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Original Scientific paper

#### NEW PROSPECTIVE VARIETY OF THE ORIENTAL YAKA TOBACCO

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#### ABSTRACT

Yv 125/3 has been used in the production of Yaka tobacco for over 25 years, as a dominant commercial oriental variety grown in eastern and southeastern regions of Macedonia. So far, the variety has been well adapted to agroecological conditions of the above regions and it meets the market requirements. However, the increasing needs of traditional market for this type of tobacco make it necessary to introduce new, more productive oriental varieties with superior quality compared to the standard. The research work of Tobacco Institute was also headed in this direction, resulting in creation of many lines and varieties of this tobacco type in the last decade, with considerably higher quality. The most prospective among them by its productivity and quality characteristics is the recognised variety Yaka b 65 - 82/1. In terms of morpho-biological, productional and technological traits, this variety is typical representative of Yaka tobacco, which can meet the requirements of the modern market and find application in the primary tobacco production.

Keywords: oriental tobacco, Yaka variety, Yaka 65 b - 82/1

#### НОВА ПЕРСПЕКТИВНА СОРТА ТУТУН ОД ОРИЕНТАЛСКИОТ АРОМАТИЧЕН ТИП ЈАКА

Ориенталската сорта Јв 125/3 е најзастапена во производството на тутун од типот јака повеќе од 25 години, како доминантна комерцијализирана сорта која се одгледува во тутунопроизводните реони на источна и југоисточна Македонија. Оваа сорта досега беше добро прилагодена за одгледување во агроеколошките услови на наведените реони и во голема мера ги задоволуваше потребите на пазарот. Во согласност со зголемените сегашни потреби на традиционалниот пазар од ваков вид на тутунска суровина се јави потреба од воведување на нови попродуктивни ориенталски сорти со подобри квалитетни својства во споредба со стандарната сорта Јв 125/3. Според тоа и научноистражувачката работа во Одделението за генетика и селекција при Научниот институт за тутун - Прилеп беше насочена во овој правец. Така, во последнива деценија беа создадени голем број на линии тутун од типот јака, со поквалитетни својства во однос на стандардот. Од нив по продуктивноста и квалитетните својства посебно се истакнува како мошне перспективна новосоздадената и призната сорта Јака б 65 – 82/1. Сметаме дека оваа сорта по однос на морфо-биолошките, производните и технолошките својства е типичен претставник за типот јака, која ќе може да ги задоволи современите тековни барања на пазарот и да најде примена во примарното производството на тутун.

Клучни зборови: ориенталски тутун, тип јака, сорта, Јака б 65 – 82/1

#### **INTRODUCTION**

In primary production of tobacco, the variety is a basic and very important factor which has a direct impact on yield increase and improvement of quality and effectiveness of tobacco production. Therefore, it is very important in selection of tobacco mixtures to include varieties with appropriate morphobiological, productional, technological and smoking properties typical of the given tobacco type. In recent years, however, there is high heterogeneity of varietal structure in areas where Yaka tobacco is grown, with uncontrolled presence of other varieties, especially of the type Prilep. This heterogeneity negatively affects the quality of Yaka tobacco in terms of authenticity and uniformity of the raw material, which has been also suggested by the experts and international buyers of this tobacco type. The problem can be resolved only by introducing new more productive and high quality tobacco varieties. The variety with its biological potential and quality characteristics should satisfy not only the interests of primary producers but of the market and manufacturers as well (Gelemerov 2005, Gornik 1973, Timov et al. 1974). For all this, strictly controlled assortment in the production of this tobacco type is an imperative. The introduction of new and more productive varieties will allow larger, continuous and planned production with high valued, uniform and authentic Yaka tobacco raw, intended in a first rate for the international and then for the local market, which will increase the foreign exchange inflow in the country. In the selection of new Yaka varieties, our attention was directed toward the increase of yield (to certain optimal limits) and quality improvement of raw tobacco. Of all the new lines and varieties investigated, the variety Yaka b 65 - 82/1 showed to be the most perspective. It was created in the Scientific Tobacco Institute - Prilep and registered in 2014. It is characterized by higher throughput than the standard variety Yv 125/3 and is suitable for obtaining uniform high quality standard and authentic tobacco raw of the type Yaka. The aim of this paper is to present the basic characteristics of this newly created aromatic variety.

#### SOME PHENOTYPIC, MORPHO-BIOLOGICAL AND PRODUCTIONAL TRAITS OF YAKA TOBACCO VARIETY Yk b 65-82 /1

The newly created variety Yaka b 65-82/1 was registered in the list of new varieties of domestic agricultural crops in 2014, by the Ministry of Agriculture, Forestry and Water Economy of R. Macedonia. This variety was obtained by generative interspecies (intervarietal) hybridization. Plants have cylindrical habitus, lightly ellipsoid in the lower belt and with regular leaf arrangement. The stalk is very strong, with medium thickness and resistant to damping of. The internode length is typical of the Yaka tobacco. The height of the stalk with inflorescence is usually 110-140 cm, depending on the conditions of breeding and applied cultural

practices. The number of leaves per plant averaged 53-55 and more, depending on the conditions of breeding. The largest leaf size varies from 18 to 23 cm, the size of the middle leaf is 16-18cm and that of the top leaves is about 10 cm. Inflorescence is semioval, loose, with pink flowers. The above morphological traits were confirmed with the results obtained in the two-years investigation performed in unirrigated conditions (Table 1). It can be seen from the results that variety Yaka b 65-82/1 is somewhat higher, it also has a higher leaf number compared to the standard Yv 125/3 and the leaf size is typical of the Yaka tobacco.

Varieties	Plant with inflorescence	Leaf number per	Largest leaf size	Width
varieties	height cm	plant	Length	vv latin
Yv. 125/3 Ø	98,5	43	20,1	10,5
Yaka b 65-82/1	110,2	55	21,3	11,2

Table 1. Morphological characteristics of investigated varieties (average 2009-2010)

Yaka b 65-82 / 1 is suitable for growing in loose, light soils poorly supplied with nutrients and it gives especially good results under irrigated conditions. This variety also gives satisfactory yield and high quality in soils with medium nutrient supply and in conditions where irrigation is not possible. In such soils a small-leaf, aromatic, substantial tobacco is obtained, with typical characteristics of the type Yaka. More intensive rainfalls during the growing season can cause an increase in the size of the lower middle leaf, but the quality of the raw material remains the same. Fertilization is carried out with NPK 250 - 330 kg/ha (8 : 22 : 20), depending on the soil and previous crop. The plant spacing is 40 cm between rows and 12 cm in the row (from plant to plant). The optimal period for planting is May 5 -25.

Length of the growing season from planting to the beginning of flowering was 65-70 days. The variety is characterized by somewhat slower growth in the first 12 to 15 days, but therefore a well developed root system was formed, making it more adjustable and tolerant to drought. The leaves mature consecutively and are not inclined to overmaturation. It takes about 40 days from transplanting to ripening of the first leaves and the total period to the end of ripening lasts about 115 - 120 days. Higher number of leaves mature simultaneously. Tobacco was harvested in 6-7 primings, picking 5-8 leaves together.

The new variety shows resistance to TMV and gives satisfactory results in resistance to blue mold, black shank and green spot or "bassara" diseases. Resistance to TMV was confirmed in the two-year investigations (2009-2010) with the standard variety Yv 125/3 and Yaka b 65-82/1 (Table 2). Two estimations were made on disease occurrence and spread in top leaves and suckers in all investigated plants and no symptoms of TMV disease were recorded during the field trial. Under the same conditions, standard variety Yv 125/3 appeared to be susceptible to TMV. In both investigation years it showed visible symptoms of TMV disease, having high percentage of infestation (70.90% and 66.66%). Dimitrieski et al. (2005) reported the highest intensity of TMV attack among the seven varieties and lines investigated in the standard variety Yv 125/3, which is in accordance with our investigations.

			I estimation				I I estimation			
Varieties	Years	Total number of observed plants	Total number of infested plants	Intensity of attack %	Response to TMV	Total number of observed plants	Total number of infested plants	Intensity of attack %	Response to TMV	
Yv 125/3Ø	2009	165	82	49,69	+	165	117	70,90	+	
IV 123/30	2010	171	91	53,21	+	171	114	66,66	+	
Yaka b	2009	181	0	0,00	-	181	0	0,00	-	
65-82/1	2010	168	0	0,00	-	168	0	0,00	-	

Table 2. Resistance of Yaka tobacco varieties to TMV

(+)= susceptible65-82/1

(-) = resistant

The dry tobacco yield usually ranges from 2300 to 3500 kg/ha, depending on environmental conditions, mode of cultivation and applied cultural practices (Dimitrieski et al.2009, Dimitrieski et al.2010). Data on productional characteristics of the newly created variety under unirrigated conditions (Table 3), compared to the standard Yv 125/3, confirm the above-mentioned values of the new variety. Higher average yield was recorded in Yaka b 65-82/1 (2343 kg/ha), which in relative amount is 51.16% above the standard variety Yv 125/3 (2531 kg/ha). Higher average purchase price was achieved with variety Yaka b 65-82/1 (2.34  $\notin$ /kg), which is 14,20 % higher than the standard Yv 125/3 (1.76  $\notin$ /kg).

Also, variety Yaka b 65-82/1 achieved higher gross income (4.772,00  $\notin$ /ha), which is 74.42% increase compared to the standard variety Yv 125/3 (2.735,80  $\notin$ /ha).

Yaka b 65-82/1 is a small-leaf aromatic variety with uniform raw material typical for Yaka tobacco. The dry leaves tissue is fine, substantial, with yellow-orange color of the middle leaves and orange to light red upper leaves. It is characterized by exceptionally high grade mixture and favorable chemical composition of tobacco. It is a well-formed medium strong tobacco, with sweetish and pleasant taste and intensive aroma.

Table 3. Productional	l characteristics	of investigated varieties	
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Varieties	Yield kg/ha		Averag	ge purchas	Average economiceffect			
	2009	2010	Average	%	€/kg	%	€/ha	%
Yv. 125/3 Ø	110,2	1628	1550	100,00	1,76	100,00	2.735,80	100,00
Yaka b 65-82/1	110,2	2502	2343	151,16	2,34	114,20	4.772,00	174,42



Foto 1. Yv 125/3



Foto 2. Yk b 65-82/1

CONCLUSION

The results of investigations lead to the following conclusions:

- According to its morphological traits, the new variety is typical representative of Yaka tobacco. It is characterized by higher plants and higher leaf number per plant (53-55) compared to the standard variety Yv 125/3.
- Yaka b 65-82/1 shows complete

resistance to TMV in field conditions, unlike the standard variety which is susceptible to the disease.

- The new variety achieved higher average yield per hectare, which in relative amounts is an increase of 51.16% compared to the standard variety.
- The newly created perspective variety

Yaka b 65-82/1 achieved higher average purchase price per 1 kg, as well as higher economic effect expressed in  $\notin$ /

ha, which is 74.42% up compared to the standard variety Yv 125/3.

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Original Scientific paper

#### STABILITY OF THE DRY YIELD IN SOME NEW AND OLD DOMESTIC TOBAC-CO VARIETIES IN THE REPUBLIC OF MACEDONIA

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#### ABSTRACT

Investigations were carried out with five commercial oriental varieties of Prilep tobacco (P-23, P-84, NS-72, P-66-9/7 and P-79-94) and five old domestic oriental tobaccos of the types: Prilep (P 10-3/2 and P 12-2/1), Djebel (Dj  $N_{\rm D}$  1) and Yaka (YK 7-4/2 and KY), to study dry mass yield per stalk. The trial was set up in the Experimental field of Tobacco Institute-Prilep in 2011, 2012, 2013 and 2014, in randomized block design with three replications, using traditional agricultural practices.

The aim of investigations was to evaluate the variability of some autochthonous and commercial tobacco varieties through biometric analysis of the above quantitative trait and thus to give assessment of their stability and guidance for their further spread and maintenance. The study will show the differences in yield between old and new varieties, by which it will confirm the improvements in tobacco selection.

The high significance in yield between varieties is genetical indicator of their mutual difference. Differences between the years of research are not significant, which is an indication that dry mass is highly heritable trait and varietal characteristic. Statistical parameters of variability are low, which is an indication of stable and homozygous genotypes, adapted to agro–ecological conditions of the region. Results on the standard deviation and variability coefficient were lower in 2014, because the seed sown in this crop was obtained from a single stalk for each variant isolated in 2011 and the same procedure was used in 2012 and 2013. The obtained data reveal that all investigated varieties are stable but the most stable one is P–66–9/7, which also has the highest yield. Minimal and insignificant advantage in stability was recorded in the old non-commercial varieties. The average yield of the new varieties was 173% higher than that of the old domestic varieties. This is the best confirmation of the successful work of Tobacco Institute in the selection of oriental aromatic tobaccos. By evaluation of varietal stability, the breeder gets better knowledge on their homozygosity and higher reliability in choosing parental pairs for the implementation of selection programs.

Keywords: tobacco (Nicotiana tabacum L.), yield, standard deviation, variability coefficient.

#### СТАБИЛНОСТ НА ПРИНОСОТ НА СУВ ТУТУН КАЈ НЕКОИ НОВИ И СТАРИ ДОМАШНИ СОРТИ ВО РЕПУБЛИКА МАКЕДОНИЈА

Испитувани се пет комерцијални ориенталски сорти од типот прилеп (П–23, П–84, НС–72, П–66–9/7 и П–79–94) и пет стари домашни ориенталски тутуни од типовите: прилеп (П 10–3/2 и П 12–2/1), џебел (Џ бр.1) и јака (ЈК 7–4/2 и КЈ), за приносот на сува маса по страк. Опитот беше поставен на експерименталното поле при Научниот институт за тутун–Прилеп во 2011, 2012, 2013 и 2014 година, по случаен блок–систем во три повторувања, со примена на традиционални агротехнички мерки.

Целта на истражувањата е да се процени варијабилноста на горенаведеното квантитативно својство кај комерцијалните и автохтоните сорти тутун со примена на биометричка анализа, а со тоа да се добијат сознанија за нивната стабилност и напатствија за нивно натамошно ширење и одржување. Проучувањата ќе ги покажат разликите во приносот помеѓу новите и старите сорти, со што ќе се потврди напредокот во селекцијата на тутунот.

Високата сигнификантност во приносот помеѓу сортите е генетски показател за нивната меѓусебна различност. Разликите помеѓу годините на истражување не се сигнификантни, што значи дека приносот на сува маса е високонаследно својство и сортова одлика. Статистичките параметри на варијабилност се ниски, што е показател за стабилноста и хомозиготноста на генотиповите, адаптирани на агроеколошките услови во регионот. Резултатите за стандардната девијација и коефициентот на варијабилност се пониски во 2014 година, бидејќи семето посеано во оваа реколта беше добиено од еден страк за секоја варијанта изолиран во 2011, и на ист начин во 2012 и 2013 година. Добиените податоци откриваат дека сите испитани сорти се стабилни, но најстабилна е П-66-9/7, која исто така има највисок принос. Минимална и незначителна предност во стабилноста е откриена кај старите некомерцијални сорти. Просечниот принос на новите сорти е 173% повисок од оној на старите домашни сорти. Овој показател е најдобра потврда за успешната работа на Институтот за тутун во селекцијата на ориенталски ароматични тутуни. Преку евалуација на стабилноста на сортите, селекционерот добива поголемо сознание за нивната хомозиготност и поголема сигурност во изборот на родителски парови за имплементирање на селекционите програми.

Клучни зборови: тутун (Nicotiana tabacum L.), принос, стандардна девијација, коефициент на варијабилност.

#### **INTRODUCTION**

Since the beginning of mankind and cultivation of wild species, man makes efforts to change their traits in order to produce desired characteristics. The principal aim of the breeders has been to improve agricultural crops towards higher yield, better quality and higher resistance to diseases, pests and ambient stresses. Scientific Institute of Tobacco–Prilep was established in 1924 and its main breeding activity is selection of oriental, small-leaf, aromatic tobaccos. The aim of this paper is to study the yield and variability of some domestic and new commercial tobacco varieties, and to get a vision on progress in the selection of oriental tobacco in the country.

#### **MATERIAL AND METHODS**

Investigations were made on dry mass yield per stalk in five new commercial varieties of the type Prilep (P–23, P–84, NS–72, P–66– 9/7 and P–79–94) and five old domestic varieties of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj  $N_{2}$  1) and Yaka (YK 7–4/2 and KY–Kishinska Yaka), and also on variability of this trait by calculating the standard deviation and coefficient of variation (Najcevska, 2002).

