berlandieri x Riparia CO4 rootstock.

4.

There had been a comparative study of irrigation by sprinkling and micro-sprinkling and drip irrigation with micro-sprinkling for cooling watering and micro-sprinkling. The objective of this paper is to make an economic assessment of the tested irrigation methods, based on a system of natural and value indicators – average yield, production costs, total output, net income, prime cost and rate of return.

The best economic results were obtained for the variant of drip irrigation with micro-sprinkling where the rate of premium vines was the highest – 57.5%.

It justified that variant of vine water supply

57,5%.

to be preferred while micro-sprinkling to be used to regulate the microclimate in the nursery.

**Key words:** vine propagation material, nursery, irrigation regime, economic effect

## INTRODUCTION

The irrigation regime, as a part of vine propagation material production technology is of great importance for obtaining a high percentage of premium grafted rooted vines (Tsvetanov and Kumanov, 2010; Tsvetanov and Kumanov, 2011).

The most commonly used in nursery practice irrigation methods for vine nursery irrigation are gravity, sprinkling and drip irrigation.

The studies of Magriso et al. (1968) showed that the effect of sprinkling for wetting the active soil layer and the ridge was equal to that of gravity irrigation, as far as there were no observed differences in the rate of obtained premium vines.

The advantages pointed in literature of sprinkling to gravity irrigation consisted of increased air humidity, favouring good callus formation between the graft and the rootstock; more rational distribution of irrigation water; ensuring suitable microclimate in the grafted zone, resulting from

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 ( , 1983;  
 , 1968;  
 , 1984).  
 -  
 ,  
 - 2,5 3,6 ,  
 ,  
 ( , 1968;  
 , 1983).  
 35

better wetting of the surface soil layer; limiting the adverse effects on soil structure; increasing the opportunities for mechanizing the main processes in the vine nursery (Magriso et al., 1968; Nikov et al., 1983; Radulov, 1984).

Economically, the benefits of sprinkling irrigation included lower prime cost of watering, higher efficiency of labour - by 2.5 to 3.6 times, depending on the rain intensity and increased economic profit resulting from the higher percentage of usable area (Magriso et al., 1968; Nikov et al., 1983).

In the last 35 years micro-irrigation systems - drip and micro-sprinkling has been introduced in practice. The advantage of drip irrigation in nurseries to the gravity and sprinkling ones resulted from the higher efficiency of irrigation water and the opportunities for savings, as well as the simultaneous limiting of weeds development.

A disadvantage of drip irrigation was that it could not maintain high air humidity, particularly important for the vines in the conditions of extremely high temperatures

during the hottest hours of the day.

45

There is a variant combining the methods of sprinkling and drip irrigation, as sprinkling is applied during the first 45 days after planting of the grafted cuttings to ensure air humidity in the period of coalescence between the graft and the rootstock while drip irrigation is applied to wet the active soil layer to the limit soil water capacity (LSWC).

Thus the advantages of both methods for irrigation in the nursery could be used however the application of this variant was limited due to the high capital costs for the two irrigation systems.

Besides the high air humidity (vital to the vines development) and the good subsurface distribution of irrigation water in the soil, micro-sprinkling as an irrigation method in vine nursery provided the opportunities for introducing with irrigation water of plant protection chemicals, fertilizers and bio-stimulators, as well as continuous operation during the hottest hours of the day under low intensity of rain.

That variety of irrigation methods raises the question of a method of choice to be applied in the vine propagation material

production technology ensuring a high rate of premium vines to be obtained, as well as increasing the economic efficiency of the production process.

The objective of this study is to make a comparative economic assessment of the results from the application of different methods of irrigation in the vine nursery.

### MATERIAL AND METHODS

The research work was carried out in 2006-2007 in the vine nursery of the Production and Experimental Base of the Institute of Viticulture and Enology-Pleven.

In 2006, a comparative study of sprinkling and micro-sprinkling irrigation was performed for determining the suitability of both methods for vine nursery irrigation. The trial was conducted in the following variants:

Variant 1 – irrigation with S-Mmark II sprinklers, with flow rate  $q = 31 \text{ L min}^{-1}$  ( $1860 \text{ L h}^{-1}$ ), at 0.3 pressure and radius of operation  $r = 15 \text{ m}$ . A square pattern of placement was used with distance between the sprinklers  $= 1.42r = 21 \text{ m}$ , thus the irrigated area by one sprinkler was  $F_i = 2r^2 = 450 \text{ m}^2$ , and the intensity  $i = q/F_i = 4.13 \text{ mm/h}$ .

Variant 2 – irrigation with

2006-2007 .

2006

1 – „S-Mmark II”.  
 $q = 31 \text{ L min}^{-1}$  ( $1860 \text{ L h}^{-1}$ ),  
 0.3  
 $r = 15 \text{ m}$ .  
 $= 1,42 r = 21$   
 $F = 2r^2 = 450 \text{ m}^2$ ,  
 $i = q/F = 4,13 \text{ mm/h}$ .  
 2 –

„Water Bird VI Clasic”  
 156 L h<sup>-1</sup>, 0.2  
 q =  
 r = 5.0 m.  
 = 1,42r = 7 m,  
 F = 2r<sup>2</sup> = 50 m<sup>2</sup>,  
 i=q/F = 3,12 mm/h.

:  
 $m = 10Hr(S_{LSWC} - S_{LLOMP}) C(spr.)$ ,  
 m<sup>3</sup>.da<sup>-1</sup>, ( , 1980),  
 (m);  
 (g cm<sup>-3</sup>);  
 e (%)  
 (%) ( .) -  
 ( .)= 20%.

10-15  
 60 cm.  
 10 cm  
 60 cm  
 -  
 ( )  
 70-80%

Water Bird VI Clasic micro-sprinklers, with flow rate q = 156 L h<sup>-1</sup>, at 0.2 pressure and radius of operation r = 5.0 m. A square pattern of placement was used with distance between the sprinklers = 1.42r = 7 m, thus the irrigated area by one sprinkler was Fi = 2r<sup>2</sup> = 50 m<sup>2</sup>, and the intensity i=q/Fi = 3.12 mm/h.

The calculation of the irrigation rates was done by the formula:

$m = 10Hr(S_{LSWC} - S_{LLOMP}) C(spr.)$ ,  
 m<sup>3</sup>.da<sup>-1</sup>, (Dzhuninski, 1980), where  
 is the active soil layer depth (m);  
 is the bulk soil density (g cm<sup>-3</sup>);  
 LSWC is the limit soil water capacity (%)  
 LLOMP is the lower limit of the optimum moisture provision (%)  
 C(spr.) - coefficient of evaporation losses during sprinkling,  
 C(spr.)=20%.

Soil moisture was monitored in dynamics, at intervals of 10-15 days for all variants and for active soil layer to 60 cm depth.

Samples were taken in triplicate, layer by layer each 10 cm to 60 cm depth and were processed by the conventional weight-thermostatic method. It was observed the lower limit of the optimum moisture provision ( LLOMP) to be between 70-80% of LSWC.

The refreshing watering was performed twice or three times per



1-2 m<sup>3</sup>/da.  
2007

1 – „Water Bird VI Clasic”.

2 – „Water Bird VI Clasic”  
q=156 L h<sup>-1</sup>, 0.2  
r = 5.0 m.  
= 1,42r = 7 m,  
F = 2r<sup>2</sup> = 50 m<sup>2</sup>,  
i=q/F = 3,12 mm/h.  
1

15 cm  
1.0 Lh<sup>-1</sup>.

day, in the morning, at lunchtime and in the afternoon in the amount of 1-2 m<sup>3</sup>/da.

In 2007 the experiment was continued by comparative testing of drip irrigation in combination with micro-sprinkling for refreshing watering and irrigation only with micro-sprinkling. The trial variants were as follows:

Variant 1 – drip irrigation in combination with micro-sprinkling for refreshing watering with Water Bird VI Clasic micro-sprinklers.

Variant 2 – irrigation with Water Bird VI Clasic micro-sprinklers, with flow rate q = 156 L h<sup>-1</sup>, at 0.2 pressure and radius of operation r = 5.0 m. A square pattern of placement was used with distance between the sprinklers = 1.42r = 7 m, thus the irrigated area by one sprinkler was F<sub>i</sub> = 2r<sup>2</sup> = 50 m<sup>2</sup>, and the intensity i=q/F<sub>i</sub> = 3.12 mm/h.

In variant 1, the grafted cuttings were watered by drip irrigation system with one lateral per ridge, located between the two rows of vines.