During tobacco vegetation in field (May – September) in 2011, mean monthly

temperature was 19,040C, number of rainy days was 32 and total precipitation was 180 mm. In the same period in 2012, mean monthly temperature was 20,30C, number of rainy days 26 and total precipitation 180 mm. In 2013 mean monthly temperature was 19,40C, number of rainy days 34 and total precipitation 153 mm, and in 2014 mean monthly temperature was 18,30C, number of rainy days 33 and total precipitation 223 mm.

#### General characteristics of the new tobacco varieties

The new commercial sun-cured varieties

created in the Scientific Tobacco Institute-

Prilep present a high quality raw material for the domestic and world market. Due to their pleasant aroma and harmonious chemical composition they enter in the mixtures of the highest–quality cigarette brands.

**Prilep P–23** – created by Kostadin Nikoloski and Milan Mitreski, through hybridization and selection in Tobacco Institute–Prilep; recognized by the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia in 1995 (Korubin– Aleksoska, 2004). It has elliptical–conical habitus, with 50–55 leaves densely arranged on stem (Figure 1).

**Prilep P–84** – created by Kiril Naumovski and Ana Korubin–Aleksoska, through hybridization and selection; recognized in 1988 in former Yugoslavia, as one of the first varieties of Prilep tobacco. Characterized by cylindrical–elliptical habitus with approximately 40–42 sessile leaves, elliptical in shape (Figure 2)

**Prilep NS-72** – created by Dushko Boceski and Simeon Karayankov; recognized in 1984 in former Yugoslavia as one of the first varieties of Prilep tobacco obtained by crossing (Korubin–Aleksoska et al., 2012); characterized by cylindrical–elliptical habitus with approximately 50 sessile leaves, elliptical in shape (Figure 3).

**Prilep P–66–9/7** – created in Tobacco Institute–Prilep by Miroslav Dimitrieski and Gordana Miceska; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2004 (Dimitrieski, Miceska, 2011; Korubin– Aleksoska et al., 2012); characterized by elliptical–conical habitus, with 54–60 ovate leaves, sessile and evenly distributed on the stem. It has been the most frequently grown tobacco variety in R. Macedonia in recent years (Figure 4).

**Prilep P–79–94** – created in Tobacco Institute–Prilep by Milan Bogdanceski; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2001 (Korubin–Aleksoska, 2004); characterized by cylindrical– elliptical habitus with 56–60 sessile leaves densely distributed, especially in the upper part of stem (Figure 5).



Fig. 1. P-23



Fig. 2. P–84



Fig. 3. NS-72

Fig. 4. P-66-9/7

Fig. 5. P-79-94

#### General characteristics of the old domestic tobacco varieties

The cultivation of old tobacco varieties in this region began long ago, during the Ottoman Empire. The centuries—long presence led to their adaptation to the present agro—ecological conditions. Through successive natural selection they have acquired resistance to drought and diseases and can rightly be called autochthonous. Today, the old varieties make a valuable material for breeding activity in the Institute.

Prilep P 10–3/2 – created by Rudolf Gornik from Tobacco Institute–Prilep (Горник, 1973); characterized by cup-like habitus, with 30–36 sessile leaves (Figure 6).

Prilep P 12–2/1 – created by R. Gornik; characterized by cup–like habitus, with 34–38 sessile leaves (Figure 7).

P 10–3/2 and P12–2/1 – produced since the 30–ies of the last century; phenotypic and genotypic characters are very similar; derived by individual selection (Borojević, 1981), from the local tobacco variety Djumaj–bale from Gorna Djumaja–Bulgaria.

Djebel Dj  $N_{2}$  1 – created by R. Gornik, in the first half of the last century; derived by individual selection from the local variety Xanthian Yaka grown in the Djebel tobacco producing region in Bulgaria; characterized by a cylindrical habitus, with 26–30 sessile leaves erected toward the stalk (Figure 8). Yaka YK 7–4/2 – created by R. Gornik and released in mass production in 1932. Derived by mass selection from Xanthian Yaka originating from Xanthy–Greece; aplant with narrow, spindle shaped–elliptic habitus; with 26–32 sessile leaves (Figure 9).

KY (Kishinska Yaka) is believed to be introduced from Moldova (Uzunoski, 1985). Environmental conditions had a great influence on the morphology and chemistry of this genotype and it took decades–long selection to create this uniform and stable variety. It is characterized by elongated–elliptic habitus; with about 40 sessile leaves (Figure 10).



Fig. 6. P 10–3/2



Fig. 7. P 12–2/1



Fig. 8. Dj № 1







Fig. 10. KY

#### **RESULTS AND DISCUSSION**

The highest dry mass yield per stalk among the investigated tobacco genotypes was found in P-66-9/7. The lowest yield among the new varieties was recorded in P-23. The yield of the old varieties was 175% lower compared to the newly-created. The highest yield among them was recorded in Kishinska Yaka and the lowest in Dj No 1. In 2014, dry mass yield in P-66-9/7 was 24,47 g/stalk, which is 353%

higher than the yield of Dj  $\mathbb{N}_{2}$  1 – 6,93 g/stalk in 2012 (Table 1).

The four-year biometric investigations of the new and old domestic tobacco varieties for the investigated quantitative trait show low standard deviation and low degree of variability, which indicates stability and uniformity as a result of their homozygotness.

				Dr	y mass	s yield	per stalk (g)					
Tobacco	20	)11		20	12		20	13		20	14	
varieties	$\overline{x} \pm s\overline{x}$	δ	V	$\overline{x} \pm s\overline{x}$	δ	V	$\overline{x} \pm s\overline{x}$	δ	V	$\overline{x} \pm s\overline{x}$	δ	V
			(%)			(%)			(%)			(%)
New comme	ercial tobacco	variet	ies									
1. P-23	16,72 0,44	4,14	7,95	17,15 0,39	1,76	5,15	17,11 0,16	0,72	6,37	17,61 0,23	0,51	5,07
2. P-84	18,92 0,35	3,32	5,72	19,73 0,30	1,35	3,64	19,23 0.17	0,75	6,12	19,86 0,21	0,63	5,18
3. NS-72	19,21 0,62	5,92	6,66	19,75 0,43	1,91	6,38	19,34 0,12	0,54	6,92	21,88 0.97	0,24	5,79
4. P-66-9/7	21,82 0,67	6,33	6,27	24,18 0,38	1,71	5,99	24,37 0,12	0,54	6,23	24,47 0,55	0,14	5,23
5. P-79-94	17,12 0,85	8,10	6,81	17,58 0,42	1,87	4,45	17,99 0,12	0,52	3,53	18,48 0,81	0,31	4,55
Old domesti	c tobacco var	ieties										
6. P 10-3/2	10,57 0,15	0,72	6,37	10,72 0,13	0,59	5,81	10,54 0,12	0,55	5,71	11,48 0,12	0,38	5,21
7. P 12-2/1	12,73 0,25	0,75	6,12	13,12 0,17	0,66	5,06	12,97 0,11	0,46	5,16	13,65 0,14	0,26	4,86
8. Dj № 1	7,04 0,12	0,54	6,92	6,93 0,11	0,47	6,93	7,39 0,07	0,44	6,03	8,02 0,09	0,17	5,78
9. YK7-3/2	9,88 0,12	0,54	6,23	10,18 0,17	0,51	4,96	9,55 0,12	0,56	4,36	10,83 0,13	0,21	4,25
10. KY	14,71 0,14	0,52	3,53	15,25 0,12	0,52	3,26	15,01 0,10	0,50	3,79	15,91 0,13	0,45	3,11
LSD0,05 = 0	,66											

 Table 1. Mean value and variability of the dry mass yield per stalk in new commercial and old domestic tobacco varieties from the Republic of Macedonia

LSD0,05 = 0,66

LSD0,01 = 1,18

According to the data presented in Table 2 and comparison by years, in 37 out of 60 combinations, differences in yield for each variety are not significant, in 14 the significance is 5% and in 9 there is 1% significance. Comparing 2011 with 2012 and 2011 with 2013, highly significant difference was found only in P–66–9/7 and 5% significance in P–84 (2011-2012) and in P–79–94 (2011–2013). Difference in yield between 2012 and 2013 has no statistical significance. Highly significant differences between 2012

and 2014 were found in NS–72, and between 2013 and 2014 in NS–72 and YK 7–4/2. Highly significant differences were recorded between 2011 and 2014, where the significance of 1% occurred in NS–72, P–66–9/7, P–79–94 and KY, and significance of the remaining varieties is 5%. According to the above results, somewhat higher yield was obtained in 2014, due to the higher precipitation during the growing season. General conclusion would be that yield is highly heritable trait and varietal characteristic.

 Table 2. Significance of differences in dry mass yield per stalk between years (from the values in Table 1)

			(ii oin the tu	ides in Table 1)			
Variation			Ye	ears			
Varieties	2011 - 2012	2011 - 2013	2011 - 2014	2012 - 2013	2012 - 2014	2013 - 2014	
1.	P-23	(0,43)	(0,39)	(0,89)*	(0,04)	(0,46)	(0,50)
2.	P-84	(0,81)*	(0,31)	(0,94)*	(0,50)	(0,13)	(0,63)
3.	NS-72	(0,54)	(0,13)	(2,67)**	(0,41)	(2,13)**	(2,54)**
4.	P-66-9/7	(2,36)**	(2,55)**	(2,65)**	(0,19)	(0,29)	(0,10)
5.	P-79-94	(0,46)	(0,87)*	(1,36)**	(0,41)	(0,90)*	(0,49)
6.	P 10-3/2	(0,15)	(0,03)	(0,91)*	(0,18)	(0,76)*	(0,94)*
7.	P 12-2/1	(0,39)	(0,24)	(0,92)*	(0,46)	(0,53)	(0,68)*
8.	Dj № 1	(0,11)	(0,35)	(0,98)*	(0,63)	(1,09)*	(0,63)
9.	YK 7-3/2	(0,30)	(0,33)	(0,95)*	(0,33)	(0,65)	(1,28)**
10.	KY	(0,54)	(0,50)	(1,20)**	(0,04)	(0,66)	(0,70)*

Differences in yield between varieties presented in Table 3 show high significance in 42 out of 45 combinations, 5% significance in one combination (P  $10-3/2 \times YK 7-4/2$ ) and no significance in two combinations (P–23 x P–79-94 and P–84 x NS–72). This indicates that the investigated varieties significantly differ among themselves in dry mass yield per stalk.

Table 3. Significance of differences in dry mass yield per stalk between varieties
(from the values in Table 1)

	Combinations	Differences		Combinations	Differences
1.	P-23 – P-84	(2,28)**	24.	NS-72 – KY	(4,77)**
2.	P-23 – NS-72	(2,89)**	25.	P-66-9/7 – P-79-94	(5,92)**
3.	P-23 - P-66-9/7	(6,56)**	26.	P-66-9/7 – P 10-3/2	(12,88)**
4.	P-23 – P-79-94	(0,64)	27.	Р-66-9/7 – Р 12-2/1	(10,59)**
5.	P-23 – P 10-3/2	(6,32)**	28.	P-66-9/7 – Dj № 1	(16,37)**
6.	P-23 – P 12-2/1	(4,03)**	29.	P-66-9/7 – YK 7-3/2	(13,60)**
7.	P-23 – Dj № 1	(9,81)**	30.	P-66-9/7 - KY	(8,44)**
8.	P-23 – YK 7-3/2	(7,04)**	31.	P-79-94– P 10-3/2	(6,96)**
9.	P-23 – KY	(1,88)**	32.	P-79-94 – P 12-2/1	(4,67)**
10.	P-84 – NS-72	(0,61)	33.	P-79-94 – Dj № 1	(10,45)**
11.	P-84 – P-66-9/7	(4,28)**	34.	P-79-94 – YK 7-3/2	(7,68)**
12.	P-84 – P-79-94	(1,64)**	35.	P-79-94 - KY	(2,52)**
13.	P-84 - P 10-3/2	(8,60)**	36.	P 10-3/2 - P 12-2/1	(2,29)**
14.	P-84 – P 12-2/1	(6,31)**	37.	P 10-3/2− Dj № 1	(3,49)**
15.	P-84 – Dj № 1	(12,09)**	38.	P 10-3/2- YK 7-3/2	(0,72)*
16.	P-84 – YK 7-3/2	(9,32)**	39.	P 10-3/2 – KY	(4,44)**
17.	P-84 – KY	(4,16)**	40.	P 12-2/1 – Dj № 1	(5,78)**
18.	NS-72 – P-66-9/7	(3,67)**	41.	P 12-2/1 – YK 7-3/2	(3,01)**
19.	NS-72 – P-79-94	(2,25)**	42.	Р 12-2/1 – КҮ	(2,15)**
20.	NS-72 – P 10-3/2	(9,21)**	43.	Dj № 1 – YK 7-3/2	(2,77)**
21.	NS-72 – P 12-2/1	(6,92)**	44.	Dj № 1 – KY	(7,93)**
22.	NS-72 – Dj № 1	(12,7)**	45.	YK 7-3/2 – KY	(5,16)**
23.	NS-72 – YK 7-3/2	(9,93)**			

#### CONCLUSION

- Standard deviation and coefficient of variation have minimum values in some new commercial varieties (P-23, P-84, NS-72, P-66-9/7, P-79-94) and old domestic varieties (P 10-3/2, P 12-2/1, Dj № 1, YK 7-4/2, KY Kishinska Yaka), indicating low variability i.e. high stability of dry mass yield per stalk, which confirms the presence of homozygosity and high genetic stability.
- The newly–created varieties have 175%

higher yield than the old domestic varieties, as a result of the successful selection work of Tobacco Institute– Prilep, with special reference to quality, which is inversely proportional to yield and which breeders must not neglect in the selection of tobacco.

• Data about stability of tobacco varieties presented in this paper mean higher safety in their selection and inclusion in future selective programs of the Institute.

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#### **PERSPECTIVE LINE OF BURLEY TOBACCO VARIETY - LINE 1334**

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#### ABSTRACT

The new perspective line of Burley tobacco has been studied. The results show that Line 1334 has the most favorable values for all biometrical identifiers. It is formed as variant with the shortest vegetative period. In the period of research, Line 1334 gave the highest yield per hectare and can be defined as high-yielding. Line 1334 and variety Burley 1317 produce the highest percentage of first class. Of all investigated variants, Line 1334 gives the lowest percentage of third class. According to the requirements of Burley tobacco, only Line 1334 possesses balanced chemical composition. It is the variant with the most favorable technological parameters. Line 1334 significantly outperforms the standard variety Burley 21 and the control variety Burley 1317 in all investigated parameters. It has many advantages and can be offered for testing and recognition as a new Burley tobacco variety.

Keywords: Burley tobacco, biometrical indices, yield, chemical composition, technology assessment

#### ПЕРСПЕКТИВНА ЛИНИЈА ТУТУН ОД ТИПОТ БЕРЛЕЈ – ЛИНИЈА 1334

Испитувана е новосоздадената перспективна линија тутун од типот берлеј. Добиените резултати покажуваат дека Линија 1334 поседува најдобри вредности во однос на сите биометриски показатели. Таа е оформува како варијанта со најкраток вегетациски период. Во периодот на истражување, Линија 1334 постигна највисок просечен принос по хектар и може да се определи како високоприносна. Од Линија 1334 и сортата Берлеј 1317 добиен е највисок процент на прва класа. Линија 1334 дава најмал процент на трета класа од сите испитувани варијанти. Согласно со барањата на типот Берлеј, само Линија 1334 поседува избалансиран хемиски состав. Таа е и варијантата со најповолни технолошки својства. Линија 1334 значително ја надминува стандардната сорта Берлеј 21 и контролата Берлеј 1317 во сите испитани својства. Таа има многу предности и може да биде предложена во ИАСАС за испитување и признавање како нова сорта тутун од типот Берлеј.

Клучни зборови: тутун берлеј, биометриски показатели, принос, хемиски состав, технолошка оценка

#### **INTRODUCTION**

With regard to yield and quality, Burley tobacco production in Bulgaria is seriously inferior to that in most other producing countries. One of the main reasons for that is the inefficient varietal structure (Dyulgerski, 2011; Mutafchieva 2009). The implemented measures in production of Virginia and Burley varieties to date do not meet modern requirements, neither of the farmers nor of the tobacco industry (Kirkova, 2005). Of all variety groups, Burley tobacco is the one that is most poorly represented in the country. That requires creation and implementation of new varieties which will meet the needs of both producers and consumers (Dimanov and Masheva, 2011, Risteski et al., 2007). The lack of high-quality tobacco varieties prevents Bulgaria to be presented as a

In the period 2003 - 2010, in the experimental field of TTPI Markovo investigations were made with Line 1334 of the Burley variety group. Variety Burley 1317 was used as control, the most widespread in the production, and variety Burley 21 was used as a standard for Burley tobacco by 2010. Line 1334 is also comparable with variety Tennessee 86 and Line 1104, which are its parental components.

The investigations included biometric measurements, necessary phenological observations, productional manipulation, technological expert evaluation and chemical analysis. Complete competitive manufacturer worldwide (Turner, 1989). This proves the necessity to strengthen the selection-research work in order to improve the varietal composition of Burley tobacco (Dyulgerski, 2011, Snell, 2006).

The purpose of this study is to present a complete characterization of the Line 1334 in view of the possibility for recognition as a variety for deployment in the production of Burley tobacco.

MATERIALS AND METODS

characterization was made of the biological morphology, properties. yield, quality, chemical composition and technological specifications of the new line. Mathematical processing of data was made by inserting the SPSS products and STATUSTUCA, as are calculated:

- the arithmetic mean x •
- standard error of the arithmetic mean -Sx
- coefficient of variation VC %

To detect differences between the variants we used the ANOVA test and Duncan's range test (1995).

# **Brief characteristics of the Line 1334**

Line 1334 is a hydride combination between the variety Tennessee 86 introduced from the U.S.A. and Line 1104, which is identical with the selection formula of variety Burley 1000. It has a typical habit hybrids with powerful growth. It develops the largest leaves and has the highest thickness of the stalk of all tested lines and varieties. The length of the vegetation period it is in accordance with all remaining lines and varieties tested, except for variety Burley 1344. The seedlings sprout first, but it is difficult to grow in this phase. The studies performed in TTPI show that this line is

resistant to PVY and TMV and moderately resistant to Alternaria (Yonchev et al., 2011). The line is less susceptible to stolbur and TSWV. The leaves are elliptical, slightly wavy with a smooth surface, symmetrical and rounded tip. The raceme is like an umbrella, the corolla color is dark pink to red. The line is not hygrophyte but withstands prolonged drought. The line is well aligned vegetatively and morphologically. Due to the large stalk it is harder to dry and not fully adapted for harvesting and curing as a whole plant.

#### **RESULTS AND DISCUSSION**

The results of the biometric measurements showed that all tested variants provide the optimal plant height for Burley tobacco. Line 1334 has almost identical values with those of the variety Burley 1317 (Table 1). These two variants are distinguished by the height of plants (167.3 cm and 168.7 cm). Regarding the number of leaves, Line 1334 has the most favorable values (31.5 leaves). This line greatly surpasses this important indicator compared to other varieties, while it has the lowest values of variational coefficient VC% (8.8 %). The difference from the next in the ranking by the number of leaves - variety Burley 1317 is 4 leaves. The lowest results were recorded in the standard variety Burley 21.

 Table 1. Average biometric data of the investigated varieties and lines over the period of study – plant height and leaf number

VARIETY/	Plant height	Leaf number			
LINE	$\overline{x}\pm s\overline{x}$	VC %	$\overline{x}\pm s\overline{x}$	VC %	
Burley 21	$160,6\pm 0,44$	10,8	$25,2 \pm 0,34$	13,1	
Burley 1317	167,3 ±0,63	9,7	$26{,}8\pm0{,}28$	11,4	
Line 1104	$156,8 \pm 0,41$	6,8	$26,3 \pm 0,24$	9,5	
Tennessee 86	$165,5 \pm 0,47$	10,2	$25{,}8\pm0{,}41$	12,9	
Line 1334	$168,7 \pm 0,43$	6,9	$31,5\pm 0,22$	8,8	

The data on leaf size in lower harvesting belt of Line 1334 are favorable compared to other variants (Table 2). Regarding the leaf length (62.4 cm), it seriously outperforms other varieties and lines included in the experiment. With respect to leaf width, it also achieved the highest levels and is slightly superior to variety Tennessee 86 (32.4 cm). The lowest values for this trait were recorded in variety Burley 21 (29.3 cm).

 Table 2. Average biometric data of the investigated varieties and lines over the period of study – size of the leaves from the lower harvesting belt

VARIETY/	Leng	th	Width		
LINE	$\overline{x} \pm s\overline{x}$	VC %	$\overline{x}\pm s\overline{x}$	VC %	
Burley 21	58,5±0,28	18,6	29,3±0,21	17,1	
Burley 1317	60,4±0,25	17,9	$30,0 \pm 0,29$	17,3	
Line 1104	59,2±0,21	14,4	$31,6 \pm 0,18$	14,8	
Tennessee 86	60,7±0,40	18,1	$32,2 \pm 0,34$	17,7	
Line 1334	62,4±0,23	13,7	$32,4 \pm 0,12$	14,0	

The results for the size of the mid harvesting belt of Line 1334, which is most important for Burley tobacco, are also the most favourable (Table 3). It greatly exceeds the results obtained in other variants both in terms of leaf length and width (64.7 cm and 34,5 cm, respectively). In this case, however, the difference from the other varieties and lines is even more pronounced. Again, the lowest values were recorded in the standard variety Burley 21 ( 60,5 cm length and 30,4 width).

VARIETY/	Leng	th	Width	1
LINE	$\overline{x} \pm s\overline{x}$	VC %	$\overline{x} \pm s\overline{x}$	VC %
Burley 21	60,5±0,34	18,4	30,4 ± 0,19	17,1
Burley 1317	61,6±0,38	17,9	31,6 ± 0,22	16,7
Line 1104	60,7±0,20	14,7	$32,6 \pm 0,22$	13,2
Tennessee 86	60,6±0,42	18,6	$32,4 \pm 0,39$	18,7
Line1334	64,7±0,22	13,8	34,5 ± 0,15	12,5

 Table 3. Average biometric data of the investigated varieties and lines over the period of study – size of the middle belt leaf

With respect to leaf size of the upper harvesting belt (length and width), the results obtained for Line 1334 are in accordance with those for the middle belt (Table 4) and are again the most favourable, exceeding other variants with a pronounced difference (54,5 cm length and 25,4 cm width). In this harvesting belt, only the results of this line may be considered favorable. In Line 1334 no small leaves were observed in the upper belt, which is the case with other variants and which presents a big problem in the selection of Burley tobacco.

Table 4. Average biometric data of the investigated varieties and lines over the period of study – size of the
leaves from the upper harvesting belt

VARIETY/	Leng	th	Width	l
LINE	$\overline{x} \pm s\overline{x}$	VC %	$\overline{x}\pm s\overline{x}$	VC %
Burley 21	50,6±0,43	15,1	$20,2 \pm 0,34$	14,0
Burley 1317	52,1±0,37	14,4	$20,4 \pm 0,37$	13,3
Line 1104	50,5±0,34	11,9	$22,3 \pm 0,31$	10,7
Tennessee 86	51,4±0,47	14,8	$22,7 \pm 0,40$	14,5
Line 1334	54,5±0,51	11,5	$25,4 \pm 0,43$	10,5

In all three belts, the coefficient VC% of Line 1334 for leaf length and width is lower (11,5 % and 10,5 %), which is a great advantage for the breeder.

All biometric identifiers of Line 1334 have the most favourable values, which is also optimal according to the standard requirements of Burley tobacco.

#### Length of the vegetative period

Regarding the length of the vegetative period, Line 1334 outperforms other variants both in seedling stage and in the field (Table 5). This line has 9 days shorter vegetation in field than the standard Burley 21. The data for this line are superior to the results obtained for parental varieties. It is formed as a variety with the shortest period of vegetation, which is its major advantage, and its variation coefficient VC% is 1,8% in seedlings and 4,9% in field.

VARIETY/	seedings		Length of the veget field	ative period –
LINE	$\overline{x} \pm s\overline{x}$	VC %	$\overline{x} \pm s\overline{x}$	VC %
Burley 21	71,7±0,36	2,5	$82,0 \pm 0,40$	8,8
Burley 1317	66,3±0,28	1,8	$75,5 \pm 0,32$	6,3
Line 1104	69,5±0,31	2,0	$79,7\pm 0,29$	5,1
Tennessee 86	72,3±0,34	2,6	$81,5 \pm 0,37$	9,3
Line1334	65,0±0,22	1,8	$73,0\pm 0,26$	4,9

Table 5. Data on the average length of the vegetative period in the seedling phase and in the field for the
period of study (in days)

#### Yield and percentage of classes

In the period of research, Line 1334 gave the highest average yield - 3345 kg/ha (Table 6 and 7). These results are highly superior to those of the next ranking - variety Burley 1317. The yield of Line 1334 exceeded that of the standard variety Burley 21 by over 20%. This line is characterized by a high yield, as evidenced in our other research (Dyulgerski, 2011). The lowest yield was recorded in its parent component, variety Tennessee 86.

In terms of percentage of high classes, data obtained for Line 1334 and variety Burley 1317 are almost equal (Table 7). These two variants gave the most favorable results with regard to this indicator. They achieved the same percentage of first-class (42%), but Line 1334 gave higher percentage of the second class (50%) and lower percentage of the third class (8%). It gave the lowest percentage of third class compared to all investigated variants. Only in this line, the percentage of third class was below 10%. Low quality was recorded in variety Tennessee 86. The standard variety Burley 21also gave unsatisfactory results.

Although the highest in terms of percentage of high classes, the results obtained in Line 1334 should be considered satisfactory. It provides a high rate of the second class, although less than that of the first class. It should be considered its weakness.

Source of Variation	Sum of Square	DF	Mean Square	Sig of F
Variants	15688,700	4	3922,175	2082,571
Years	28,250	15	1,883	,386
2- way interactions	15716,950	19	3924,058	

Table 6. Analysis of variance for cured tobacco yield

Table 7. Average yield and percentage of high classes of Burley tobacco varieties and lines included in the
trial for the period of study

VARIETY/	Yield			
LINE	kg/ha	Ι	II	III
Burley 21	2673 <sup>d</sup>	33	46	21
Burley 1317	3117 <sup>ь</sup>	42	47	11
Line 1104	3032°	40	46	14
Tennessee 86	2610 <sup>e</sup>	25	52	23
Line 1334	3345 <sup>a</sup>	42	50	8
LSD 5%	46,4			

### **Chemical composition**

The results for chemical composition showed that Line 1334 significantly exceeds the other variants in all six investigated indicators (Table 8). Only in this line the content of nicotine and total nitrogen was lower than 3%. The results obtained for sugar content (1,21%) can also be considered as very favorable. This trait can be defined as satisfactory in the variety Burley 21. The laboratory tests show that only line 1334 has a balanced chemical composition typical for Burley tobacco, which is another significant advantage of this line.

VARIETY/ LINE	Nicotine %	Sugars %	Total nitrogen %	Ashes %	Ammonia%	Proteins %
Burley 21	2,66	0,85	2,84	17, 40	0,32	10,8
Burley 1317	2,59	1,04	2,65	16,73	0,33	8,6
Line 1104	1,91	1,13	2,17	15,81	0,34	7,7
Tennessee 86	1,86	1,21	2,32	16,46	0,34	13,7
Line 1334	3,18	0,52	3,29	18,85	0,31	10,2

#### **Technological parameters**

In general, all physical and technological parameters of investigated variants conform to the standards of Burley tobacco (Table 9). Line 1334, however, has the lowest percentage of stalk, the lowest leaf density and, especially, the highest utilization in cigarettes. Only in Line 1334, the number of cigarettes obtained from 1 kg of tobacco exceeds1785.

Variety/ Line	Leaf num- ber/ kg. tobacco	Midrib%	Length cm	Width cm	Weight unit leaf area g/cm <sup>2</sup>	Density of tobacco leaves g/cm <sup>3</sup>	Density of cut tobacco g/cm <sup>3</sup>	Conditional yield Number of cigarettes / kg tobacco
B 21	167	28,6	48,5	19,7	0,0051	0,426	0,178	1656
B 1317	165	28,8	49,2	20,9	0,0054	0,423	0,173	1633
L 1104	169	29,7	48,9	21,8	0,0055	0,438	0,181	1585
Tenn. 86	172	30,1	45,9	23,3	0,0058	0,449	0,186	1511
L 1334	154	26,3	53,3	22,8	0,0043	0,0402	0,168	1785

#### CONCLUSION

Line 1334 has the most favorable values for all biometric identifiers, which is optimal according to the standard requirements for Burley tobacco. This variant has the shortest vegetative period, which is its big advantage.

Average data for the period of investigation show that Line 1334 gives the highest yield per hectare and can be considered as a highyielding variety.

Line 1334 yields the highest percentage of first class (42%) and the lowest percentage of third class (8%) compared to all other variants investigated.

Only Line 1334 has a balanced chemical compositioninaccordance with requirements of Burley tobacco. It is a variant with the most favorable technological parameters. Also, only in Line 1334, the number of cigarettes obtained from 1 kg of tobacco exceeds 1785.

Line 1334 considerably outperforms the standard variety Burley 21 and the control variety Burley 1317 in all investigated parameters. It has many advantages and can be offered for testing and recognition as a new variety of Burley tobacco.

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# SOME ECOLOGICALLY FRIENDLY METHODS FOR CONTROL OF PATHOGENIC FUNGUS *ALTERNARIA ALTERNATA* ON TOBACCO

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#### ABSTRACT

Contemporary trends in plant protection are directed to use ecological sustainable methods that will make possible limited use of chemical pesticides.

The influence of *Trichoderma harzianum* (pure culture) and a bioproduct Trilogy 70 EK (1%) were investigated vs. the contact fungicide Antracol WP-70 (0.25%).

Some variants were involved: the treatment several times during the vegatation, 48 hours before inoculation and immediately after inoculation, while the fungicide, only just after inoculation.

The lowest intensity of the disease was observed in the variant with treatment of plants with *T. harzianum* several times during vegetation. These results are better than those of fungicides Antracol WP-70.

A bioproduct Trilogy 70 EK (1%) has shown more weakly results, but the same as Antracol WP-70.

Biological method is a good alternative to chemical method of control the parasitic fungus *Alternaria alternata*, the causing agent of the brown spot on tobacco

Key words: Alternaria alternata, biocontrol, biocontrol agent, biopreparate

#### НЕКОИ ЕКОЛОШКИ ПРИФАТЛИВИ МЕТОДИ ЗА СУЗБИВАЊЕ НА ПАТОГЕНАТА ГАБА *ALTERNARIA ALTERNATA* НА ТУТУНОТ

Современите трендови во заштитата на растенијата се насочени кон примена на еколошки прифатливи методи кои ќе овозможат намалување на стандардните хемиски средства.

Испитувано беше влијанието на *Trichoderma harzianum* (чиста култура) и биопрепаратот Trilogy 70 EK (1%), наспроти контактниот фунгицид Antracol WP-70 (0,25%).

Вклучени беа неколку варијанти: третирање неколку пати во текот на вегатацијата, 48 часа пред инокулацијата и непосредно по инокулацијата, додека кај фунгицидот- само по инокулација.

Најмал интензитет на напад од болеста беше констатиран кај варијантата каде се вршеше третирање на растенијата со *T. harzianum* неколку пати во текот на вегетацијата. Овие резултати се подобри во споредба со тие кај фунгицидот Antracol WP-70.

Биопрепаратот Trilogy 70 EK (1%) даде нешто послаби резлтати, но исти како и Antracol WP-70.

Биолошкиот метод претставува добра алтернатива за хемискиот начин на сузбивање на паразитната габа *Alternaria alternata*, предизвикувач на кафената дамкавост кај тутунот

Клучни зборови: Alternaria alternata, биолошка борба, биоконтролен агенс, биопрепарат

# INTRODUCTION

Contemporary trends in plant protection are focused on application of environmentally safe methods that would allow reduce standard chemical pesticides. Integral Protection (IPM), despite preventive measures include biological control as an effective, modern method of protection that ensures a healthy and clean environment.

Biological control of pathogens is based not only on application of commercial products and bioagents, but also on their multiplication. Lately, there has been increasing interest in biochemical-based products and antagonistic relations among microorganisms.

Considering the fact that biological control does not offer general solution, it develops separately from crop to crop and from pathogen to pathogen.

The causing agent of the brown spot disease is a parasitic fungus *Alternaria alternata*. There are effective chemical agents for its control. But, efforts to reduce the number of treatments, in periods with increased possibility of its occurrence, implies the application of certain ecological methods of preventing the disease.

Fungi of the genus *Trichoderma* are the most powerful biocontrol agents. There are numerous mechanisms involved in biocontrol against plant pathogens (Harman et al., 2004; Harman, 2006). Biological control of *Trichoderma* is confirmed in more pathogens on tobacco (Gveroska, 2013 a). This is the strongest in soil pathogens. Hence, these agents can be used in control of the the damping off disease on tobacco seedling (Gveroska, 2013 b). However, their application in control of foliar pathogens

also give excellent results. *T. harzianum* is a biocontrol agent used in control of *A. alternata* (Monte, 2001; Roco and Perez, 2001; Sempere and Santamarina, 2007). Sustainable Agricultural Production strives

to apply other bioproducts, based on plant extracts or other biochemical components. There are many essential oils that show insecticidal and fungicidal activity to suppress important plant pathogens (Isman, 2000). Their potential for crop protection and positive impact on healthy environment ensure a commercialization of these biopesticides.