The laterals had built-in drop-formation units every 15 cm with flow rate of 1.0 Lh<sup>-1</sup>. The volume of the supplied irrigation water was controlled by means of water-meter installed at the beginning of the system. The refreshing watering was performed twice or

1-2 m<sup>3</sup>/da.  
 „Water Bird VI Clasic”  
 $q = 156 \text{ L h}^{-1}$ , 0.2  
 $r = 5.0 \text{ m}$ .  
 $= 1.42 r = 7 \text{ m}$ ,  
 $F = 2r^2 = 50 \text{ m}^2$ ,  
 $i = q/F = 3.12 \text{ mm/h}$ .

400  
 100

7-8 cm  
 2 m

1  
 ( . )

7-14  
 60 cm.  
 10 cm

three times per day, in the morning, at lunchtime and in the afternoon in the amount of 1-2 m<sup>3</sup>/da. Water Bird VI Clasic micro-sprinklers were used with flow rate  $q = 156 \text{ L h}^{-1}$ , at 0.2 pressure and radius of operation  $r = 5.0 \text{ m}$ . The sprinkles were located in a square pattern with a distance between them  $= 1.42r = 7 \text{ m}$ , thus the irrigated area by one sprinkler was  $F_i = 2r^2 = 50 \text{ m}^2$ , and the intensity was  $i = q/F_i = 3.12 \text{ mm/h}$ .

The trials in both years were carried out by the block method with two variants in four replicas, as each variant consisted of 400 grafted cuttings or 100 grafted cuttings in one replica. The grafted cuttings were planted in ridges at a distance of 7-8 cm between the cuttings in the ridge and 2 m between the ridges.

The quantity of watering and irrigation rates was determined by the same method used in the comparative testing of sprinkling and micro-sprinkling, as from the formula in Variant 1,  $C(\text{spr.})$  - coefficient of evaporation losses during sprinkling was dropped.

The soil moisture dynamics was monitored at intervals of 7-14 days to 60 cm depth. Samples were taken in triple replicates at 10 cm and were processed by the conventional weight-thermostatic method.

The mathematical data

$t = 5,0$  %,  $t = 1,0$  %  $t = 0,1$  %  
 ( , 1999).

( ./ha),  
 (BGN/ha),  
 (BGN/ha),  
 (BGN/ .),  
 , %.

( , 1997).

2014 .,

2,28

1 .,

2006 .

processing was carried out by the method of analysis of variance at confidentiality levels of the differences (Student's criteria)  $p = 5.0\%$ ,  $p = 1.0\%$  and  $p = 0.1\%$  (Dimova and Marinkov, 1999).

The economic assessment was made on the basis of the values of the following indicators: average yield (pc./ha), total production (BGN/ha), production costs (BGN/ha), net income (BGN/ha), prime cost (BGN/pc.), rate of profitability, %. For determining the production costs, detailed technological charts were worked out based on the estimated constructive method (Nikolov, 1997).

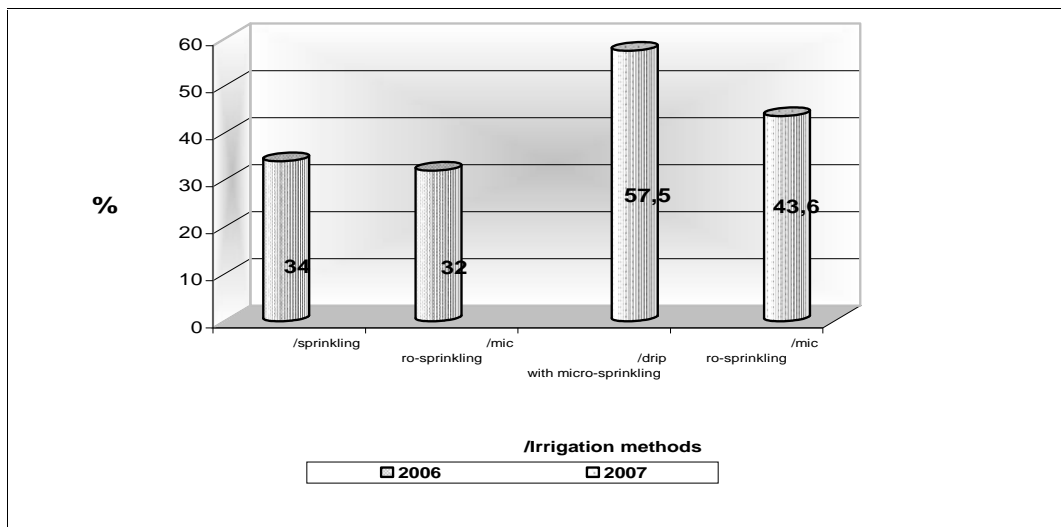
The material investments were evaluated at the current market prices by December 2014, and the labour costs – based on rates and wages used in IVE - Pleven. The estimated value of premium grafted rooted vines production was done at the average market price of 2.28 BGN for 1 pc, according to information of the National Statistical Institute.

## RESULTS AND DISCUSSION

The rate of premium vines was the most important indicator in the production of grafted rooted vines. The indicator values obtained from the comparative testing of sprinkling and micro-sprinkling in 2006 were very close, respectively 34.0% and 32.0%

34,0% 32,0% ( . 1).

(Fig. 1). The difference was not statistically proven, meaning that for maintaining optimal regime of irrigation in vine nursery both methods could be applied.



. 1.

2006 . 2007 .

**Fig. 1. Obtained rate of premium vine propagation material during the comparative testing of irrigation methods in vine nursery in 2006 and 2007**

- The ensuring of the necessary irrigation regime in growing grafted vine cuttings in the nursery was related to capital investments for the acquisition of an irrigation system, as well as operating expenses in connection with the annual installation and removal of the system.

It was assumed that the lifespan of the irrigation system was 15 years, which formed the depreciation rate of 7%.

15

7%.

1

5,7%

( 2400 ./ha),

27,3%),

The value of irrigation water was calculated based on the cost of supply by own drilled water well.

The data in Table 1 showed that the amount of operating costs for irrigation, incurred for micro-sprinkling exceeded by 5.7% the amount of the realized investment for the variant of sprinkling.

However, the total production costs in the first method of irrigation were less, as a result of the lower yield of premium grafted vines (2400 pcs./ha), respectively the lower cost of labour for harvesting the production.

The logical result was the lower value of the realized net income with micro-sprinkling (by 27.3%), calculated as the difference between the value of the total production and the production costs, compared to the variant irrigated by sprinkling. The integrated indicator accounting the economic impact of irrigation - the rate of return, had similar values for the two variants of the study, which did not form a significant difference in terms of the economic viability of the compared methods.

The wide range of factors influencing the rate of premium vine propagation material

- determined the extremely low level of profit established on the basis of direct production costs.

1.

2006

**Table 1 Economic effect of sprinkling and micro-sprinkling as irrigation methods in vine nursery, tested in 2006**

Indicators	2006	
	Tested irrigation methods in 2006	
	sprinkling	micro-sprinkling
Average yield, pc./ha	40800	38400
Total production, BGN/ha	93024	87552
Production costs, BGN/ha	73011	72998
including for irrigation:	3624	3832
-	414	552
- material	3000	3000
- labour force	210	280
- depreciation	20013	14554
Net income, BGN/ha	1,79	1,90
Prime cost, BGN per 1 vine	27,4	19,9
Rate of profitability, %		

2007

- Given the widely used drip irrigation in practice, in 2007 the experimental work continued with a comparative study of drip irrigation, in combination with micro-sprinkling and only micro-sprinkling for irrigation in the vine nursery.

- The choice of experimental variants was based on the

opportunity of synergies, combining the benefits of both methods. The obtained rate of premium vines in variant 1 (drip irrigation combined with micro-sprinkling) exceeded by 13.9% the rate of premium vine propagation material in the variant irrigated by micro-sprinkling (Fig. 1).

The difference was statistically proven justifying the assumption that drip irrigation had provided a higher rate of premium vine propagation material.

The combined application of drip irrigation with micro-sprinkling for refreshing watering was related to increasing the amount of capital investment and the higher labour costs for the annual installing and removing of the system (Table. 2).

As a result, the amount of operating costs for irrigation in this variant was higher by 47.6% of the total expenses for the variant of growing the rooted cuttings under the conditions of micro-sprinkling.

That difference was due to the increase in depreciation costs of irrigation facilities - by 35.0%, labour costs - by 33.4% and the cost of supplying irrigation water - by 107.5%.

The yield of premium grafted vines obtained from a unit of area in the first variant of irrigation

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That difference was due to the increase in depreciation costs of irrigation facilities - by 35.0%, labour costs - by 33.4% and the cost of supplying irrigation water - by 107.5%.

The yield of premium grafted vines obtained from a unit of area in the first variant of irrigation

31,9% , exceeded by 31.9% the yield from the variant with micro-sprinkling.