There are proven antifungal components in Neem oil (Azadirachta indica) - it is a mixture of tetranortriterpenoids that the antifungal activity express in the highest degree as mixture, which suggess on additional / synergistic effect (Govindachari et al., 1998). Azadirachtin is the most powerful tetranotriterpenoid of the Neem tree and by toxicological tests, use as a biopesticide, dose of 500, 1000 and 1500 mg / kg / day for 90 days caused no sign of toxicity, mortality, changes in tissue weight or pathological changes in blood parameters. The highest dose (1500 mg/kg)can be taken as the basal dose to calculate the safe limits (Raizada et al., 2001).

Data presented defined the aim of our research - to examine some alternative, ecologically friendly methods of preventing the tobacco from the brown spot disease. That is, to examine the effect of *Trichoderma harzianum* and bioproduct Trilogy 70 EK on *Alternaria alternata* comparing them with chemical control - contact chemical fungicide Antracol WP-70.

# MATERIAL AND METHODS

Investigations were made with variety P 23, grown in usual way in biological laboratory. Twenty plants per each variant and the check were planted .

Fresh tobacco leaves with expressed symptoms of Brown spot disease were used for inoculation.

Inoculation with the pathogen was

performed at the end of the growing season, as the most suitable period for infection and disease occurrence.

*Trichoderma harzianum* was used as **biocontrol agent** because of its remarkable reducing effect on most tobacco pathogens, including *A. alternata*.

Suspension of pure culture was used for plant treatment (spraying of plants) -2 Petry dishes in 250 ml distiled water.

**Bioproduct** Trilogy 70 EK is an extract of Neem oil, obtained from the Neem tree (Azadirachta indica) which grows in India. It was applied in the recommended rate of 1%.

Several variants were included in the biocontrol agent and in bioproduct:

- 3 treatments during the growing season

- 1 treatment 48 hours prior to inoculation

- 1 treatment immediately after inoculation

$$i = \frac{\sum n \cdot k}{N \cdot K} x100$$

n - leaf number in corresponding category k - category

Where:

According the aim of investigations, the **contact fungicide** Antracol WP-70 (propineb 70%) in the rate of 0.25% was also included for chemical treatment, immediately after inoculation.

The test plants were sprayed only with water and treated in the same way as other plants. Plants were covered with polyethilene bags and kept 10 days in noncontrolled conditions in biological laboratory. Estimation was made on detached tobacco leaves, categorized on a scale from 0 to 5:

- 0 no symptoms of disease
- 1 1 spot on the leaf
- 2 2-5 spots
- 3 6 10 spots
- 4 11 25 spots

5 - over 50% of leaf surface infected *Disease intensity* was calculated using the formula of *McKinney*:

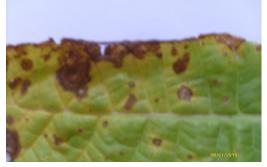
N - total number of analyzed leaves K - total number of categories

#### **RESULTS AND DISCUSSION**

Brown spot disease is manifested by the appearance of brown spots on the leaf surface, by which it was named (Fig. 1, 2). Its appearance depends on weather conditions and the proper agrotechnical operations, primarily timely harvest.

It appears first on the lower and spreads to the upper leaves and attacks the flower buds and shoots, too (Fig. 3).

The causing agent of the disease is



Ph. 1 Brown spot disease in tobacco

pathogenic fungus *A. alternata*. In artificial inoculation with the pathogen we can get similar symptoms (Ph. 4). Artificial inoculation is a good way of testing the intensity of attack by the disease. After a period of incubation and categorizing the leaves in the corresponding category of the scale, intensity of attack for each variant is calculated (Table 1 and 2).



Ph. 2 Brown spot disease in oriental tobacco leaves



Ph. 3 Spreading of disease from the lower to upper leaves

The contact chemical fungicide Antracol WP-70 showed weak results in control of the pathogen *A. alternata*.

Amomg variants with the biocontrol agent, the lowest intensity of attack by the disease (in both years of testing) is performed in the variant were plants were treated with *T*. *harzianum* several times during vegetation (19.31 and 21.09%). What is important is that these results are better than those of the contact fungicide Antracol WP-70 (Table 1 and 2).

According to Quarles (2000), the beneficial microorganisms are effective only at high relative humidity (often 60 to 80%) and their effect is limited in biolaboratories or areas with low relative humidity during the vegetation season. The bacteria are less sensitive to moisture and therefore they have a wide range of application. These data justify multiple application of the fungus *T. harzianum*.

The multiple application of this biocontrol agent enables its rapid multiplication. On the other hand, it is possible constantly acting and development of the three main mechanisms of biocontrol competition, antibiosis, mycoparasitism etc. Also, development of other biocontrol



Ph. 4 Symptoms of disease- artificial inoculation

mechanisms that act synergistically, which certainly influences on the such little intensity of attack by disease.

Most biofungicides are applying as a preventive measure and act as a barrier between the pathogenic fungus and plant tissues and it is necessary to apply before the emergence of new leaves or sensitive part of the plant, at the first signs of the disease or in the presence of favorable climatic conditions for the emergence of the disease (Quarles, 2000). In the constant presence of the biocontrol agent with multiple treatment, there is a covering of all moments in preventing the disease.

Treating plants with T. harzianum 48 hours before and immediately after inoculation gave significantly poorer results (Table 1 and 2). The average values of disease intensity in these two variants are - 44.16 and 42,55% (Graph 1). In this case there are favorable conditions for the growth of the pathogen and the host plant and the time of 48 hours is very little to release certain substances that limit the growth of the pathogen. Similar findings were presented by the BPIA (2014). Fungi require specific environmental conditions to proliferate and their means of affecting the target organisms are diverse.

Variant	Total number of leaves	% of infecte leaves	Intensity of attack (%)
Check Ø	676	98,52	58,48
Antracol WP-70 + <i>A. alternata</i>	210	65,71	31,43
<i>T. harzianum</i> (several times )+ <i>A. alternata</i>	359	58,50	19,31
<i>T. harzianum</i> (before 48 hours) + <i>A. alternata</i>	537	81,19	42,61
T. harzianum.+ A. alternata	520	78,65	40,51
Trilogy 70 EK (several times ) + A. alternata	360	85,56	46,94
Trilogy 70 EK (before 48 hours) + A. alternata	333	75,08	42,54
Trilogy 70 EK + A. alternata	417	71,94	30,77

 Table 1. The influence of biological and chemical way for control of

 Alternaria alternata (I st year)

Neem oil has an expressive fungicidal properties and can be used to effective control of the leaf pathogens that cause spots. Among the most fungi that act, there is *A. tenuis* (Govindachari et al., 1998). The content of Azadirachtin affects the fungus and its spreading among susceptible plants. For effective control of the brown spot disease, it needs spraying once in 10 days (Teaser, 2015). According to Bozukov (2005) Trilogy 70 EK is bioproduct with high efficiency and prolonged effect

on supression the tobacco pathogens - *Peronospora tabacina* and *Erysiphe cichoracearum*. But in our research these treatments had failed.

Neem oil affects mitochondrial oxidative phosphorylation, which inhibit the respiratory chain (Biswas et al., 2002). The mechanism of action is thus causes strong drying / suffocating of the pathogen. Therefore, the greatest effect has applied immediately after inoculation (average intensity of attack - 31,83% (Graph 1).

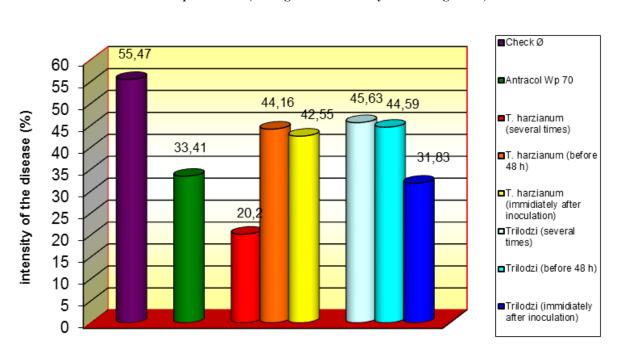
Table 2. The influence of biological and chemical way for control of
Alternaria alternata (II <sup>nd</sup> year)

Variant	Total number of leaves	% of infecte leaves	Intensity of attack (%)
Контрола Ø	570	87,72	52,46
Antracol WP-70 + A. alternata	195	64,10	35,38
<i>T. harzianum</i> (several times )+ <i>A. alternata</i>	331	58,01	21,09
<i>T. harzianum</i> (before 48 hours) + <i>A. alternata</i>	350	86,00	45,71
T. harzianum.+ A. alternata	487	85,22	44,59
Trilogy 70 EK (several times) + A. alternata	252	85,56	44,31

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Trilogy 70 EK (before 48 hours) + A. alternata	357	82,54	46,63	
Trilogy 70 EK + A. alternata	335	73,13	32,89	

The other two variants with Trilogy 70 EK gave poor results (averages - 45.63 and 44.59%). Its effect has the bigest expression on the surface of leaves. According to the declaration for Neem oil (Annonimus, 2015), thorough coverage of the leaves provides good protection because prevents

contact and adhesion of the fungus on the leaf. This means that aplication several times or 48 hours before inoculation can not prevent the penetration of this endotrophic fungus, which results in higher values of intensity of attack by disease.



#### Graph 1. Effect of use the alternative methods in control of the brown spot disease (average values of two year investigations)



- There is a possibility to apply ecologically friendly methods in the control of pathogenic fungus *Alternaria alternata*.

- The best results in our investigation were obtained with the variant treated with *Trichoderma. harzianum* several times during the growing season.

- These results were better compared to chemical control with the contact fungicide Antracol WP-70.

- Bioproduct Trilogy 70 EK (1%) gave somewhat poorer results, but its best activity

was achieved when applied immediately after inoculation (as well as a chemical fungicide). - Since the results obtained with this bioproduct were identical to those of Antracol WP-70, it can be recommended as a substitute for chemical-based products.

- Comparing the biological way of protection by chemical, biological is a good alternative to the use of chemical fungicides in the control of *Alternaria alternata*, the causing agent of Brown spot disease on tobacco.

- The application of these methods, certainly

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supported by the facts on the effectiveness of this way of protection, on the one hand, and eligibility to human health, on the other, would increase the ecologically sense of producers at a higher level.

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#### CHAMAEMYIIDAE (DIPTERA) - PREDATORS OF APHIDS ON TOBACCO

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#### ABSTRACT

The use of natural enemies is the basic means of biological control of arthropods, vertebrates, weeds and plant pathogens.

Chamaemyiidae, also known as silver flies or silver aphids, are larval predators of Homoptera found in a wide range of cultural plants, trees and weeds.

Observation of silver flies was made during 2010-2012 in the region of Prilep, on 20 tobacco stalks and 100 tobacco leaves.

The collected material was further analysed under laboratory conditions.

During the investigations, the aphidophagous species *Leucopis* sp. (Chamaemyiidae) was determined as predator of *Myzus persicae* Sulz. on tobacco.

Females lay their eggs singly on the underside of tobacco leaves, among the aphid colonies. The larva of this species is considerably greedier during the second, and particularly during the third larval stage. Larvae are transformed into pupae in the same plants on which they feed. Adults are mobile and they are able to locate the prey quickly.

*Leucopis* sp. survival on *M. persicae* and its quantitative representation was significantly higher in 2011-2012 than 2010. The most massive occurrence of silver flies is recorded in August.

Chamaemyiidae can be used in biological control of aphids, as a part of integrated protection against these pests.

Key words: tobacco, leaf aphids, predator, Chamaemyiidae, Leucopis sp.

#### СНАМАЕМУНДАЕ (DIPTERA) ПРЕДАТОРИ НА ЛИСНИТЕ ВОШКИ НА ТУТУНОТ

Основа на биолошката контрола на членконогите, 'рбетниците, плевелите и растителните патогени е употребата на природни непријатели.

Видовите од фамилија Chamaemyiidae се познати како сребрени муви. Ларвите се предатори на Homoptera, кои живеат во широк спектар на културни растенија, дрвја и плевели.

Проучувањата на сребрените муви беа спроведени на тутунот во околината на Прилеп во текот на 2010-2012 година, со примена на следниве методи на ловење: проверка на сите лисја од 20 тутунски страка и проверка на 100 тутунски лисја.

Собраниот материјал беше дополнително одгледуван и анализиран во лабораториски услови.

При истражувањата го детерминиравме афидофагниот вид *Leucopis* sp. (Chamaemyiidae), како предатор на *Myzus persicae* Sulz. на тутунот.

Женката ги полага јајцата поединечно, меѓу колониите од лисните вошки, на долната страна на тутунските листови. Лакомоста на ларвите е зголемена за време на вториот, а посебно во третиот ларвен степен.

Ларвите се куклат, на истите растенија каде што се хранеле. Возрасните се мобилни и може брзо да го лоцираат пленот.

Опстанокот на видот *Leucopis* sp. на *M persicae* и неговата квантитативна застапеност е значително повисока во 2011-2012 година отколку во 2010 година. Најмасовна застапеност на сребрените муви има во август.

Chamaemyiidae може да се користат во биолошка контрола на растителните вошки и во склоп на интегралната заштита против овие штетници.

Клучни зборови: тутун, лисни вошки, предатор, Chamaemyiidae, *Leucopis* sp.

# INTRODUCTION

Chamaemyiidae (Diptera) are small cosmopolitan flies. Immature Chamaemyiidae are predators of scale insects, mealybugs and aphids. They are important means of biological pest control. According to Gaimari and Turner (1996), the family Chamaemyiidae is represented by a small flies (1-4 mm) whose larvae are predators on soft bodied Homoptera.

Adults occur in various grassland habitats, in reeds, in mixed or deciduous forests, in lowlands, but also in montane habitats (Barták and Papp, 2009).

According to Evenhuis (1992), they are recorded as predators of aphids and coccids. Colless and Mcalpine (1991), also recorded them as predators of psyllids.

In Quebec, Canada, silver fly *Leucopis spp*. (Chamaemyiidae: Diptera) larvae are commonly encountered within apple aphid

colonies (Fréchette et al., 2008).

Silver fly is the specific host for *H. pruni* that feeds only on *Phragmites spp*. (Rakhshani *et al.*, 2010).

Some species of *Neoleucopis* and *Leucopis* were introduced into North America from Europe, for control of Dreyfusia spruce (Tanasijtshuk, 1997).

Thalji (1988, 1992), obtained Chamaemyiidae as predator of aphids on leaf sunflower in Serbia -Vojvodina.

Our first report of Chamaemyiidae as predator of aphids in Macedonia was in 1996. Silver flies are found within aphid colonies of tobacco in the region of Prilep (Krsteska, 2002).

However, very little is known about the ecology and biology of most of the members of this group.

#### **MATERIAL AND METHODS**

Investigations were carried out during 2010-2012, on tobacco plants in the area of Prilep.

The observations of silver flies were made with application of the following methods of catching: check of all leaves from 20 tobacco stalks and check of 100 tobacco leaves (Davies method).. Monitoring and collecting of material from tobacco seedlings was performed during tobacco vegetation, from May until the end of September, at 10-day intervals.

The collected material was further nourished, cultivated and analysed under laboratory conditions. For research of Chamaemyiidae in laboratory conditions and for investigation of their biology, standard methodology was applied. Adults were fed on a mixture of honey and yeast in cages, while larvae were reared on tobacco leaves infested with *M. persicae* in Petri dishes. Once formed pupae can be put in tubes where the emergence of adults can be easily observed.

Weight of silver flies in various stages of growth was measured on Sartorius BL 210 S analytical balance (d=0.1 mg), while length and width on Carl Zeiss Jena binocular (25 x 5). Body length was measured by adding the length of the head (without antennae) through

the thorax to the abdominal length, to account

for differential curling of the abdomens.

# **RESULTS AND DISCUSSION**

The Chamaemyiidae (Diptera) family, commonly known as silver or aphid flies, represents a group of larval predators attacking aphids. adelgids, scales. mealybugs and are thought to be potentially useful as biological agents of these insects. Chamaemyiidae The family belongs to the kingdom Animalia, subkingdom Eumetazoa, phylum Arthropoda, subphylum Hexapoda, class Insecta, order Diptera, suborder Brachycera, division Muscomorpha, Schizophora, Acalyptratae, superfamily Lauxanioidea.

It is divided into three subfamilies: Chamaemyiinae, Cremifaniinae, Leucopinae.

Although the species of some chamaemyiid genera are quite general in their feeding habits, many genera are restricted to a particular host taxon (Gaimari, 2012). In all years of our investigations silver flies were recorded as predator on *M. persicae* in tobacco fields.