38030 BGN/ha. - That determined the higher amount of total production - with 38030 BGN/ha. The increase compensated in full the higher costs for the implementation of the irrigation regime under the conditions of drip irrigation in combination with micro-sprinkling and the higher labour costs for harvesting the additionally obtained production.

2.

**Table 2. Economic effect of drip irrigation in combination with micro-sprinkling and micro-sprinkling as irrigation methods in vine nursery, tested in 2007**

Indicators	2007 . Tested irrigation methods in 2007	
	Drip with micro-sprinkling	micro-sprinkling
Average yield, pc./ha	69000	52320
Total production, BGN/ha	157320	119290
Production costs, BGN/ha	76397	75712
Including for irrigation:	5982	4053
-	1604	773
- material	4000	3000
- labour force	378	280
- depreciation	80923	43578
Net income, BGN/ha	1,11	1,45
Prime cost, BGN per 1 vine	105,9	57,6
Rate of profitability, %		





refreshing watering as the most cost-effective economic variant for irrigation in the vine nursery.

The combination of both methods allowed the disadvantages of using only drip irrigation in the nursery for maintaining the air humidity to be overcome, which created conditions for increasing the economic impact of production activity.

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**FTA (WHATMAN)**

**PPV**

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\*E-mail: [janos.adam@uni-corvinus.hu](mailto:janos.adam@uni-corvinus.hu)

**USE OF FTA® (WHATMAN) MEMBRANE FOR COLLECTION AND DETECTION OF BULGARIAN PPV ISOLATES**

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**SUMMARY**

*Plum pox virus* is the causative agent of the most devastating viral disease on *Prunus* species, called Sharka. It has caused millions of Euro loss since its first appearance in 1918. The first scientific report was published by Atanasoff in 1932.

The economically important host plants are the plum, peach and apricot but studies show some disturbing occurrences on cherry and sour cherry as well. Recent studies show that nine molecularly different PPV strains have been discovered.

For better understanding of the virus, 64 samples were collected from different parts of Bulgaria and were

*Plum pox virus*  
 -  
*Prunus*,  
 1918.  
 1932.  
 PPV.  
 -  
 64

32

, 32

FTA

FTA

PPV-D, M, Rec

FTA

FTA®,

: PPV = Plum pox virus; FTA® = Flinders Technology Associates; RT = ; PCR =

*Plum pox virus* (+ssRNA)  
*Potyviridae*,  
(

1917/18

1932

(1948), Szirmai  
Husz Klement

posted by conventional mail to Hungary on FTA membranes. 32 samples were originated from flower tissue, 32 samples were from leaf tissue. The detection and the storage conditions were tested by using FTA membrane.

According to the results PPV-D, M, Rec isolates and mixed infections were identified from the samples. The leaf samples are currently under further studies. A few advantages of the FTA Cards are easy sample collection, rapid detection and convenient sample forwarding even to other countries. However this method has limits, since during the experiment some negative results also occurred, further studies are necessary to establish a reliable protocol.

**Key words:** PPV, strains, FTA®, RNA, sample collection

**Abbreviations:** PPV = Plum pox virus; FTA® = Flinders Technology Associates; RT = reverse transcription; PCR = polymerase chain reaction

## INTRODUCTION

*Plum pox virus* (+ssRNA) is a member of the *Potyviridae* family, transmitted by aphids in a non-persistent manner and it is the most devastating viral causative agent on stone fruit trees.

The first symptoms were observed in 1917-18 on plums in Bulgaria, and the first report was taken in 1932 by Atanasoff. The first report from the economically important stone fruits in Hungary was taken by Szirmai from apricot (1948), by Husz and Klement from plum (1950) and by Németh from peach

(1950) Németh (1963). PPV (Pribék et al., 2001), (Salamon and Palkovics, 2002), (Cambra et al., 2006).

o PPV. (PPV-M, PPV-D PPV-Rec) (Dallot et al., 1998; Myrta et al., 1998; Glasa et al., 2004),

FTA® (Flinders Technology Associates) Card (WHATMAN)

. FTA

(Fujita and Kubo, 2006), (Gustavsson et al., 2009), (Owor et al., 2007; Grund et al., 2010).

(1963). PPV infects not only stone fruits but almond (Pribék et al., 2001) and blackthorn (Salamon and Palkovics, 2002) as well, which is a natural wild host species endanger orchards as a reservoir.

Sharka disease heavily determines the world's stone fruit production, and causes loss of Euro millions in every year (Cambra et al., 2006).

Recent studies have demonstrated the occurrence of nine molecularly different PPV strains. Three strains (PPV-M, PPV-D and PPV-Rec) are in majority (Dallot et al., 1998; Myrta et al., 1998; Glasa et al., 2004) and the other six have specific geographical locations or infrequent occurrence.

The FTA® (Flinders Technology Associates) Card (WHATMAN) is a chemically treated filter paper designed for the collection and room temperature storage of biological samples for DNA analysis. The FTA Cards are widely used for DNA sampling and storage. The adaptability of this technology covers the fields of forensic, like sexual offence cases (Fujita and Kubo, 2006), medical fields as human virology (Gustavsson et al., 2009), and plant pathology (Owor et al., 2007; Grund et al., 2010) as well.

It can be applied to a wide range of

			biological sources such as different body fluids, plant tissues and bacterial cultures.
			Many kinds of biological specimen have been already tested to sample and process several plant pathogens in PCR and RT-PCR.
	PCR	RT-PCR.	
			The storability of the DNA samples, are much better on room temperature, then the RNA samples'. The degradation of the RNA depends on the storage temperature and the type of the cell which contains the nucleic acid. RNA in mammalian cells is stable for two or three month on room temperature, but in plant cells this period of time is only five to ten days (Natarajan et al., 2000).
		(Natarajan et al., 2000).	
	Roy Nassuth (2005),		On the other hand, according to Roy and Nassuth (2005) the detection of RNA viruses from plant tissue prints is possible after months of storage in FTA Card on room temperature.
		FTA	
			Sixty-four plant tissue samples were collected from different parts of Bulgaria and were posted by conventional mail to Hungary on FTA Cards. 32 samples were originated from flower tissue, 32 samples were collected from leaves. These samples were investigated to the presence of PPV.
FTA	. 32	, 32	
	PPV.		

## MATERIAL AND METHODS

*Prunus domestica*  
 2013 2014.  
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 ,  
 .  
 1x PBS  
 ( -  
 , pH 7.4),  
 100 µl  
 FTA  
 (Whatman)  
 .  
 ,  
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 2  
 mm , Harris  
 Uni-Core Micro Punch,  
 200 µl FTA  
 PCR  
 .  
 5  
 TE-1  
 ,  
 60  
 RT-PCR.  
 (RT)  
 (Maiss et al., 1989)  
 M4T  
 , 3'  
 - .  
 PPV, M4 (rev) Sprimer ( )  
*Potyvirus*  
 PCR  
 3'N1b-5'CP

Samples were prepared from flowers and leaves of different *Prunus domestica* cultivars during 2013 and 2014. The different samples were originated from variant locations of Bulgaria and the time of collections were different as well. The samples were ground in 1x PBS buffer (Phosphate-buffered saline, pH 7.4), and 100 µl plant homogenate were applied on the FTA Classic Card (Whatman) for RNA collection and storage. The cards were dried at room temperature, and were sent by conventional mail in an envelope to Budapest, Hungary. The cards were stored on room temperature. For cDNA preparation 2 mm disc was taken from the Card by the Harris Uni-Core Micro Punch and two-time washed with 200 µl of FTA Purification Reagent in the PCR tube. After the washing steps the samples were incubated at 5 min in TE<sup>-1</sup> buffer at room temperature, dried 60 min at room temperature and directly used in the RT-PCR. RT (Maiss et al., 1989) was conducted with M4T reverse orientated primer, located at the 3' end, at the polyA tail of the virus. To confirm the presence of PPV, M4 (rev) and Sprimer (for) *Potyvirus* specific primers were used for the PCR targeted the 3'N1b-5'CP region (Chen and Adams, 2001).