Larvae of silver flies were usually found among colonies of green peach aphid -*M*. *persicae*. The role they play in the long term regulation of aphid populations can be important, because their larvae are feeding with them.

According to our investigations in the region of Prilep, aphid infestations are inevitable and occur every year, ranging from medium to large. We collected Chamaemyiidae eggs and larvae from tobacco leaves infested with *M. persicae*. The collected material was further nourished, cultivated and analyzed under laboratory conditions.



Fig. 1 Leucopis sp.

During investigations we identified the aphidophagous genus *Leucopis* and studied the life history of *Leucopis* sp. (Fig. 1), as an aphid predator on tobacco.

Eggs were laid in or near colonies of the host on the underside of tobacco leaves. They are laid singly among *M. persicae* colonies. Acording to Clausen (1940a), they are laid singly among the egg masses or colonies of the host. Tracewski (1983), observed that *Leucopis* sp. *nr. albipuncta* eggs are usually laid in a group of 2–3 under apple leaves in New Hampshire (cit. Fréchette *et al.*, 2008). According to Ghadiri *et. al.* (2003), the average fecundity was 35.7 eggs.

The eggs are tiny, almost invisible, 0.35-0.38 mm long and are pearly white, with the surface bearing longitudinal ridges. They have two ends, the first one rounded and the anterior end is a bit pointed. Eggs are usually deposited horizontally on the leaf. In our investigations, the duration of egg stage was 2.5–3.5 days, similar to the data reported by Clausen (1940a) - 3-4 days. The average incubation period of *Leucopis glyphinivora* Tanas is 2.7 (Ghadiri *et. al.*, 2003).

As the embryo develops, the color of the egg turns from white to brown. By contraction and spreading, larva tears the chorion and then gently slides off the egg shell.

After hatching, the larva is of transparent white color and is still egg-shaped. The larvae are somewhat restricted by their body size, being able only to subdue prey smaller than themselves. First larval instar was exclusively feeding on 1st and 2nd nymphal instars of aphid.

During their growth, a larva shed two times and passes through three larval instars and becomes more and more mobile. The larva is very similar to those of the family Syrphidae, in the transition from the first to the second stage. Larvae vary in rear spiracles: in Syrphidae rear spiracles are found together, and in silver flies they are distributed along the edges of the last segment, more or less protruding.

As the larvae of *Leucopis sp.* grow, the integument is bare, they become larger and they turn reddish (Fig. 2). Larval respiration can be clearly observed through the cuticle. In third larval stage (L3), larvae have the average weight ranging from 4 mg to 6 mg, and the length from 4.5 mm to 5 mm. According to Clausen (1940a), third instar larvae of *Leucopis bella* are 5.00 mm long and clothed only with tiny setae.

Larvae are tapered toward the head, broadest in the abdominal region, and bluntly rounded posteriorly, with caudal spiracles. L3 is dorsally slightly curved and ventrally slightly flattened.



Fig. 2 Larva

They have strong mouth-hooks, suitable for catching the prey, sharp mouthparts like a dagger, strong pharynx and head muscles which help them to stab and suck the prey. Their greed increases during the second, and particularly during the third larval stage.

According to Sandhu and Kaushal (1977), fly larvae are eating 40-60 aphids during their development.

When they hunt aphids they do not disperse them, which provide them constantly with food. During the 3rd instar larvae are more mobile, even move between aphid colonies and plants (Fréchette *et al.*, 2008).

In our investigations the duration of larval stage is 6-7 days, which is less than findings of Sandhu and Kaushal (1977), according to whom this stage lasts 10-12 days. Larvae of *Leucopis verticalis* Malloch complete their development in 12 to 15 days (Sluss and Foote, 1971).

Larvae do not leave excrements frequently, but before pupation. The great quantity of mucilaginous substance darkens quickly and attaches the puparium to the substratum. The black excrements signalize that some larva in the laboratory or in field was transformed into pupa. In the nature, larvae are transformed into pupae in the same plants on which they feed, among the host: in the underside of tobacco leaves, in leaf sleeve or hidden among flowers and seed capsules (Fig. 3). In Petri dishes, larvae seek for suitable place for pupation in reverse side of leaves or flowers, in hidden places far from light. Puparium is formed from the last larval skin and its color and patterns resembles of the 3rd stage. Immediately after pupation, the pupa is soft and its inside is still pulsating. Gradually, the skin of the pupae becomes firmer. The puparium is reddish brown, the front part of the pupae is rounded and adults eclode from there. Posterior spiracles of the mature larva persist unchanged.



Fig. 3 Pupa and wrinkled, dark aphids

The average pupal size is 3.5 mm and the average weight is 5.5 mg. In our investigations, duration of pupal stage was 6-7 days. Sandhu and Kaushal (1977) reported pupal duration of 15-20 days. Puparium duration of *Leucopis glyphinivora* Tanas. averaged 8.45 days (Ghadiri *et. al.*, 2003). The pupal period of

non-diapausing pupae of *Leucopis verticalis* Malloch. requires about 12 days (Sluss and Foote, 1971).

Before the eclosion of the imago, the pupa becomes darker. As the head of imago presses the puparium, it cracks and the imago comes out of the pupa (Fig. 4 and 5).



Fig. 4 Pupa before eclosion

Adult insects are small, winged, considerably short and silvery grayish, with dark grey spots on the head, thorax and abdomen. The wings are moderately broad, costa is continuous, vein Sc is complete, striking crossveins and wing costal area darkened. The head is almost round and wider than the thorax. The facetae are dark brown. Ocelli are present. Antennas are black, third limb of the antenna is the largest and arista is black and bare. Frons is widened anteriorly, clypeus is small. On mesonotum golden brownish lateral stripes and bristles are present (Fig. 6).



Fig. 5 Eclosion of imago



Fig. 6 Golden brownish lateral stripes and bristles on mesonotum

The body is 3 mm long. Scattered small and large bristles and microtrichia are present. Typical for this species is that their body is somewhat curved in the lower part, i.e. the abdomen is curved downward. Legs are black. Femur apices, some part of tibia and tarsus is yellow. Male genitalia are symmetrical. The species is distinguishable using male characteristically genitalia. Female has flexible tubular ovipositor.

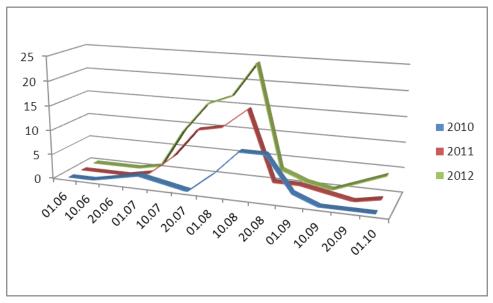
In laboratory conditions, the imago lives 6-7 days. Adults are mobile and able to

locate the prey. Reproductively mature females will found the prey while looking for oviposition sites.

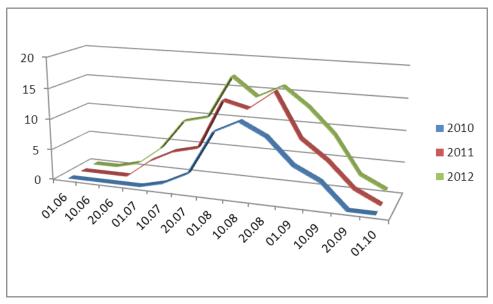
In our investigations, the growth of one generation from egg to imago was 21-24 days. In nature, if no diapause intervenes, the entire life cycle of *Leucopis verticalis* Malloch. can be completed in 33–42 days (Sluss and Foote, 1971). Martelli (1908), reported that the cycle from egg to adult takes 30 days. In temperate regions, overwintering is occurring as diapausing pupae

(Sluss and Foote, 1971). Leucopis sp. survival on *M. persicae* and

quantitative representation was significantly higher during the 2011-2012 than 2010. Their abundance is highly correlated with aphid density. On graph 1 and 2 are shown the results of quantitative representation of silver flies on tobacco.



Graph 1. Quantitative representation of silver flies on tobacco (method of Davies)



Graph 2. Quantitative representation of silver flies on tobacco (survey of 20 tobacco stalks)

The greatest quantitative representations of Chamaemyiidae are noticed in August.

# CONCLUSIONS

The eggs are tiny, almost invisible. The eggs are pearly white, 0.35-0.38 mm long, with longitudinally ribbed surface and with two ends, the first one is rounded and the

anterior end is a bit pointed. Duration of the egg stage was 2.5-3.5 days.

After hatching, larva is of transparent white color and is still egg shaped. During its

growth, the larva shed two times, passing through three instars and becoming more and more mobile. As the larvae of *Leucopis* sp. grow, the integument is bare, they become larger and they turn reddish. In the third larval stage (L3), larvae have the average weight ranging from 4 mg to 6 mg and are 4.5 mm to 5 mm long.

Larvae are tapered toward the head, broadest in the abdominal region, and bluntly rounded posteriorly, with caudal spiracles. L3 was dorsally slightly curved and ventrally slightly flattened. Duration of larval stage was 6-7 days.

The puparium is reddish brown, the front part of pupae is rounded and adults eclode from there. Posterior spiracles of the mature larva persist unchanged. The average pupal size was 3.5 mm and the average weight was 5.5 mg. Duration of pupal stage was 6-7 days.

Adult insects are small, winged, considerably short and silvery grayish, with dark grey spots on the head, thorax and abdomen. Scattered small and large bristles and microtrichia are present. The body is 3 mm long. In laboratory conditions, the imago lives 6-7 days.

In our investigations, the growth of one generation from egg to imago was 21-24 days

The role that they have in the long term regulation of aphid populations can be important. They may be used for biological control of aphids and in the scope of integrated pest management against these pests.

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#### INFLUENCE OF SOME FOLIAR SUBSTANCES IN THE PRODUCTION OF TO-BACCO SEEDLINGS

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## ABSTRACT

Study on the effect of some foliar products in tobacco seedlings production was conducted in 2012 at the Experimental field of Tobacco Institute - Prilep. Trials were performed with four organic foliar products (Slavol, SCD Probiotics – EMa, Bioflor and Energy) combined with one fertilization with ammonium-nitrate and two fertilizations with foliar products, including three fertilizations with foliar products only.

The investigated products have a positive effect on plants with roots, increasing their length from 20.00% to 33.68% and on plants without roots, which were 35.84% to 56.22% longer compared to the check.

They also have positive effect on the number of plants suitable for transplanting. In the variant fertilized with ammonium nitrate and Bioflor, the number of these plants was 38.33% higher compared to the check.

The investigated foliar products did not give positive results in the increase of the root system length.

It can be stated that investigated organic products (Slavol, Bioflor, SCD Probiotics – EMa and Energy) can be used for foliar fertilization of tobacco seedlings, individually or in combination with easily soluble mineral fertilizer ammonium nitrate.

Keywords: tobacco seedlings, foliar fertilization, Slavol, SCD Probiotics -EMa, Bioflor

## ВЛИЈАНИЕ НА НЕКОИ ФОЛИЈАРНИ СРЕДСТВА ВО ПРОИЗВОДСТВОТО НА ТУТУНСКИ РАСАД

Испитувањата за влијанието на некои фолијарни средства во производството на тутунски расад се извршија во 2012 година на Опитното поле од Научниот институт за тутун – Прилеп. Испитувањата се направени со четири органски фолијарни средства (Slavol, SCD Probiotics – EMa, Bioflor и Energy во комбинација со едно прихранување на амониум-нитрат и две прихранувања од фолијарните средства, како и три фолијарни прихранувања само со фолијарните средства.

Испитуваните средства за прихранување имаат позитивно влијание врз зголемувањето на должината на растенијата со корен од 20,00% до 33,68%, а растенијата без корен од 35,84 до 56,22% во однос на контролата. Тие влијаат позитивно и врз зголемувањето на бројот на растенија погодни за расадување. Кај варијантата прихранувана со амониум нитрат и со фолијарното средство Bioflor, бројот е зголемен за 38,33% во однос на контролата.

Испитуваните фолијарни средства недале позитивни резултати во зголемувањето на должината на кореновиот систем во однос на контролата.

Од испитувањата се констатира дека органските средства Slavol, Bioflor, SCD Probiotics – EMa и Energy може да се употребуваат за фолијарно прихранување на тутунскиот расад, посебно или во комбинација со лесно растворливото минерално ѓубре амониум нитрат.

Клучни зборови: тутунски расад, фолијарно прихранување, Slavol, SCDProbiotics -EMa, Bioflor

#### **INTRODUCTION**

Foliar fertilization is a technique of feeding plants through the leaves. It is not a substitute for basic or initial fertilization, but allows rapid compensation of plant needs for some important nutrients. With foliar fertilization, utilization of nutrients increases to almost 90% and with soil fertilization it is only 10%. All nutrients that can be received through the root can also be received through the leaf. Scientific research on foliar fertilization dates from about 40 years ago.

In recent years, foliar fertilizers have been obtained by extraction of organic fertilizers from Californian worms, applying the technology of beneficial microorganisms, along with addition of biostimulators, plant hormones, certain mineral elements and natural components for protection from diseases and pests. These fertilizers directly affect the biochemical and physiological processes in plants and indirectly the increase of microbiological activity of soil, which results in increase of nutrient uptake by the plants.

Little research has been done on the use of organic foliar fertilizers in the production of tobacco seedlings. For this reason, the subject of this paper is to study the application of several foliar fertilizers, separately or in combination with easily soluble nitrogen fertilizer. Our aim was to determine the effects of their application in obtaining healthy and good quality seedlings, as a condition for stable and efficient tobacco production. The available literature data mainly refers to nutrition of tobacco seedlings with easily soluble nitrogen fertilizers (Chile saltpeter NaNO3 and nitromonkal KAN), but also to the number and time of their application.

Benkovic (1964) recommends feeding of tobacco seedlings with KAN and states that the best time for application is when young seedlings develop the first pair of true leaves with approximate size of a nail. Then it is necessary to apply a single fertilization with 150 - 200 g/10 m<sup>2</sup> KAN per seedbed. However, previously non-fertilized soils need a second fertilization with 150g KAN per seedbed. The second fertilization usually takes place seven days after the first one. Doney (1981) reports that the first feeding should be performed with  $10 \text{ g/m}^2$  ammonium nitrate when the seedbed is completely green, the second prior to the rapid growth stage with 15-20  $g/m^2$  and the third one with the same amount of fertilizers, depending on the condition of seedlings. Seedlings should not be fertilized the last ten days before transplanting in field. Dražic (1995) recommends the first feeding to be done with the emergence of the first pair of leaves and the second one 7 to 10 days after, with 15-20 g/m<sup>2</sup> amount of KAN.

Dimitrov et al. (2005) recommend the first feeding to be made in the 4th-leaf stage with 15 g/m<sup>2</sup>, the second one in the stage of rapid growth with 20-25 g/m<sup>2</sup> and the third with 10-15 g/m<sup>2</sup> ammonium nitrate.

Gveroska at al., (2012) reported that tobacco seedlings treated with probiotic ProBio Origina <sup>tm</sup> have the highest length of the root system, which is particularly important for successful transplanting.

#### MATERIAL AND METHODS

Aromatic oriental tobacco Prilep P-66-9/7 was used as material for work. Investigations were carried out at the Experimental field of the Scientific Tobacco Institute - Prilep in 2012 with the following variants: 1. Check  $(\emptyset)$  – non-fertilized 2. Ammonium nitrate (34.4%) –first feeding with 10 g/m<sup>2</sup>, second feeding with 15 g/m<sup>2</sup> and third feeding with 20 g/m<sup>2</sup>

3. Ammonium nitrate (34.4%) - one feeding with 10 g/m<sup>2</sup> + two foliar feedings with 1% Slavol solution

4. Ammonium nitrate (34.4%) - one feeding with 10 g/m<sup>2</sup> + two foliar feedings with 0.1% Energy solution

5. Ammonium-nitrate (34.4%) – one feeding with 10 g/m<sup>2</sup> + two foliar feedings with 10% Bioflor solution

6. Ammonium nitrate (34.4%) - one feeding with 10 g/m<sup>2</sup> + two foliar feedings with 10% SCD Probiotics -EMa solution 7. Slavol - three foliar feedings with 1% solution

8. Energy - three foliar feedings with 0.1% solution

9. Bioflor - three foliar feedings with 10% solution

10. SCD Probiotics -EMa - three foliar feedings with 10% solution

The first foliar feeding of seedlings was applied on 29.4.2012, the second on 7.5.2012 and the third one on 16.5.2012. The required preventive treatments were also applied to protect seedlings from diseases and pests.

Total length of seedlings (stalk and root), length of seedlings without root, length of root, seedlings weight, leaf number of the seedlings, number of plants suitable for transplanting and length of the period of seedlings production were the subject of

Foliar feeding is often called "strengthening" of tobacco seedlings. According to the investigations, all tested products show stimulating effects on growth and quality of tobacco seedlings and on the number of plants suitable for transplanting. The this research.