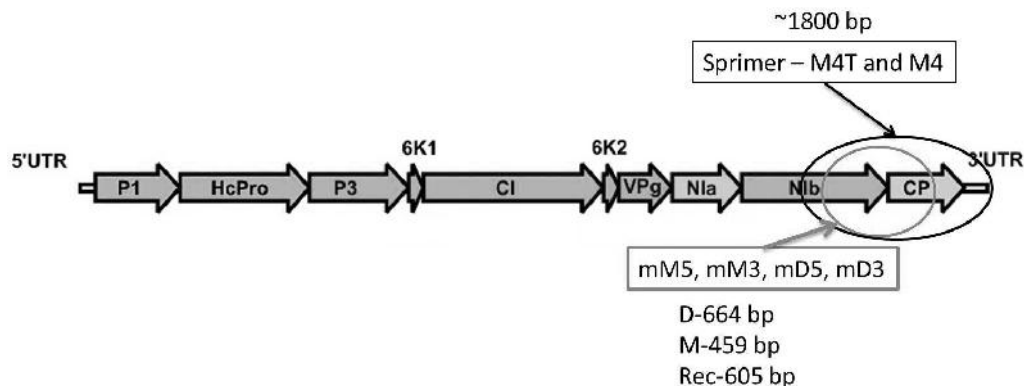
(Chen and Adams, 2001).

mM5, mD5, mM3  
 mD3 (Šubr et al., 2004)  
 nested PCR,  
 PCR Sprimer-M4 PCR,  
 N1b ( 1, . 1).  
 ( . 2).

For the strain identification the mM5, mD5, mM3 and the mD3 primers were used (Šubr et al., 2004) in a nested PCR applied on the PCR product of the Sprimer-M4 PCR, targeted the recombination breakpoint located in the 3' end of the N1b gene (Table 1, Fig. 1). The results were checked by gel electrophoresis in 2.5 % agarose gel (Fig. 2).

1.  
**Table 1. The primer orientations, the sequences and the targeted genomic regions.**

Primer (orientation)	Sequence (5'-3')	Genomic region
M4T (-)	GTTTTCCCAGTCACGACT <sub>(15)</sub>	polyA tail
Sprimer (+)	GGNAAYAAAYAGYGGNCARCC	3'N1b-CP
M4 (-)	GTTTTCCCAGTCACGAC	3'N1b-3'CP
mM5 (+)	GCTACAAAGAACTGCTGAGAG	3'N1b-5'CP
mM3 (-)	CATTCCATAAACTCCAAAAGAC	3'N1b-5'CP
mD5 (+)	TATGTCACATAAAGCGTTTCTC	3'N1b-5'CP
mD3 (-)	GACGTCCCTGTCTCTGTTTG	3'N1b-5'CP



1.  
**Fig. 1. The studied genomic regions and the scheme of the nested PCR.**

## RESULTS AND DISCUSSION

The results of the study indicate that the *Plum pox virus* was present in the flower samples







(FTA ) | inoculation an herbaceous host  
- | plant.

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## PHYSIOLOGICAL AND POMOLOGICAL PROPERTIES OF INTENSE-COLOURING SPORTS OF 'GALA' AND 'ELSTAR' APPLE CULTIVARS

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### SUMMARY

The paper presents the results of a three-year study on physiological (phenophases of flowering and fruit ripening) and pomological properties (morphometric, chemical and organoleptic) of 'Gala Must' and 'Red Elstar', the well-known intense-colouring sports of 'Gala' and 'Elstar' respectively, which were grown under agro-environmental conditions of a ak, Republic of Serbia.

Regarding flowering and fruit ripening phenophases, both assessed sports are classified as mid-late flowering and belong to the group of autumn cultivars ('Gala Must' 18<sup>th</sup> August; 'Red Elstar' 2<sup>nd</sup> September).

The 'Gala Must' had larger fruits (average weight – 157.56 g; height and width – 61.33 mm and 69.94 mm, respectively).

The better fruit quality, measured by the chemical composition, was found in the 'Red Elstar' (soluble solids content – 14.29%; total sugars and acids content – 11.84% and 0.52%, respectively).

– 11.84% 0.52%).  
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 ( 19.40).  
 " " "  
 : Malus ×  
 domestica,

Based on the overall evaluation of the examined organoleptic traits, the 'Gala Must' fruit was given better marks (total organoleptic assessment 19.40).

Therefore, these sports may greatly contribute to the advancement of the Serbian apple assortment, as well as that 'Gala Must' can be recommended for commercial production.

**Key words:** *Malus × domestica*, cultivar, sport, physiological properties, pomological properties

## INTRODUCTION

(*Malus × domestica* Borkh.)  
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 43,250 ha,  
 259,671  
 (2009-2013;  
<http://faostat.fao.org>).  
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 50%  
 (Luki , 2006;  
 Milatovi et al., 2009).  
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 O'Rourke (2001)  
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 3%

Apple (*Malus × domestica* Borkh.) is the most significant fruit species in the Republic of Serbia, second only to plum, with the total cultivated area reaching around 43,250 ha and the average production of 259,671 tons (for the period 2009-2013; <http://faostat.fao.org>). The most important apple-growing regions of our country feature the cultivars of the medium fruit quality, among which the 'Idared' is still the most popular cultivar, representing around 50% in the assortment structure (Luki , 2006; Milatovi et al., 2009). Based on an analysis of the assortment structure of 34 leading global apple producers (excluding China), O'Rourke (2001) established that 'Idared' occupied the eighth position at the global level, i.e. accounting for around 3% of the intensive apple plantations.

The high cost of modern

apple production requires that a cultivar have consistent yields and commercial-quality fruit, be suitable to manipulation, storage and shipping and generate high consumer demand.

Based on their share in the global output, 'Gala' and 'Elstar' belong to the group of 12 apple cultivars that are currently the most important in the world trade (Hampson and Kemp 2003).

Both cultivars are prone to producing red colour sports, which vary considerably in stability. Even with adequate size, intense-colouring fruit can result in upgrading fruit and is generally associated with good visual appearance and high consumer acceptance.

Although red colour does not affect eating quality, it influences consumer decisions to buy apples (Crassweller and Hollender, 1989) and the profits of growers (Iglesias and Alegre, 2006).

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Although red colour does not affect eating quality, it influences consumer decisions to buy apples (Crassweller and Hollender, 1989) and the profits of growers (Iglesias and Alegre, 2006).

Taking into consideration the necessity for permanent changes in the structure of the apple assortment, as well as the fact that climatic conditions have a significant impact on the weight and quality of the apple fruit, the aim of this paper was to examine the physiological and pomological characteristics of the darker-red mutants of 'Gala' ('Gala Must') and

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 (2007-2009):

'Elstar' ('Red Elstar') cultivars in the region of a ak (Western Serbia).

- Based on the results of this paper, it will be established whether our agro-ecological conditions are suitable for their growing, i.e. to be
- able to recommend the best clone for commercial growing, from the practical point of view.

## MATERIAL AND METHODS

### *Plant material*

- The research was conducted at the experimental apple plantation at the Preljinsko brdo facility of the Fruit Research Institute in a ak, Republic of Serbia.

The plantation was set up in spring 2005, using the 'knip' seedlings with 5 and more lateral branches, grafted onto the M9 rootstock, at the 4 x 1 m planting distance.

- The training system was the slender spindle, and the plantation was supplied with a drip-irrigation system, supported by
- implementation of modern agro-technical and pomo-technical measures.

Two of the intense-colouring mutants of 'Gala' ('Gala Must') and 'Elstar' ('Red Elstar') cultivars were examined over the three-year period (2007 2009):





(5-10% ),  
 ( 90% (85-  
 ) 90% bloom  
 ).  
 (5), (4),  
 (3), (2), (1)  
 (0).

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(g),  
 (mm),  
 (mm),  
 (mm)

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examined by observing and recording the onset of flowering (5–10% of flowers opened), full bloom (over 90% of flowers opened) and end of flowering (85–90% of petals fallen off). The bloom abundance was expressed in marks: excellent (5), very good (4), good (3), weak (2), poor (1) and no bloom (0). The harvesting time was determined using the iodine-starch test.

*Pomological properties.*

- Morphometric characteristics of the fruits were determined by monitoring the following parameters: fruit weight (g), fruit height (mm), fruit width (mm), fruit shape index, length of stalk (mm) and number of seeds per fruit.

- The examined parameters were determined using standard morphometric methods, on a sample consisting of 75 fruits. The values of the fruit shape index were obtained by calculating the ratio between the fruit height and width.

The following parameters were established as a means of determining the chemical composition of the fruit: content of soluble solids (using the 'Carl Zeiss' binocular refractometer); content of total and invert sugars (according to the Luff-Schoorl method); sucrose content (calculated as the difference between the total and invert

(  
 0.1 N NaOH  
 ); pH  
 (CyberScan 510 pH  
 ).

[  
 (0-5)  
 (0-6)]  
 [ (0-4), (0-6) (0-4)],

(  
 ).  
 (0-20)

(ANOVA)  
 F  $P \leq 0,05$   
 $P \leq 0,01$ .

sugars, multiplied by coefficient of 0.95); content of total acids, expressed in malic acid (titration of 0.1 N NaOH with the presence of phenolphthalein as indicator); pH value of the fruit juice (CyberScan 510 pH meter).