The following mineral and organic-mineral fertilizers were used in the research: Ammonium nitrate - the <u>nitrate</u> salt of <u>ammonium</u>, with chemical formula  $NH_{4NO3}$ . It contains 34-35% nitrogen, half of which is in the form of ammonium (NH4), and the other half in the form of nitrate (NO3). Producer – Russia.

**ENERGY** – organic foliar fertilizer rich in organic bioactive substances extracted from the mineraloid leonardite. It is composed of humic acid, fulvo acid, plant extracts and natural biostimulators (vitamins, ferments). Producer – Agromarket Igor, Strumica, R. Macedonia.

**BIOFLOR** – liquid organic microbiological fertilizer. The basis of Bioflor is the manure obtained from Californian worms. Producer – Indus Dooel, Skopje, R.Macedonia

**SCD PROBIOTICS - EMa -** liquid organic microbiological fertilizer. composed of natural ingredients, based on the principle of "effective microorganisms (EM). Presently, it consists of four groups of microorganisms: lactic acid bacteria, photosynthetic bacteria, yeasts and actinomycetes. Producer – SCD Probiotics, USA.

**SLAVOL** - natural bioorganic fertilizer containing bacteria (nitrogen-fixing bacteria and phosphate-mineralizators), natural vitamins and growth stimulators. It is universally certified fertilizer that can be used in organic and traditional farming. Producer – Agrounik, doo Beograd, Serbia.

# **RESULTS AND DISCUSSION**

presented data represent the average values obtained from 10 replicates. The obtained results of foliar application will be compared with variant 1 (non-fertilized check) and variant 2 (traditional mode of feeding).

## Total length of seedlings (stalk and root)

The applied foliar products have a positive impact on seedlings length, i.e. on their

growth and development.

The length of seedlings ranged from

approximately 19.0 cm in the check variant 1 (non-fertilized) to 22.8 cm in variant

2, fertilized only with ammonium nitrate (Table 1).

No.	Variants		%	%	%
1	Non-fertilized check (Ø)	19.0	100.00	83.33	-
2	$AN^{(0)} 34.4\%$ - $F^{(3)}$	22.8	120.00	100.00	-
3	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Slavol- $F^{(2)}$	23.8	125.26	104.38	100.00
4	AN <sup>0)</sup> 34.4% -F <sup>1)</sup> + SCD Probiotics –Ema–F <sup>2</sup>	23.5	123.68	103.07	100.00
5	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Bioflor- $F^{(2)}$	22.8	120.00	100.00	100.00
6	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Energy- $F^{(2)}$	25.4	133.68	111.40	100.00
7	Slavol –F <sup>3)</sup>	24.7	130.00	108.33	103.78
8	SCD Probiotics –Ema – F <sup>3)</sup>	24.9	131.05	109.21	105.96
9	Bioflor – F <sup>3)</sup>	24.2	127.36	106.14	106.14
10	Energy – F <sup>3)</sup>	23.2	122.10	101.75	91.34
AN	<sup>0)</sup> –Ammonium nitrate	LSD			
$F^{1}$ - One feeding		5% = 0.73 cm			
$F^{2)}$ – Two feedings		1% = 0.97 cm			

Table 1. The effect of foliar feeding on seedlings length (cm)

F <sup>2)</sup> – Two feedings	1% = 0.97 cm
F <sup>3)</sup> –Three feedings	0.1% = 1.26 cm

Compared to the check, the length of seedlings in variants with combined fertilization increases from 20.00% in variant 5 to 33.68% in variant 6; compared to variant 2 the increase of length ranges from 3.07% in variant 4 to 11.40% in variant 6.

Variants 7,8,9 and 10 fertilized only with foliar products also increased the seedlings length from 22.10% in variant treated with Energy to 31.05% in variant treated with SCD Probiotics-Ema. Compared to variant 2, the increase ranged from 1.75% with Energy

to 9.21 % with SCD Probiotics-Ema.

Compared to combined fertilization, fetilization with foliar products only increased the length of the seedlings from 3.78% with Slavol to 6.14 % with Bioflor, and with application of Energy the length decreased for 8.66%. The highest increase was obtained in variant 6, fertilized with combination of ammonium nitrate and Energy.

The statistical analysis of data shows significant differences in all variants fertilized with foliar product compared to the check.

## Length of seedlings without root

The length of tobacco seedlings without root is another important trait for determination of quality. Some of the seedlings are stunted in growth, with unusually developed root. These plants should not be transplanted in field because they give lower yields. From the presented data on the length of tobacco seedlings without root (Table 2), the same conclusion can be made as for the seedlings with root: in all variants where foliar treatment was applied, the length of the stalk with leaves is greater compared to the check variant.

No.	Variants		%	%	%
1	Non-fertilized check (Ø)	13.2	100.00	72.52	-
2	$AN^{0}$ 34.4% - $F^{3}$	18.2	137.35	100.00	-
3	$AN^{(0)}$ 34.4% - $F^{(1)}$ + $Slavol - F^{(2)}$	19.2	144.90	105.49	100.00
4	AN <sup>0)</sup> 34.4% - $F^{1)}$ + SCD Probiotics –Ema – $F^{2)}$	19.7	148.67	108.24	100.00
5	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Bioflor - $F^{(2)}$	18.0	135.84	98.90	100.00
6	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Energy - $F^{(2)}$	20.0	150.94	109.89	100.00
7	Slavol – F <sup>3)</sup>	20.7	156.22	113.73	107.81
8	SCD Probiotics –Ema – F <sup>3)</sup>	19.8	149.43	108.79	110.00
9	Bioflor $-F^{3)}$	19.4	146.41	106.59	107.78
10	Energy $-F^{3}$	18.9	142.64	103.84	94.50

Table 2. The effect of foliar feeding on the length of seedlings without root (cm)

AN<sup>0)</sup> - Ammonium nitrate

F<sup>1)</sup>- One feeding

F<sup>2)</sup> – Two feedings

F<sup>3)</sup> - Three feedings

The length of seedlings without root ranges from 13.25 cm in the check to 18.2 cm in variant 2. So, in this variant, the feeding resulted in 37.35% higher average length of the stalks compared to the check, which means that nitrogen application has a significant effect on growth and development of tobacco plants.

In combined feeding, the length increase in plants without root ranges from 35.84%(variant 5,  $AN^{0}$  + Bioflor) to 50.94%(variant 6,  $AN^{0}$  + Energy), and in variant 2 the increase ranges from 5.49% in variant 3 to 9.89% in variant 6. Only in variant 5 ( $AN^{0}$  + Energy) plants without root showed 1.10% lower length compared to the plants

## LSD

5% = 1.17 cm

1% = 1.55 cm

0.1% = 2.01 cm

in variant 2.

Compared to the check, feeding only with foliar products increased the length of seedlings from 42.64% in variant 10 (Energy) to 56.22% in variant 7 (Slavol). Compared to variant 2, the increase of seedlings length ranges from 3.84% in variant 10 to 13.73% in variant 7.

The analysis of variance and LSD test show high statistical significance of the values obtained in all investigated variants compared to the check, indicating that differences in length of the above-ground part of plants between the check and fertilized variants are result of the activity of applied products.

#### Length of root

The aim of feeding is to provide fast and good quality nutrition to tobacco seedling, i.e. to allow normal growth and development both of its above-ground parts and the root system. In our trials (Table 3), the highest average root length of 5.8 cm was obtained in the check variant. This was expected, because the availability of nutrients in the soil affects the development of the root system. In the opposite case, when the amount of nutrients is not sufficient, plants are forced to develop their roots in lower layers of soil in search for food, as is the case with the check (non-fertilized variant).

In variant 2, the average root length is 4.6 cm, which is 1.15 cm ( about 20.00%) less than the check variant,.

No.	Variants		%	%	%
1	Non-fertilized check (Ø)	5.75	100.00	125.0	_
2	$AN^{(0)}$ 34.4% - $F^{(3)}$	4.6	80.00	100.00	-
3	$AN^{(0)}$ 34.4% - $F^{(1)}$ + $Slavol - F^{(2)}$	4.4	76.52	95.65	100.00
4	AN <sup>0)</sup> 34.4% - $F^{1)}$ + SCD Probiotics –Ema – $F^{2)}$	5.3	92.17	115.22	100.00
5	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Bioflor - $F^{(2)}$	4.6	80.00	100.00	100.00
6	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Energy - $F^{(2)}$	5.5	95.65	119.56	100.00
7	$Slavol - F^{3}$	5.6	97.39	121.74	127.27
8	SCD Probiotics –Ema – F <sup>3)</sup>	5.1	88.69	110.87	96.23
9	Bioflor $-F^{3)}$	4.9	85.21	106.52	106.52
10	Energy $-F^{3)}$	4.3	74.78	93.48	78.18

Table 3. The effect of foliar feeding of seedlings on root length (cm)

AN<sup>0)</sup> - Ammonium nitrate

- F<sup>1)</sup>- One feeding
- F<sup>2)</sup> Two feedings
- F<sup>3)</sup> Three feedings

The root length in variants with combined fertilization ranged from 4.4 cm in the variant with ammonium nitrate + Slavol, to 5.5 cm in the variant with ammonium nitrate + Energy. Of all variants with combined fertilization, the best results were obtained in variant 6 ( $AN^{0}$  + Energy). The average root length in this variant was 5.5 cm, which is higher than in the other variants (3, 4, 5)where combined fertilization was applied. In all variants with foliar feeding a decrease of the root length was observed. The lowest decrease compared to the check was estimated in variant 7 (2.61%) and the highest in variant 10 (25.22%). Comparison between variants with foliar feeding and LSD

1% = 0.68 cm

0.1% = 0.88 cm

variant 2 shows that the use of the above products, except for variant 10, gave better results and increased the root length from 6:52% in variant 9 to 21.74% in variant 7. Comparison between the variants with combined feeding and those with foliar application reveals that the best results were obtained in variant 7, where the increase of root length was 27.27%. The decrease of root length in tobacco seedlings as a result of feeding was confirmed through statistical processing of data by the analysis of variance and LSD test. The decrease of root length ranged from 0.1% in variants 2, 3, 5 and 10 to 1% in variant 9.

#### Seedlings weight

Weight of the seedlings is a parameter which indicates that tobacco plants, under the influence of fertilization, formed a well developed plant mass with adequate supply of nutrients, which promises successful transplanting and acceptance of tobacco in field (Table 1).

The lowest amount of organic matter was formed in the check variant, with an average weight of 5.09 g/plant. Positive impact on formation of organic matter was observed in Variant 2, which had an average weight of 6.04 g/plant, or 18.66% higher than the check.

Higher average weight of seedlings was observed in all variants with combined fertilization. The increase of seedlings weight in relation to the check ranges from 16.30% in variant 5 to 30.45% in variant 3. Foliar feeding also showed a positive effect on seedlings weight. Compared to the check, the increase of weight ranged from

16.64% (variant 7) to 25.73% (variant 10).

No.	No. Variants		%	%	%	
1	Non-fertilized check (Ø)		100.00	84.27	-	
2	$AN^{0}$ 34.4% - $F^{3)}$	6.04	118.66	100.00	-	
3	$AN^{0)} \ 34.4\% \ \ - \ F^{1)} + \ Slavol - F^{2)}$	6.64	130.45	109.93	100.00	
4	AN <sup>0)</sup> 34.4% - $F^{1)}$ + SCD Probiotics –Ema – $F^{2)}$	5.98	117.48	99.00	100.00	
5	$AN^{(0)} 34.4\%$ - $F^{(1)}$ + Bioflor - $F^{(2)}$	5.92	116.30	98.01	100.00	
6	$AN^{(0)} 34.4\% - F^{(1)} + Energy - F^{(2)}$	6.18	121.41	102.31	100.00	
7	Slavol – F <sup>3)</sup>	6.09	116.64	100.82	91.72	
8	SCD Probiotics –Ema – F <sup>3)</sup>	6.37	125.14	105.46	106.52	
9	Bioflor – $F^{3)}$	5.77	113.35	95.52	97.47	
10	Energy – F <sup>3)</sup>	6.40	125.73	105.96	103.56	
AN	<sup>(0)</sup> - Ammonium nitrate	LSD				
F <sup>1)</sup> -	One feeding	5% = 0.68  cm				
F <sup>2)</sup>	– Two feedings	1% = 0.73g				
F <sup>3)</sup>	- Three feedings	0.1% = 0.95g				
and v 8 and better	barison between variants 3,4,5 and 6 ariants 7,8,9 and 10 shows that variants 10 with applied foliar feeding showed results in the increase of seedlings at than variants 4 and 6 with combined	The statis a presence indicating	et than varia stical analy se of stat that fertilize that the act	sis of da istical si ed plants h	ata shows gnificance, ave higher	

#### Table 4. The effect of foliar feeding on seedlings weight (g)

Leaf number of the seedlings

products.

Important trait in the production of tobacco seedlings is the number of leaves formed on the stalk in the period prior to transplanting. The average number of leaves in the check variant is 6.4, and in variant 2 it is 6.8. In all variants with combined feeding as well as in those fertilized with foliar product, the

fertilization, while variants 7 and 9 showed

average leaf number is 6.8 (in variants 3, 4, 5, 6, 7 and 9) and 6.7 (variants 8 and 10). The presented data show that the effect of feeding is negligible but, compared to the check, there is still some increase in the number of leaves, ranging from 4.68% to 6.25%.

No.	Variants		%	%	%
1	Non-fertilized check (Ø)	6.4	100.00	94.11	-
2	$AN^{0}$ 34.4% - $F^{3}$	6.8	106.25	100.00	-
3	$AN^{(0)}$ 34.4% - $F^{(1)}$ + $Slavol - F^{(2)}$	6.8	106.25	100.00	100.00
4	$AN^{0}$ 34.4% - $F^{1}$ + SCD Probiotics –Ema – $F^{2)}$	6.8	106.25	100.00	100.00
5	$AN^{(0)}$ 34.4% - $F^{(1)}$ + Bioflor - $F^{(2)}$	6.8	106.25	100.00	100.00

Table 5. The effect of foliar feeding on leaf number of the seedlings

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6 AN <sup>0)</sup> 34.4% - $F^{1)}$ + Energy – $F^{2)}$	6.8	106.25	100.00	100.00
7 Slavol – $F^{3}$	6.8	106.25	100.00	100.00
8 SCD Probiotics –Ema – F <sup>3)</sup>	6.7	104.68	98.52	98.53
9 Bioflor – $F^{3}$	6.8	106.25	100.00	100.00
10 Energy $-F^{3}$	6.7	104.68	98.52	98.53
AN <sup>0)</sup> - Ammonium nitrate	LSD			
F <sup>1)</sup> - One feeding	5% = n.s.			
F <sup>2)</sup> – Two feedings	1% = 0.73g			
F <sup>3)</sup> - Three feedings	0.1% = 0.95g	3		
In addition, it should be noted that leaf	seedlings wi	hich is dif	fcult to ch	ange even

In addition, it should be noted that leaf number is a distinctive trait of tobacco

seedlings which is difficult to change, even with better nutrition of plants.

#### Number of plants suitable for transplanting

The lowest number of suitable plants was found in the check-9600 plants/10m<sup>2</sup> (Table6). In variant fertilized with ammonium nitrate,

the average number of suitable plants was  $11680/10m^2$ , which is 21.66% higher than the check.

No.	Variants	10m <sup>2</sup> (1 seedbed)	%	%	%
1	Non-fertilized check (Ø)	9600	100.00	82.19	_
2	$AN^{0}$ 34.4% - $F^{3}$	11680	121.66	100.00	-
3	$AN^{0}$ 34.4% - $F^{1}$ + $Slavol - F^{2}$	11360	118.33	97.26	100.00
4	AN <sup>0)</sup> 34.4% - $F^{1)}$ + SCD Probiotics –Ema – $F^{2)}$	11520	120.00	98.63	100.00
5	$AN^{0}$ 34.4% - $F^{1}$ + Bioflor – $F^{2}$	13280	138.33	113.70	100.00
6	$AN^{0}$ 34.4% - $F^{1}$ + Energy - $F^{2}$	12800	133.33	109.59	100.00
7	Slavol – F <sup>3)</sup>	12000	125.00	102.74	105.63
8	SCD Probiotics –Ema – F <sup>3)</sup>	11840	123.33	101.37	102.78
9	Bioflor $-F^{3)}$	12480	130.00	106.85	91.76
10	Energy – F <sup>3)</sup>	12160	126.66	104.11	95.00

AN<sup>0)</sup> - Ammonium nitrate

- F<sup>1)</sup>- One feeding
- F<sup>2)</sup> Two feedings
- F<sup>3)</sup> Three feedings

With combined fertilization, the number of plants suitable for transplanting increased from 18:33% (variant 3) to 38.33% (variant 5) compared to the check, while feeding with foliar products increased it from 23.33% to 30.00%.