The organoleptic assessment of the fruit quality was performed based on the parameters of the fruit appearance [intensity of over colour (0 5) and the attractiveness (0 6)] and quality of fruit flesh [consistency (0 4), taste (0 6) and aroma (0 4)], with points awarded by five tasters, in accordance with the methods stipulated by instructions for testing apple usability (Regulations of Ministry of Agriculture, Forestry and Water Management of Republic of Serbia).

The overall organoleptic mark of the fruit quality of the assessed cultivars (0 20) has been obtained by summing up the individual points.

*Statistical analysis of data.* The statistical significance of the quantitative values has been determined using the Fisher model of variance analysis (ANOVA) of two-way factorial experiment, applying the F test for  $P \leq 0,05$  and  $P \leq 0,01$ . In the cases when the F test revealed significance, differences of arithmetical means

(LSD test)  
 $P \leq 0,05$   $P \leq 0,01$ .  
 SPSS (SPSS. Inc.,  
 Chicago, IL).

and their interaction effect were  
 - further tested using the test of least  
 - significant differences (LSD test) for  
 - significance threshold of  $P \leq 0,05$   
 and  $P \leq 0,01$ . The data analysis was  
 performed using the SPSS statistical  
 - software package (SPSS. Inc.,  
 - Chicago, IL).

## RESULTS AND DISCUSSION

### *Physiological properties of assessed apple cultivars*

In the course of the period  
 under consideration, 'Gala Must'  
 recorded a flowering time that was  
 on average two days earlier  
 compared to 'Red Elstar', whereas  
 it also demonstrated a higher  
 bloom abundance and a longer  
 flowering time (Table 1). The time  
 and rate of flowering observed in  
 'Gala Must' are in accordance with  
 the results reported for this cultivar  
 by Luki et al. (2011), as well as  
 the data published by Milošević et  
 al. (2007) referring to the flowering  
 phenophase of 'Mondial Gala' and  
 'Galaxy' clones in the same agro-  
 ecologic conditions. However,  
 'Gala Must' has a flowering time in  
 the conditions of West Serbia that  
 is on average 10-12 days earlier  
 compared to clones 'Schniga<sup>®</sup>',  
 'Brookfield<sup>®</sup>', 'Buckeye<sup>®</sup> Gala' and  
 'Galaxy<sup>®</sup>' in the conditions of  
 Southern Tirol (Guerra and Knoll  
 2007).

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 'Galaxy<sup>®</sup>' in the conditions of  
 Southern Tirol (Guerra and Knoll  
 2007).

The average onset of flowering for  
 'Red Elstar' established in our

11-  
 Nenadovi -Mratini et al. (2001)  
 3.0  
 4.3  
 18<sup>th</sup>  
 2<sup>nd</sup>  
 14<sup>th</sup> 23<sup>rd</sup>  
 Milatovi  
 Luki et al. (2011)  
 Miloševi  
 (2007),  
 a

research was 11<sup>th</sup> April, which is in agreement with the results of the three-year study published by Nenadovi -Mratini et al. (2001) for 'Elstar' in the region of Belgrade.

The average bloom abundance of 'Red Elstar' was 3.0 points, which is lower than the mark awarded to 'Gala Must'. The high bloom abundance of 'Gala Must' was marked with 4.3 points, indicating the marked cropping potential of this cultivar. In the second experimental year, both cultivars recorded an earlier flowering time and reduced bloom abundance.

From the aspect of the fruit ripening time ('Gala Must' – 18<sup>th</sup> August; 'Red Elstar' – 2<sup>nd</sup> September), both cultivars belong to the group of autumn apple cultivars. The 'Gala Must' fruits ripened in the period from 14<sup>th</sup> until 23<sup>rd</sup> August in the corresponding years of the study, which is in accordance with the data stated by Milatovi et al. (2009) for 'Galaxy', 'Gala Must' and 'Royal Gala' in the region of central Šumadija, as well as with the results reported by Luki et al. (2011) in earlier research. However, according to Miloševi et al. (2007), the ripening time of 'Mondial Gala' and 'Galaxy' is the first decade of September, which is around ten days later compared to the clone of 'Gala Must'. The same authors reported

" TM",  
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 " ",  
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 " "

a later average ripening time of 'Elista<sup>TM</sup>', one of the 'Elstar' clones, compared to the ripening time of 'Red Elstar', as established in this study.

1.

**Table 1. Physiological properties of the assessed apple cultivars**

Cultivar	Year	/Blooming time					
		Onset	Full bloom	End	Abundance (0–5)	Duration	Harvest time
Gala Must'	I	10. 04.	18. 04.	24. 04.	5.0	14.0	14. 08.
	II	09. 04.	15. 04.	24. 04.	3.0	15.0	18. 08.
	III	09. 04.	15. 04.	23. 04.	4.0	14.0	23. 08.
	/Mean	09. 04.	16. 04.	24. 04.	4.3	14.3	18. 08.
Red Elstar'	I	11. 04.	16. 04.	23. 04.	4.0	12.0	29. 08.
	II	10. 04.	14. 04.	24. 04.	1.0	14.0	03. 09.
	III	12. 04.	18. 04.	25. 04.	4.0	13.0	05. 09.
	/Mean	11. 04.	16. 04.	24. 04.	3.0	13.0	02. 09.

*Pomological properties of the assessed apple cultivars*

Testing of parameters of the fruit morphometric characteristics revealed statistically significant differences among the examined apple cultivars. 'Gala Must' had a statistically larger fruit weight (157.56 g) than 'Red Elstar' (149.42 g) (Table 2). The average fruit weight of 'Gala Must' was higher than the values reported by Milatovi et al. (2009) for the same cultivar (144 g). However, according to Rutkowski et al. (2005), the fruit weight of 'Gala Must' ranged between 144 g and 218 g in the course of the three-year study. Blažek and Hlušíková (2007) have reported slightly higher values of fruit weight than the ones determined for the

examined cultivars in this study, i.e. 'Gala Must' (162.8 g) and 'Elstar' (158.3 g).  
 According to the results published by Milatovi et al. (2009), 'Elista' has medium-size fruits (128.9 g), which is lower than the values established by this study.  
 Based on the average fruit weight, 'Gala Must' and 'Red Elstar' can be classified as cultivars with medium-size fruits. The fruit height was larger in 'Gala Must' (61.33 mm), whereas a large fruit width was determined in 'Red Elstar' (71.80 mm).  
 Consequently, the fruit shape index indicates the elongated (conic) shape of 'Gala Must', i.e. the elongated-flattened shape of 'Red Elstar' fruit. 'Gala Must' had a statistically significant larger length of the fruit stalk (30.77 mm), which is important from the aspect of the strength of the bond between the fruit and the bearing branch, especially in the conditions of non-existent chemical thinning of flowers/fruits.  
 A higher number of seeds was determined in the fruit of 'Red Elstar' (9.16), as a parameter that has an impact on the dimensions and weight of the fruit, according

examined cultivars in this study, i.e. 'Gala Must' (162.8 g) and 'Elstar' (158.3 g).

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Uemura et al. (2001).

to Uemura et al. (2001).

" -  $r^2 = 0.99$ ; "  
"  $r^2 = 0.92$ ).

A high positive linear correlation was determined between the number of seeds in the fruit and the fruit weight, in both assessed cultivars ('Gala Must' -  $r^2 = 0.99$ ; 'Red Elstar' -  $r^2 = 0.92$ ). When observed according to the year of the trial, the only aspect with no statistically significant differences was that of the fruit shape index, whereas all of the other parameters showed dependence on the experimental year.

## 2.

**Table 2. Morphometrical properties of fruits of the assessed apple cultivars**

Parameter	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Fruit shape index	St lk length (mm)	Number of seeds	
( ) / Cultivar (A)							
'Gala Must'	157.56	61.33	69.94	0.88	30.77	7.26	
'Red Elstar'	149.42	58.62	71.80	0.82	25.44	9.16	
(B)/Year (B)							
I	134.53	56.90	69.11	0.83	25.90	7.67	
II	170.32	62.93	73.64	0.86	31.17	8.72	
III	155.62	60.10	69.87	0.86	27.25	8,25	
(A × B) / Cultivar × Year (A × B)							
I	145.13	57.97	68.10	0.85	30.43	6.71	
'Gala Must'	II	170.87	65.03	73.07	0.89	32.93	7.85
III	156.67	61.00	68.67	0.89	28.93	7.22	
I	123.93	55.83	70.12	0.80	21.37	8.62	
'Red Elstar'	II	169.77	60.83	74.20	0.82	29.40	9.59
III	154.57	59.20	71.07	0.83	25.57	9.27	
ANOVA							
A	**	*	*	**	**	**	
B	**	**	**	ns	**	**	
A × B	ns	ns	ns	*	**	ns	

## 3).

Examination of parameters of the chemical composition of the fruit revealed statistically significant differences among the assessed cultivars (Table 3). 'Red Elstar' cultivar recorded a higher content of soluble solids (14.29%),



(14.29%),  
 (11.84%  
 3.84%)  
 ".  
 Blažek and Hlušíková (2007),  
 . . 13.6% 14.2%  
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 " ". Zdunek  
 Cybulska (2011),  
 " "  
 13.9%  
 " "  
 14.4%  
 - "  
 " (0.52%),  
 pH  
 (" " - 4.06;  
 " - 3.73).  
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as well as the higher content of total sugars and sucrose (11.84% and 3.84%, resp.) compared to 'Gala Must'.

The average values of the soluble solids contents in the studied cultivars were at the levels reported by Blažek and Hlušíková (2007), i.e. 13.6% and 14.2% of soluble solids content for 'Gala Must' and 'Elstar', respectively.

Zdunek and Cybulska (2011) reported that in the period of harvest 'Gala' fruit had the content of soluble solids of 13.9%, while 'Elstar' recorded the value of 14.4% for the same parameter.

The total acids content was higher in 'Red Elstar' (0.52%), whereas the pH value for the fruit juice recorded the opposite tendency ('Gala Must' – 4.06; 'Red Elstar' – 3.73).

Based on the total acids content in the fruit, 'Gala Must' can be classified as a sweet-fruit cultivar, whereas the 'Red Elstar' cultivar belongs to the class of cultivars with a mildly acid fruit. When observed according to the year of the trial, the differences among the tested parameters were statistically significant, with the exception of the sucrose content levels.

### 3.

**Table 3. Chemical properties of fruits of the assessed apple cultivars**

Parameter	Soluble solids (%)	Sugar content (%)			Total acids (%)	pH Fruit juice pH value	
		Total	Inverted	Sucrose			
( ) / Cultivar (A)							
'Gala Must'	13.54	10.96	8.05	2.75	0.28	4.06	
'Red Elstar'	14.29	11.84	7.76	3.84	0.52	3.73	
(B)/Year (B)							
I	14.95	12.29	8.72	3.39	0.51	3.79	
II	13.05	10.51	7.18	3.14	0.36	3.90	
III	13.77	11.36	7.82	3.36	0.34	4.00	
(A × B) / Cultivar × Year (A × B)							
'Gala Must'	I	14.70	11.95	9.08	2.73	0.32	4.02
	II	12.07	9.23	6.67	2.38	0.28	4.03
	III	13.87	11.70	8.39	3.14	0.25	4.12
'Red Elstar'	I	15.20	12.62	8.35	4.05	0.69	3.55
	II	14.03	11.78	7.68	3.89	0.43	3.77
	III	13.66	11.01	7.24	3.58	0.42	3.87
ANOVA							
A	**	**	*	**	**	**	
B	**	**	**	ns	**	**	
A × B	ns	ns	ns	**	**	ns	

As regards the parameters of the fruit appearance and quality of the flesh, the study has revealed significant differences among the examined cultivars (Table 4).

In all the tested parameters, the best marks were awarded to 'Gala Must' (over-colour intensity – 4.01 and attractiveness – 4.94), whereas 'Red Elstar' scored higher in the parameters of the fruit flesh quality (taste – 5.27, aroma – 2.94 and consistency – 2.89).

These results correspond to the data stated by Nenadovi -Mratini et al. (2001), reporting that among the eight tested autumn apple cultivars, 'Elstar' was awarded with

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– 4.01  
4.94),  
(  
– 5.27,  
– 2.94  
– 2.89).  
Nenadovi -  
Mratini et al. (2001),

the highest score for the fruit taste (4.4 on a 0–5 scale). However, with the overall organoleptic assessment of the fruit quality of 19.40 points, 'Gala Must' scored higher than 'Red Elstar', with its overall grade of 18.03 points. When observed according to the year of the trial, the differences among the tested parameters were statistically significant. The highest average intensity of the over colour and the attractiveness of fruit in both cultivars were recorded in the second year, whereas the best taste, aroma and consistency of the fruit were established in the first year of the study.

#### 4.

**Table 4. Fruit organoleptic properties of the assessed apple cultivars**

Parameter	Over colour intensity (0–5)	Attractiveness (0–6)	Taste (0–6)	Aroma (0–4)	Consistency (0–4)	Total (0–25)	
( )/Cultivar (A)							
'Gala Must'	4.01	4.94	5.02	2.69	2.74	19.40	
'Red Elstar'	2.96	3.97	5.27	2.94	2.89	18.03	
I	3.49	4.17	5.33	2.92	2.93	18.84	
II	3.62	4.62	5.02	2.80	2.79	18.84	
III	3.35	4.59	5.09	2.74	2.73	18.49	
(A × B)/Cultivar × Year (A × B)							
'Gala Must'	I	4.00	4.57	5.13	2.77	2.83	19.30
	II	4.07	5.20	4.83	2.73	2.77	19.60
	III	3.97	5.07	5.10	2.57	2.63	19.34
'Red Elstar'	I	2.97	3.77	5.53	3.07	3.03	18.37
	II	3.17	4.03	5.20	2.87	2.80	18.07
	III	2.73	4.10	5.07	2.90	2.83	17.63
A	**	**	*	**	*		
B	*	*	*	*	*		
A × B	ns	ns	ns	ns	ns		

## CONCLUSIONS

- Based on the study of the physiological and pomological properties of 'Gala Must' and 'Red Elstar' cultivars, it is possible to reach the following conclusions:

- 'Gala Must' and 'Red Elstar' belong to the group of medium-late flowering, autumn apple cultivars;

- Based on their morphometric characteristics, both cultivars can be categorised within the group of cultivars with medium-large and elongated, i.e. elongated and elongated-flattened fruits, with 'Gala Must' recording the higher average fruit weight and length;

- Parameters of the chemical composition and organoleptic assessment of quality of the fruit flesh point to the better fruit quality of 'Red Elstar', whereas the 'Gala Must' was awarded significantly higher marks for the fruit appearance parameters.

- The examined intense-colouring sports of the 'Gala' and 'Elstar' have demonstrated good results in the agro-ecological conditions of a ak, and may greatly contribute to the advancement of the apple assortment structure in the Republic of Serbia.

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31064.

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## CHARACTERIZATION OF AUTOCHTHONOUS APPLE GENOTYPES FROM THE COLLECTION OF FRUIT RESEARCH INSTITUTE > A AK

Sla ana Mari \*, Milan Luki

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### SUMMARY

The paper presents some biological and agronomic properties of 20 indigenous apple genotypes from Fruit Collection of Fruit Research Institute – a ak.

In accordance with Apple Descriptors, the following properties of the genotypes were assessed: tree habit and vigour; flowering season; bearing habit; harvest maturity; fruit size, shape and attractiveness; ground colour; over colour and type of over colour; amount and type of russeting; texture; eating quality; susceptibility to bitter pit; disease susceptibility to *Venturia inaequalis* (Cooke) Wint., *Podosphaera leucotricha* (Ell. & Ev.) and *Erwinia amylovora* (Burnill) (on scale from 1 to 9).

Regarding the full flowering and harvest maturity, the genotypes were classified into nine (from extremely early – 'Petrova a' to extremely late – 'Šumnjaja')

20

(Apple Descriptors – . .)

*Venturia inaequalis* (Cooke) Wint., *Podosphaera leucotricha* (Ell. & Ev.) *Erwinia amylovora* (Burnill) (1-9):



(Hancock et al. 2008).

Collecting, evaluation, characterization and utilization of old local apple genotypes, adapted to abiotic and biotic stress due to different environments characterizing the region of Serbia, can potentially provide a rich and useful genetic variability, especially for resistance and quality traits.

Indigenous cultivars need to be evaluated to find new sources of polygenic resistance for breeding new cultivars with stable and durable field resistance. Some of the genotypes appear to carry useful genes and alleles that, if not preserved, may no longer be available.

Fruit Research Institute – a ak (FRI) has a long tradition of collecting new as well as investigating genotypes in the existing apple collection. The FRI apple collection comprises 592 apple genotypes.

The recognition of the need for collection and utilization of autochthonous apple genotypes with their agronomically important properties has existed in Serbia for some time (Mratini 2005; Mari et al. 2007; Mratini and Fotiri -Akši 2011), e.g. good storage capability and fruit quality (Mari et al. 2005).

This work was undertaken primarily to investigate biological

(Mratini 2005; Mari et al. 2007; Mratini and Fotiri -Akši 2011),

(Mari et al. 2005).