The increased number of seedlings suitable for

transplanting is a result of feeding, either by soil or foliar application, because availability of nutrients allows higher photosynthetic activity and thereby better growth and development of higher number of plants per unit area.

## .Length of the period of seedlings production

The length of the period of seedlings production (from sowing the seed to transplanting) is very important for production of a good quality tobacco. In our investigations, the seedlings were ready for transplanting in about 44 days, except for the non-fertilized check which took another 4-6 days to reach the appropriate size for transplanting.

# CONCLUSIONS

- Feeding the seedlings with ammoniumnitrate and organic foliar products (separately or in combination) has a significant impact on the increase of seedlings length. The best results for this parameter were obtained in variant 6, fertilized with ammonium nitrate and Energy.
- Feeding of tobacco seedlings also resulted in increase of their weight, which is evident in all the varieties investigated. The best effect on this trait was achieved in variant 3, fertilized with ammonium nitrate and Slavol, which gave 30.45% increase of weight compared to the check.
- Feeding with foliar products had no effect on the increase of root system

length. The best results for this trait were obtained in the check variant.

- Feeding of tobacco seedlings has a positive impact on the increase of the number of plants per unit area that are suitable for transplanting. Most of the suitable plants were obtained in variant 5, fertilized with ammonium nitrate and foliar product Bioflor.
- According to the obtained results, the investigated organic foliar products Slavol, Bioflor, SCD Probiotics-EMa and Energy can be used for foliar feeding of tobacco seedlings separately or in combination with easily soluble mineral nitrogen fertilizers such as ammonium nitrate.

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Original Scientific paper

#### THE PHYSICAL CHARACTERISTICS OF RAW TOBACCO- PRILEP 66 - 9/7

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#### ABSTRACT

Prilep 66 - 9/7 is the most common variety in production of oriental tobacco in Prilep area. This variety provides the raw material with high quality and flavor, typical for oriental tobacco. Experiment was placed in the region of Prilep in the period 2013-2014. Oriental variety Prilep 66 9/7 was used as a testing material. The aim of this study was to demonstrate the quality of materials, expressed through the physical characteristics of the raw material, which is obtained by application of appropriate agro-technical measures in various meteorological years of tobacco production.

Keywords: physical characteristics, raw tobacco, Prilep 66-9/7

#### ФИЗИЧКИ КАРАКТЕРИСТИКИ НА ТУТУНСКАТА СУРОВИНА - ПРИЛЕП 66 - 9/7

Во производството на ориенталски тип тутун во прилепскиот реон, најзастапена е сортата Прилеп 669/7. Суровината од овој тип тутун е со висок квалитет, карактеристична за ароматичен ориенталски тип тутун. Опитот беше поставен во реонот на Прилеп, во 2013 и 2014 година. Како материјал во испитувањето беше користена сортата Прилеп 66 - 9/7. Целта на ова истражување беше да се демонстрира квалитетот на материјалот, изразен преку физичките карактеристики на тутунската суровина, добиена со користење на соодветни агротехнички мерки во различни метеоролошки години на производство.

Клучни зборови: физички карактеристики, тутунска суровина, Прилеп 66-9/7

#### **INTRODUCTION**

Tobacco (*Nicotiana tabacum L.*) has been cultivated almost in all districts in Macedonia with ages, especially when it comes to production of aromatic type of tobacco. It is traditional agricultural crop where labor force has a great importance. A large part of work activities are carried out manually, but recently, more attention is paid to implementation of new technical achievements in production process. So, most of the working operations are carried out mechanized (transplantation of seedlings, cultivation, irrigation with fertilization etc.). The most important thing is to increase productivity while obtaining high quality tobacco. Modified traditional practices of tobacco producers made contribution for larger productivity. Therefore, modern tobacco producers pay more attention to agro-technical measures and protection from the starting point (production of seedlings), till the end of harvest. Mainly, oriental varieties of tobacco in Macedonia are grown in accordance with soil characteristics as well as climatic conditions in the territory, so tobacco receive specific characteristics which determines the value of raw tobacco material.

# **MATERIAL AND METHODS**

The experiment was placed in the region of Prilep, at the land of individual tobacco producer. Tobacco seedlings were traditionally produced, in cold beds covered with polyethylene. For that purpose, 5 g per 10 m<sup>2</sup> certified tobacco seed from Scientific Tobacco Institute – Prilep has been used. The sowing of tobacco was made on 15 March, in 2013 and on 19 March, in 2014. All necessary agro-technical measures were applied during the period of tobacco seedlings production. In this study, the experiment was set-aside area of 7 acres (fallow land from previous year, 2012). The main processing of the soil in 2014 year is due to loosening the soil as a result of the surface preparation in the previous year. To achieve the best results, the regulation must be carried out on plug plang, and semi-spiral functions of cultivator should be regulated. In order to collect the maximum moisture of autumn and winter precipitation, a different way of plowing is done, until the spring of preparation allows the trimming the surface, in order to achieve greater grinding of lumps. Transplanting of tobacco seedling was made in the second half of May, with mechanical transplanting machine (three seats program AGROBAR Vinica). Transplanting

machine was served by five workers, from which three batteries for planting and the two positions for seedlings transplanting. Planting density of the tobacco seedling was 40x15 cm on previously prepared soil. Additional cultivation is carried out at a depth of 12-15 cm, after accepting tobacco seedlings (to reduce the growth of weeds). During the growing season in 2013 (July, August), irrigation was made several times with rotary sprinkler model "Rink". In both production years fertilization with mineral fertilizer NPK (8:22:20) was performed on the surface of about 350 kg/ ha. Tobacco harvesting was performed manually in technical maturity of leaves. Tobacco leaves were strung with threading machine (Glotsas Tobacco Machines). Tobacco was sun cured in traditional way, on scaffolds covered with polyethylene. For two production years, following physical properties were determined: leaf dimension (cm), main rib (%), leaf thickness (µm), materiality of the tissue (g/m2), as well the yield of dry weight per unit area (kg/ ha). Analysis of physical properties was performed on pre-prepared tobacco leaves, cleaned of soil, dust and other impurities.

## **RESULTS AND DISCUSSION**

The results showed that there is a need of using the respective equipment in the process of tobacco production, such is deep plowing to be executed with one aggregate composed of IMT-539 with two-raw plough, penetrating at about 25-30 cm. In order to keep on with good agricultural practice, the processing of the soil in autumn, was

controlled by on plows devices to plough arable layer on the surface on the soil rich with plant residues, but at the same time to translocate it at the bottom that will be used by the roots' system of the plant (Davchev, 2007). In the secondary management of soil, the same aggregate, tractor IMT 539 and two-raw plough were used. The results showed that if the unit is regulated, it is enough one spring plowing cultivation of the surface. When saving or reducing the working processes (instead of three main treatments, should be two treatments), up to 10 % energy per hectare area can be reduced, and thus, a lower production cost for one kilogram of tobacco can be directly saved. It is necessary to follow different conditions at the field in order implement appropriate cultivation to producing high-quality practices for tobacco. It is very important to emphasize the quality of tobacco seedlings and its manipulation, regulation of the discs itself as well, which directly depend on quality

of transplantation. According to Davchev (2007), the success of the adoption of seedlings depends on agro-meteorological conditions at the time of transplanting, the type of the soil, the extent of its preparation etc.. The frequent oscillations in temperature usually reduce the proper development of the root. Distribution of rainfalls through the vegetation period in 2013 was inadequate (May, June, July and August). Total rainfalls didn't exceed more than 178,2 mm. Accordingly, simultaneously with transplanting watering of tobacco was performed, thus allowing easier acceptance of tobacco seedlings in new conditions at the open field. Vukadinovic (1999), claims that in the period of transplantation the plant easy avoids stress as a result of particular morphological and physiological adaptations in addition with proper quantity of water. The next year (2014), irrigation of tobacco was not recommended due to excess of water (Table 1).

Month	2013	3	2014		
Wonth	Temperature	Rainfalls	Temperature	Rainfalls	
May	17.3	74.8	15.2	52.1	
June	19.8	78.0	19.7	35.0	
July	22.4	9.2	22.1	74.6	
August	23.8	16.2	22.2	68.6	

## Table 1.Meteorogical data (vegetation period 2013 -2014)

\*Source: National Hydrometeorological Services of R.Macedonia (weather station in Prilep)

During the vegetation, tobacco plants create and accumulate a certain amount of green mass in the leaves of which, after drying, it gets dry weight. This amount of tobacco depends mainly on the biological potential of the variety, the quality of tobacco seedling, meteorological conditions, largely byagro-technical measures during the vegetation, the degree of maturity of tobacco, and drying process afterward. Drying tobacco is very important postharvest practice for obtaining the best possible quality of tobacco. According to Dimitrieski&Miceska (2011), dry tobacco yield from Prilep 66 9/7, is ranged from 2000 to 3600 kg/ha. The yield is highly dependent on growing conditions and applied agricultural practices. The average yield for both years (2013-2014) amounted to 2400 kg/ha. The results of our research are in correlation with the data reported by authors. Maturity of the Tobacco and drying process have great influence on obtain dry mass. The results showed that 615 kg green mass of tobacco is required to be dried, so to be produced 100 kg of dry mass.The dimensions of the leaves depend on the variety, environmental conditions as well as agro technical measures in the vegetation period. The following data show the average values of leaf dimensions by harvest. The leaves from the top have smallest size.

Harvest	Length, cm	Width, cm	Ratio L:W
Lower	10.70	5.60	1.91
Middle	15.80	7.40	2.13
Upper	10.30	5.00	2.06
Average	12.26	6.00	2.03

Table 2. Average value of length and width of tobacco leaf (average 2013/14)

Table 2, clearly express the legality regarding the dimensions of tobacco leaves. The highest average length have leaves from middle belt (15.8 cm), and the lowest ones from the upper belt (10.3 cm). Karajankov et al. (2007), claim that oriental types of tobacco have small leaves up to 18 cm. The highest average width have the leaves from the middle (7.4 cm), and least, those from upper belt (5.0 cm). Data show that dimensions of the leaves are the result of the type of tobacco, or variety, and agroenvironmental conditions during vegetation. The ratio length:width of the leaf, actually determines the form of the leaf. In general, the form of tobacco leaf is varieties feature which depends on affiliation. It is directly affected by weather conditions of and agrotechnical measures implemented during vegetation of tobacco (Boceski, 2003). The shape of the leaf is not a significant feature in the technological processing of the leaf, because during drying it changes as a result of contraction of the leaf and reducing the surface of tissue. Data in Table 2 show that the ratio L: W of the leaves as the average value of the leaves is 2.03. It means that ratio is

favorable, if considered that the best ratio is 2: 1 (lamina is relatively symmetric with regard to the main rib). Mitreski (2012), points out that ratio green: dry mass of oriental tobacco Prilep varieties, ranges from 6.11-6.95: 1. In terms of technological characteristics of raw tobacco, or the contents of tobacco ribs, they are an important indicator of the quality of raw material. The high content of main rib is always a sign of poor quality. The content of main rib in the oriental tobacco is low and rarely exceed 20% of the total weight of the leaf (Mitreski, 2012). The thickness of tobacco leaf depends on the anatomical structure, cell size parenchyma, thickening of the membrane and intracellular spaces. For thickness, ribs have some effect. In general, as long as the leaves are thicker, the quality is even worse, and vice versa. Materiality tobacco leaves indicates the amount of dry matter  $(g / m^2)$ , which is located in the leaf tobacco per unit leaf area without main rib and without secondary ribs. Materiality is variable, but within certain limits.The obtained values of the physical properties of the raw material of Prilep 66 9/7, shown in Table 3.

Harvest	Rib, %	Leaflamina, %	Thickness, µm	Materiality,g/m <sup>2</sup>	
Lower	22.99	77.05	65.90	42.13	
Middle	18.68	81.30	73.20	46.28	
Upper	15.49	84.51	91.70	67.35	
Average	19.05	80.95	76.94	51.92	

Table 3. Physical characteristics of raw tobacco (average 2013/14)

The content of the main rib decreases from bottom to top (Table 3). According to the legality, increasing participation of leaf going from the bottom up, this is characteristic of oriental tobacco. The results show that leaf lamina is represented by 77.05% (lower leaves), 81.30% of the leaves from the middle band, and 84.51% in the top insertions. The average value (19.05%) of main rib and (80.95%) lamina as characteristic of the variety, positively influencephysical and degustative properties of raw material. The thickness of the leaves increases, going from the bottom (65.9µm) to the middle band,up to the top (91.7µm). The results fit with the materiality of raw tobacco, in the range of oriental tobacco (Uzunoski 1985). The greatest features of materiality haveupperleaves (67.35g/m<sup>2</sup>). Greater materiality in upper insertions explained as a result of their enhanced photosynthesis activity by transferring part of the plastic materials from the lower to the upper leaves (Macedonian oriental types of tobacco are characterized by moderate materiality).

# CONCLUSIONS

The yield and quality of tobacco began to be formed already in the process of production of tobacco seedlings, as a main factor for better acceptance after transplanting.

The results showed that there is a need of using the respective equipment in the process of tobacco production. The meteorological conditions have great influence on process of production.

Apart from the biological potential of the variety, the obtained yield and quality largely depend on influence of many other environmental factors, including on time solid preparation and tillage of the soil.

The ratio 6.1: 1 between green mass and dry mass of tobacco means that during the process of drying, significant physiological changes, took place in the structure of the leaves of tobacco.

Mainly, 80.95% is the leaf lamina, while the contents of the rib represents 19.5% of the entire leaf. Materiality of the Tobacco leaf is on average  $51.92 \text{ g}/\text{m}^2$ , ranges within the limits of oriental varieties of tobacco.

In order to achieve adequate yield and quality of raw material it is recommended to implement appropriate, on time agro technical practices.

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Original Scientific paper

# ECONOMIC AND CONSUMER CHARACTERISTICS OF NEW LINES BURLEY TOBACCO

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#### ABSTRACT

The aim of this study to evaluate by economic performance and consumer properties of our new lines Burley. Therefore defined economic ones, chemical indicators and the smoking properties of the newly selected seven lines of Burley tobacco. The obtained results show that with the best economic performance is Line 1362, followed by Line 1390. With respect to all new production lines superior standard Burley 21 variety, which is an indication of the success of the selection work. In complex by chemical parameters differ Line 1362, Burley 21 variety and Line 1390. Good smoking and properties revealed Line 1362 and Burley 21 variety. The overall results suggest that as a variant with the best economic features and consumer properties is presented Line 1362. Other research options are with medium quality. Very good comprehensive evaluation of Line 1362, allowing it to be offered for production testing and offering for recognition as a new variety of Burley tobacco.

Key words: Burley tobacco, new lines, yield, chemical indicators, smoking properties

#### ЕКОНОМСКИ И УПОТРЕБЛИВИ СВОЈСТВА НА НЕКОИ НОВИ ЛИНИИ ТУТУН ОД ТИПОТ БЕРЛЕЈ

Целта на ова испитување е оценка на стопанските карактеристики и употребната вредност на нашите нови линии од тутун тип Берлеј. Определени се стопански, хемиски индикатори и пушачките својства на седум нови линии тутун тип Берлеј. Добиените резултати покажуваат дека со најдобри стопански карактеристики е линија 1362, проследено со линија 1390. Сите нови линии се споредени со најдобрата стандардна сорта Берлеј 21, која е показател за успешноста на селекцијата. По комплексноста на хемиските параметри се истакнуваат линијата 136, сортата Берлеј 21 и линијата 1390. Со добри пушечки својства се одликуваат линијата 1362 и сортата Берлеј 21. Целосно резултати укажуваат на тоа дека како една варијанта со најдобри својства карактеристики и употребната вредност е линијата 1362. Други испитувани параметри се со среден квалитет. Многу добра сеопфатна проценка има добиено линијата 1362 и затоа постои можност истата да биде понудена за тестирање во производство и признавање како нова сорта од тутун од тип Берлеј.

Клучни зборови: тутун Берлеј, нови линии, принос, хемиски индикатори, пушачки својства

#### **INTRODUCTION**

Although restrictions against tobacco and smoking, recent years there is even a small

increase in the consumption of cigarettes American blend type / 0.6% year /. Tobacco

Burley type is indispensable component of cigarettes of this blend. Participate in their composition to 45% (Spears and Tones, 1981; Davis and Nielsen, 1999). Bulgaria is not a traditional producer of Burley tobacco. Therefore its cultivation is associated with a number of difficulties (Bozukov, 2012). The first place obtained yields are lower compared with the leading producing countries. The lower is percentage of first class (Turner, 1989). This requires the creation of new, high-yielding varieties adapted to our conditions and not least desirable of tobacco (Mutafchieva 2009, Palmer et al., 2007; Risteski et al., 2012).