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 Apple Descriptors (IBPGR)  
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and agronomic characteristics of 20 indigenous apple genotypes according to the Apple Descriptors (IBPGR).

It is also intended to investigate and document genotypes in the existing FRI apple collection, contributing significantly to the preservation of the apple germplasm.

### MATERIAL AND METHODS

1986,  
 m.

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Twenty autochthonous apple cultivars from Fruit Collection of FRI were analysed. They are listed in Table 1. The collection was established in 1986, at a spacing of 4 × 1.5 m. The apple accessions were grafted on M26 rootstocks and planted three trees each.

Apple Descriptors  
 (IBPGR). 50

The characterization of the assessed genotypes was done according to the Apple Descriptors (IBPGR). The samples of 50 fruits per genotype were harvested randomly at the stage of physiological ripeness. The following properties of the apple genotypes were assessed:

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 1=  
 2=  
 / , 5=  
 3=  
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-Tree habit (of branches):  
 1=Extremely upright, 2=Extremely upright/upright, 3=Upright, 5=Spreading, 6=Spreading/drooping, 7=Drooping, 9=Weeping;  
 -Tree vigour: 1=Extremely weak,

1= , 3= , 5= , 7= , 9= ;  
 - :  
 1= , 2= , 3= , 4= , 5= , 6= , 7= , 8= , 9= ;  
 - : 1=Wijcik McIntosh, 3=INRA II, 7=INRA IV; I, 5=INRA III, 9=INRA IV;  
 - :  
 1= , 2= , 3= , 4= / 5= , 6= / , 7= , 8= , 9= ;  
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 1= , 2= , 3= , 4= / 5= , 6= , 7= , 8= , 9= ;  
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 1.0= , 1.1= - , 1.2= - , 2.0= , 2.1= - ( ), 3.0= , 3.2= , 4.0= , 4.1= - ( ), 5.0= , 5.1= - , 5.2= - ;  
 - :  
 1= , 3= ,

3=Weak, 5=Intermediate, 7=Vigorous, 9=Extremely vigorous;  
 Flowering season: 1=Extremely early, 2=Very early, 3= Early, 4=Early/intermediate, 5=Intermediate, 6=Intermediate/late, 7=Late, 8=Very late, 9= Extremely late;  
 -Bearing habit: 1=Wijcik McIntosh, 3=INRA type I, 5=INRA type II, 7=INRA type III, 9=INRA type IV;  
 -Harvest maturity: 1=Extremely early, 2=Very early, 3=Early, 4=Early/mid-season, 5=Mid-season, 6=Mid-season/late, 7=Late, 8=Very late, 9=Extremely late;  
 -Fruit size: 1=Extremely small, 2=Very small, 3=Small, 4=Small/medium, 5=Medium, 6=Medium large, 7=Large, 8=Very large, 9=Extremely large;  
 -Fruit shape: 1.0=Globose, 1.1=Globose-conical, 1.2=Short-globose-conical, 2.0=Flat, 2.1=Flat-globose (oblate), 3.0=Conical, 3.2=Intermediate-conical, 4.0=Ellipsoid, 4.1=Ellipsoid-conical (ovate), 5.0=Oblong, 5.1=Oblong-conical, 5.2=Oblong-waisted;  
 -Fruit attractiveness: 1=Extremely poor, 3=Poor, 5=Intermediate,

5=	,	7=	,	7=Good, 9=Extremely good;
9=			;	Ground colour: 1=Red, 2=Orange,
	:	1=	,	3=Cream-white, 4=Yellow,
		2=	,	5=Green-yellow, 6=Green;
3=	-		,	
5=	-	6=	;	
-			:	-Over colour: 1=Orange, 2=Pink,
1=		2=	,	3=Red, 4=Dark red, 5=Purple,
4=		5=	,	6=Brown;
6=			;	
-			:	-Type of over colour: 1=Striped,
1=		2=	,	2=Streaked, 3=Mottled,
4=		5=	,	4=Splashed, 5=Slightly blushed,
6=		(	),	6=Washed-out (faded),
7=			,	7=Complete over colour, 8=Other;
8=			;	
-			:	-Russet amount: 1=0%, 2=12%,
2=	12%,	3=	25%,	3=25%, 4=37%, 5=50%, 6=62%,
4=	37%,	5=	50%,	7=75%, 8=87%, 9=100%;
6=	62%,	7=	75%,	
8=	87%,	9=	100%;	
9=	100%;			
-			:	-Russet type: 1=Extremely fine,
1=		2=	,	2=Very fine, 4=Intermediate,
	4=	6=	,	6=Coarse, 8=Scaly, 9=Cracked;
8=		9=	;	
-			:	-Eating quality: 1=Extremely poor,
1=		2=	,	2=Very poor, 3=Poor,
	3=	4=	/	4=Poor/intermediate,
5=		6=	/	5=Intermediate,
7=		8=	,	6=Intermediate/good, 7=Good,
9=			;	8=Very good, 9=Extremely good;
-		1=		-Texture: 1=Extremely coarse,
	3=	5=	,	3=Coarse, 5=Intermediate, 7=Fine,
7=		9=	;	9=Extremely fine;
-			:	-Bitter pit susceptibility: 0=None,
1=		2=	,	1=Extremely slight, 2=Very slight,
	3=	4=	,	3=Slight, 4=Slight/intermediate,
	5=	6=	,	5=Intermediate,
	7=	8=	,	6=Intermediate/severe, 7=Severe,
		9=	,	8=Very severe, 9=Extremely
			;	severe;
-			:	-Disease susceptibility to scab
( <i>Venturia</i>	<i>inaequalis</i>	(Cooke)		( <i>Venturia inaequalis</i> (Cooke)
				Wint.), powdery mildew

Wint.),  
 (*Podosphaera leucotricha* (Ell. & Ev.)  
 amylovora (Burnill))  
 9: 1=0-3%, 2=4-6%, 3=7-12%,  
 4=13-25%, 5=26-50%, 6=51-75%,  
 7=76-88%, 8= 89-99%, 9=100%.

(*Podosphaera leucotricha* (Ell. & Ev.)) and fireblight (*Erwinia amylovora* (Burnill)) on scale from 1 to 9: 1=0-3%, 2=4-6%, 3=7-12%, 4=13-25%, 5=26-50%, 6=51-75%, 7=76-88%, 8= 89-99%, 9=100%.

## RESULTS AND DISCUSSION

- Apple breeding programmes need new germplasm to improve current cultivars which are in demand by both market and industry. The backbone of successful breeding programme is availability of diverse genotypes with important and useful properties.

- In fact, apple breeding is dependent on constant use of old and new cultivars, as well as on the use of different wild species.

- It is obvious that, in this context, apple genetic resources in the region of Serbia can play an important role. In addition, this work is encouraging efforts aimed at collection and conservation of apple genetic resources. The results of the investigation of 17 characteristics in 20 apple genotypes are presented in Tables 1 and 2, and reveal differences among the genotypes.

### *Tree habit and flowering time*

, Regarding both tree habit and vigour, the genotypes can be

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 Mratini  
 and Fotiri -Akši (2011),  
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 (4)

divided into four groups, i.e. from upright (7 genotypes) to drooping (4 genotypes) and from extremely weak ('Vlahinja') to vigorous (11 genotypes).

The predominant tree habit and vigour were upright and spreading/drooping, and vigorous respectively (Table 1).

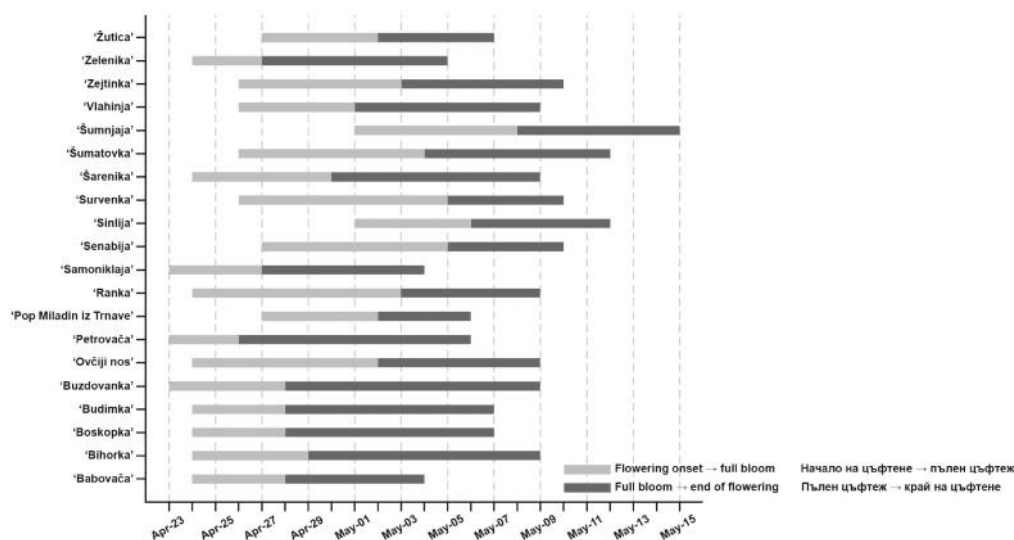
The earliest onset of flowering was recorded in cultivars 'Buzdovanka', 'Petrova a' and 'Samoniklaja' (April 23<sup>rd</sup>) and the latest in 'Sinlija' and 'Šumnjaja' (May 1<sup>st</sup>). Figure 1 shows flowering season of the evaluated accessions. There was a 12 day-difference approximately in full bloom between the earliest and latest accessions, which is in agreement with Mratini and Fotiri - Akši (2011) who reported a 16 day-difference in full bloom of the studied autochthonous apple cultivars.

Regarding full flowering, the genotypes were classified into nine groups, from extremely early – 'Petrova a' to extremely late – 'Šumnjaja'. Most of the genotypes belong to very early flowering apples (6 genotypes), while late flowering apple accessions (4) should be favoured because of their capacity to avoid frost injury.

1.

**Table 1. Major biological properties of the assessed apple accessions (concerning tree, flowering and disease susceptibility)**

Accession number	Accession name	Tree habit	Tree vigour	Flowering season	Bearing habit	Harvest maturity	Susceptibility to scab	Susceptibility to mildew	Susceptibility to fireblight
FR22	'Babova a'	5	7	2	3	7	6	2	1
FR3	'Bihorka'	6	7	3	5	7	8	2	1
FR7	'Boskopka'	6	7	2	3	7	2	2	1
FR1	'Budimka'	3	7	2	7	8	2	1	1
FR4	'Buzdovanka'	7	3	2	5	7	4	2	1
FR14	'Ov iji nos'	6	7	5	7	8	2	2	1
FR10	'Petrova a'	7	5	1	7	5	3	4	1
FR11	'Pop Miladin iz Trnave'	3	7	5	9	7	3	4	1
FR12	'Ranka'	3	5	6	5	6	4	2	1
FR23	'Samoniklaja'	7	7	2	9	7	5	3	1
FR6	'Senabija'	3	3	7	7	7	5	3	1
FR8	'Sinlija'	5	5	8	5	7	2	2	1
FR21	'Šurvenka'	7	7	7	9	8	3	2	1
FR15	'Šarenika'	5	5	3	5	8	2	2	1
FR19	'Šumatovka'	3	5	7	7	9	3	4	1
FR16	'Šumnjaja'	6	5	9	7	8	2	4	1
FR2	'Vlahinja'	6	1	4	5	9	2	2	1
FR20	'Zejtinka'	3	7	6	5	8	4	3	1
FR13	'Zelenika'	3	7	2	7	8	3	6	1
FR9	'Žutica'	6	7	5	7	7	2	4	1



1.

**Fig. 1. Flowering season of the evaluated apple accessions**



( 2).  
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 (1 ).  
 27.7 g  
 (" ") 172.0 g  
 (" " ),  
 Mratini and Fotiri -Akši (2011),  
 70.0 g 193.3 g.  
 al. (2003)  
 152.2 g.  
 (7 )  
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 (10 )  
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 ),  
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on fruit size, the accessions were classified into five groups, from very small (5 accessions) to medium large (1 accession). Actually, fruit weight ranged from 27.7 g ('Survenka') to 172.0 g ('Šarenika'), and most of the accessions were classified as small to small/medium. According to Mratini and Fotiri -Akši (2011), fruit weight of the studied autochthonous apple cultivars varied, ranging from 70.0 g to 193.3 g.

Similar findings were reported by Pirlak et al. (2003) for promising native summer apple cultivars grown in Turkey, whose fruit weight varied from 49.5 g to 152.2g.

The predominant fruit shapes in this study were globose-conical (7 accessions) and flat (5 accessions). Namely, the accessions were divided into six groups, from globose-conical to conical, and oblong fruit shape was observed in 'Ov iji nos'. The evaluated accessions were classified as poor (10 genotypes) and intermediate (7 genotypes) in terms of fruit attractiveness. Good attractiveness was observed in 'Bihorka' and 'Ov iji nos'.

Ground colour varied from yellow (2 accessions) to green (8 accessions), whereas green-yellow was observed in 10 studied genotypes. Pink, red, dark red or



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brown were determined over  
 - colours with streaked to washed-  
 - out type of over colour. The  
 - predominant over colour and type  
 - of over colour in the studied  
 - autochthonous apple accessions  
 were red/dark red (9 and 7  
 - accessions, respectively) and  
 mottled/slightly blushed (7 and 6  
 - accessions, respectively). No over  
 colour was observed in 'Petrova a'  
 and 'Zelenika'.

## 2.

**Table 2. Major fruit properties of the assessed apple accessions**

Accession name	Fruit size	Fruit shape	Fruit attractiveness	Ground colour	Over colour	Type of over colour	Russet amount/type	Eating quality	Texture
'Babova a'	3	2.0	3	5	4	3	1	4	3
'Bihorka'	4	3.0	7	6	4	2	2/2	6	5
'Boskopka'	5	2.0	1	5	3	5	8/6	7	3
'Budimka'	4	2.0	3	5	3	5	2/4	4	3
'Buzdovanka'	3	1.1	3	5	4	5	2/2	6	7
'Ov iji nos'	5	5.0	7	5	3	4	2/2	4	3
'Petrova a'	3	2.0	5	5	/	/	2/2	3	5
'Pop Miladin iz Trnave'	4	1.1	5	4	3	3	1	6	5
'Ranka'	3	2.1	5	4	2	3	2/2	4	5
'Samoniklaja'	2	1.1	5	5	3	3	1	3	3
'Senabija'	3	1.1	5	6	3	3	2/1	5	3
'Sinlija'	3	1.1	3	6	3	5	2/2	6	5
'Survenka'	2	3.0	5	5	4	2	1	3	3
'Šarenika'	6	2.1	3	6	3	5	2/6	5	3
'Šumatovka'	2	3.0	3	5	4	3	1	4	3
'Šumnjaja'	2	2.1	3	6	4	6	2/2	2	1
'Vlahinja'	3	1.2	3	6	4	3	1	3	1
'Zejtinka'	3	1.1	5	6	6	5	2/2	3	3
'Zelenika'	3	1.1	3	6	/	/	2/2	6	5
'Žutica'	2	2.0	3	5	3	2	1	4	3

(7 )

(10 12% ).

" " (

" " )

" " - "

" " ").

( 1).

( 1-9 Van der Zwet ),

4-6% (2 6 ) 88-

99% (8 ).

(13 ,

2 3 ).

6 ( 4 6

(8 ) 1 ).

Most of the assessed genotypes were non-russeting (7 accessions) or with extremely fine to very fine russet covering up to 12% of fruit surface (10 accessions). As for eating quality and texture, the accessions were classified into six (from very poor – ‘Šumnjaja’ to good – ‘Boskopka’) and four groups (from extremely coarse – ‘Šumnjaja’ and ‘Vlahinja’ to fine – ‘Buzdovanka’).

*Disease susceptibility*

All of the evaluated apple genotypes showed field resistance to fireblight and a wide range of field susceptibility to scab and powdery mildew (Table 1).

With regard to scab susceptibility (the 1–9 scale corresponds to the Van der Zwet scale and the portion of the tree blighted), the accessions can be classified into 6 groups, from 4–6% (2 on the scale) to 89–99% (8 on the scale). Most of the studied genotypes showed low field susceptibility to scab (13 genotypes, ranging from 2 to 3 on the scale). Medium and high scab susceptibility was observed in 6 (ranging from 4 to 6 on the scale) and 1 genotypes (8 on the scale), respectively.

Regarding susceptibility to powdery mildew, the genotypes

0-3% (1 )  
 51-75% (6 ).  
 20 , 14  
 ( )  
 1 3 ) 6  
 ( )  
 4 6 ).

were divided into five groups, from 0–3% (1 on the scale) to 51–75% (6 on the scale). Out of 20 genotypes studied, 14 showed low susceptibility (ranging from 1 to 3 on the scale) and 6 medium field susceptibility to mildew (rated from 4 to 6 on the scale). High field susceptibility was not observed in the evaluated apple accessions.

### CONCLUSIONS

This study has revealed that apple accessions in Serbia are rich and polymorphic in characters. Future studies aim to continue work in the field of conservation of apple genetic resources in the region of Serbia through long-term conservation, molecular fingerprints using a novel and robust method, and increase in the number of genotypes.

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