The own sake increase the quantities of Burley tobacco leads to a lack of market realization (Snell. 2006). His search is based on the specific requirement, satisfying the needs of the tobacco industry (Kirkova, 2005; Kirkova and Taskova, 2005).

Combining good economic qualities and custom properties in a variety is very difficult and long process (Dimanov and Masheva, 2011; Dimitrieski et all, 2006). The main task of the Bulgarian selection is established and implemented in producing new, high yield varieties of Burley tobacco satisfying as claims of tobacco growers and requirement of the industry (Dyulgerski 2011).

The aim of our research to do assessment by economic performance and consumer properties of our new lines Burley tobacco. On this basis, to performed selecting for production testing.

# **MATERIAL AND METHODS**

The experimental work is carried out in TTPI - Markovo in the period from 2008 to 2010. At the study are subjected created by us seven lines of Burley tobacco, namely: Line 1231, Line 1252, Line 1277, Line 1323, Line 1362, Line 1383 and Line 1390. All they have shown good biological indicators and are very well aligned morphological and vegetative. To control used Burley 21 variety officially recognized standard in Bulgaria by 2010 in Burley tobacco. For all variants is applied uniform technology of growing, harvesting and drying. Production after manipulation defined dry tobacco yield per hectare, percentage of first, second and third class. In the chemical-technological laboratory in TTPI are provided samples harvested and air-dried tobacco leaves for analysis of basic chemical indicators as follows: nicotine, sugars (soluble carbohydrates), total nitrogen, ash, ammonia, chlorine and proteins.

To determine the chemical composition, tasting evaluation, and subsequent data processing are used standardized methods. To detect differences between the versions used ANOVA and many rank test of Duncan (1995).

# **RESULTS AND DISCUSSION**

## 1. Economic indicators

Average for the period of study Line 1362 gives the highest yield per hectare (Table 1). This line is formed by high-yield. With a small margin her following Line 1390. With high yield and are distinguished Line 1383 and Line 1323. The lowest yield is presented standard Burley 21 variety.

Variety/ Line	Yield kg/ha	Percentage of classes			
		Ι	II	III	
Burley 21	2718 <sup>e</sup>	34	49	17	
Line 1231	3023°	36	51	13	
Line 1252	2937 <sup>cd</sup>	26	52	22	
Line 1277	2783°	24	56	20	
Line 1323	3178 <sup>b</sup>	28	58	14	
Line 1362	3347ª	41	44	5	
Line 1383	3117 <sup>bc</sup>	30	59	11	
Line 1390	3282ª	33	53	14	
LSD 5%	98				

 Table 1. Yield and percentage of classes of variants of Burley tobacco included in the experience average for the period of study

With regard to yield any new lines exceed the testimony of the standard. This is an indication of success in selection work regarding such an important indicator.

In terms of percentage of the classes data in Line 1362 again with the most favorable parameters (Table 1). Line 1362 gives the highest percentage of first-class, and also this line provides the lowest percentage of third class of all studied variants. Only her first class percentage of is over 40, and a third class is below 10%.

In a complex of economic indicators in the first is ranked Line 1362, followed by Line 1390. With a relatively good indication of the percent of classes are also present Line 1231, Line 1390 and Burley 21 variety. The lowest quality formed Line 1277.

The results of the new lines concerning the percentage of classes should be considered satisfactory, since all of them prevailing percentage of second class.

## 2. Chemical indicators

With the highest values for nicotine content is Line 1362 (Table 2). None variant does not detect values above 3%. In Line 1252 nicotine content is too low by a requirement of Burley tobacco. In other variants obtained results are satisfactory.

Variety/ Line	Nicotine	Sugars	Total nitro- gen	Ashes	Ammo-nia	Chlorine	Protein
Burley 21	2,67	0,83	3,25	17,88	0,32	0, 34	9,76
Line 1231	2,02	0,95	2,71	17,17	0,34	0,35	8,43
Line 1252	1,90	1,07	2,56	21,87	0,35	0,37	11,34
Line 1277	2,36	1,01	2,82	12,43	0,34	0,41	10,22
Line 1323	2,62	0,90	2,94	17,15	0,33	0,34	15,62
Line 1362	2,93	0,82	3,63	17,71	0,31	0,32	10,66
Line 1383	2,74	1,23	3,33	17,12	0,31	0,33	9,27
Line 1390	2,69	0,88	3,28	18,26	0,33	0,34	8,85

Lowest, respectively the best sugars content reveals Line 1362, followed closely by a

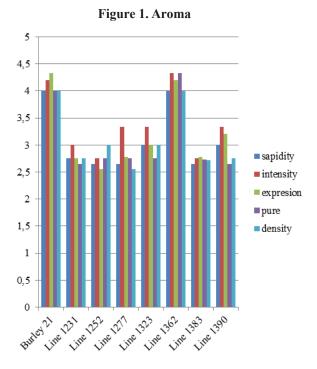
standard - Burley 21 variety. The results of the other options are satisfactory. The

sugar content in Line 1383 is too high for standards in Burley tobacco.

The content of total nitrogen for all variants is in the norms for Burley tobacco. Best results in terms highest values are found in Line 1362.

The ash content of at most the variant is at an optimum for Burley tobacco. At Line 1277 values for ashes are too low, and in Line 1252 too high.

Tasting evaluation is performed on mono cigarettes without filter segment and equal conditions. By elements of the perceptions



The results clearly show the favorable smoking properties at Line 1362 and standard Burley 21 variety.

In conclusion, it can summarize that a complex of economic and consumer properties stands out Line 1362. This variant is superior to the standard variety in all tested parameters. The lines subject

Line 1362 gives the highest yield per hectare of all studied variants. From the Line 1362 is with the lowest content of ammonia and chlorine which extremely undesirable for Burley tobacco. In Line 1383 are found good results. Unacceptably high levels of chlorine are reported in Line 1277.

Line 1323 stands too high values for protein content. In other studied variants it is within legal limits for Burley.

# 3. Smoking properties

the smoking properties of the studied lines Burley tobacco are presented in Figure 1 and Figure 2.

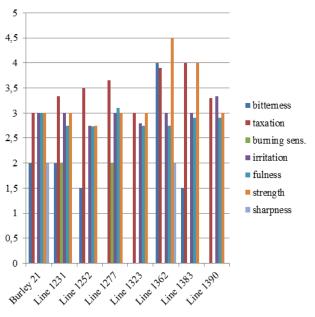


Figure 2. Taste, physiological strength and sharpness

to our research is observed other options superior standard Burley21 in economic performance, but his inferior in the smoking properties.

New created Line 1362 of varietal group Burley tobacco is with very good economic and consumer properties, allowing it to be offered for production testing.

## CONCLUSION

same line receives the highest percentage of first class.

With best economic qualities is present Line 1362. With good economic characteristics is also Line 1390.

In complex studies chemical indicators most distinguished Jine 1362. With relatively balanced chemical composition is characterized also Burley 21 variety and Line 1390.

With good smoking and properties are presented Burley 21 variety and Line 1362.

Regarding economic parameters all new lines are superior to the standard Burley 21 variety, but the most his retreat for consumer properties.

In a complex of studied parameters with the best results stands out for Line 1362. This is a prerequisite to be offered for production testing and offering for recognition as a new variety of Burley tobacco.

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# THE POSSIBILITY OF TOBBACO CLUSTER FORMING IN THE REPUBLIC OF SRPSKA

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#### ABSTRACT

The goals of clustering in the tobacco industry in the Republic of Srpska and Bosnia and Herzegovina are the creation of a modern and developed industrial structure, which will gradually fit into the economies of the integrated market and thereby contribute to the development of a more favorable economic environment. The necessity of such a development orientation stems from the facts that the economy of the region is based on the production of primary products of lower processing stages and that long-term economic growth is not possible on this basis. Therefore, the establishment and development of clusters of the tobacco industry in The Republic of Srpska and Bosnia and Herzegovina would represent a major development strategy whose positive effects would be felt in the entire underdeveloped region.

Keywords: cluster, manufacturer, survey.

#### МОЖНОСТ ЗА ФОРМИРАЊЕ НА ТУТУНСКИ КЛАСТЕРИ ВО РЕПУБЛИКА СРПСКА

Целите на кластеризација во тутунската индустрија на Република Српска и Босна и Херцеговина се создавање на модерна и развиена индустриска структура која постепено ќе се вклопува во стопанските текови на интегралниот пазар и со тоа ќе придонесува за развојот на поповолен стопански амбиент. Неопходноста од таквата развојна ориентација произлегува од фактот дека стопанството на овој регион е засновано на производство на примарни производи во пониска фаза на обработка и дека врз таа основа не може да се обезбеди долгорочен економски раст.

Поради тоа, воспоставувањето и развојот на кластери на тутун во Република Српска и Босна и Херцеговина претставува исклучително важна развојна стратегија чии позитивни ефекти ќе се рефлектираат на целиот регион, за кој со право може да се каже дека има карактеристики на неразвиеност.

Клучни зборови: кластер, производител, анкети.

#### **INTRODUCTION**

The experience of the Republic of Srpska and Western Balkan countries in the field of the clusters are more than modest. Specific examples of clusters that exist or are under development are mainly experimental trials that were mostly developed without clearly defined rules, organization, mission and vision. One of the main reasons for this is the lack of knowledge and lack of understanding of the term "cluster" and the importance of their institution for the development of the region. In the RS and the Western Balkans in general business conditions are extremely unstable. Not the end of the constructed system at the state level will encourage and facilitate development of small and medium enterprises (SMEs) and their clustering.

In this regard, the question arises: if and how capable SMEs are to continually invest in product development, technology, knowledge, and thus form the basis of development of the country or region? How realistic is it to expect that SMEs will invest some funds in a research project? Is tobacco manufacturer capable of coming independently to the European market and survive in this market without connecting with other manufacturers, carriers, etc.? Could SMEe alone meet the demands of the global market both in terms of quality and quantity and in terms of sustainable development and adaptation to the new, growing, market requirements?

The answers and solutions to all of these questions can be found by linking SMEs and creation of clusters depending on the characteristics and economic opportunities in the region. In this way, more SMEs make a large system that is able to fulfill most of the requirements of the market and still remain flexible. Companies that are included into functional or regional group or network will be more efficient than the companies which are not'.

which would be useful in the realization

of this project. This primarily refers to the

level of awareness and knowledge about

the clusters that exist among small and

medium-sized enterprises. Therefore, in the

next section we will present the results of

surveys conducted among small and medium

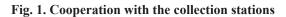
enterprises in the Republic of Srpska.

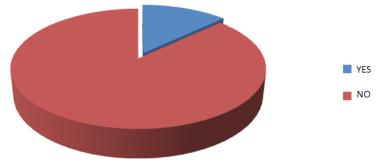
# CLUSTER APPROACH TO THE PROBLEM OF TOBACCO

The use of cluster yields is quite clear. However, to be serious in the approach to the cluster development in our region it is necessary to explore the reasons that led to a very poorly developed network of clusters. First of all it is necessary to conduct research in small and medium-size enterprises (SMEs) and based on that form some conclusions

# PRELIMINARY QUESTIONNAIRE

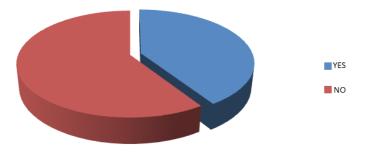
The following section will show the results of research that have been conducted on the basis of the survey of 14 companies and 15 entrepreneurs from the RS, which are associated with agricultural production, i.e. the production of tobacco. This part of the study represents preliminary questionnaire on the basis of which we had intended to present problems faced by small and medium-size enterprises in the field of the present business conditions, and based on that, we come to the basic conclusion - that by the process of clustering large part of these problems can be overcome. In Appendix 1 preliminary questionnaire is shown, and the questions were mainly related to exports, the cost of raw materials, organized education, collaboration, sales, etc.





To the question "How satisfied are you with the collaboration with collection stations" majority of respondents gave an answer that generally they were not satisfied or that such cooperation was at a very low level. The reason for such a high percentage of negative responses should be sought in the low prices of products, the criteria determining class quality products and quality group of tobacco, as well as low and irregular premiums for products, for which it can be said that they are not in the domain of buyers, but it is up to the relevant ministry. To the next question, related to the competition and its effect on the volume and quality of tobacco production, 45 % of respondents said that competition affects the production and sale of tobacco and tobacco products. It is reasonable that the reason for this is the import of tobacco into the Republic of Srpska and Bosnia and Herzegovina. The local market for agricultural products and tobacco as raw materials and finished products is filled with goods of questionable quality and questionable origin, which greatly narrows the scope for domestic producers for serious and quality production.

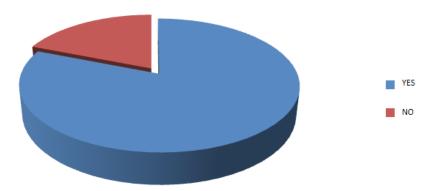
Fig. 2. Influence of competition on the scope and quality of tobacco production



Purchase price of agricultural products and tobacco is an extremely important and almost essential factor which influences the manufacturers' decision to deal with this production. That is confirmed by the fact that 93% of respondents gave a positive answer to this question. The reason for this should be sought in the fact that tobacco is commodity that is in demand and that it is less and less to offer, as well as the fact that his collection is relatively good



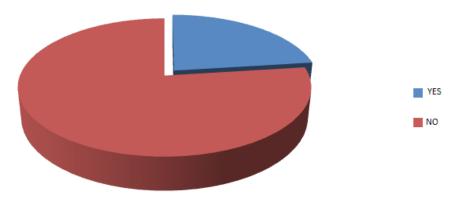
Asked whether the producers are interested to expand production, 81 % of respondents said yes. This is a very high percentage, considering that the production of agricultural products, and tobacco is associated with a lower degree of uncertainty (climate, set production, marketing) and the fact that the size of arable land for growing tobacco is usually limited. This is surely influenced by the fact that producers are thinking economically - higher production, higher yields and higher profits.



#### Fig 4. The interest of producers to expand production

Cluster, as a new form of business association, is apparently a new term and not many are familiar with its meaning. Therefore, the question of how the clusters contributed to the increase of production and marketing of tobacco in the region, was positively responded only by 23 % of the respondents. This is certainly due to the insufficient knowledge of the modes in which the clusters operate, the benefits of clustering through reducing operating costs and increasing revenues from operations, and the fact that human mentality is such that hardly accepts innovations or changes.

#### Fig 5. Contribution to the business through clusters



The general estimate based on the survey is that farmers and producers of tobacco in the Republic of Srpska and Bosnia and Herzegovina are not satisfied with the current situation and believe that the situation in this region can lead to a much higher level, where the role of the state, or the line ministry is very important, if not crucial.

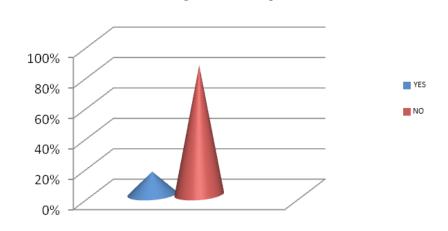
#### ANALYTICAL SURVEY

To closer perceive and analyze the problems in tobacco industry, we made a more detailed survey, primarily of the small and medium-size enterprises. During our research conducted in the region of Posavina and Semberije, where traditionally grown tobacco types are Virginia and Burley, we performed a study in three economic entities engaged in the production, purchase and sale of tobacco, as well as their subcontractors. The survey included AD ''Tobacco'' Bijeljina, ZZ ''Obudovac'', and ZZ ''Agreks'' Donji Zabar and their subcontractors.

Unlike the first part of the questionnaire related to general data, the second part was

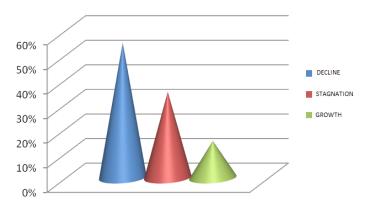
Fig 6. Tobacco exports

more focused on marketing and sales. For example, only 15 % of the total number of respondents were producing tobacco for exports and the remaining 85 % mainly formed their market sales in the area Republic of Srpska - Bosnia and Herzegovina. Such results indicate the current deficit problem, the import - export route, which is typical for developing countries.



In terms of expected earnings, results speak in favor of negative growth (55 %) and stagnation (35 %), and only a small proportion of companies (15 %) expected earnings growth in the future. The most common reason for this devastating condition, despite the economic crisis, is the very low level of competitiveness of the enterprises. In today's "harsh" business conditions, companies lose the existing market and it is very difficult for them to find new markets, because they are too small and weak in comparison to the large corporations that dominate and play a major role in today's business environment.

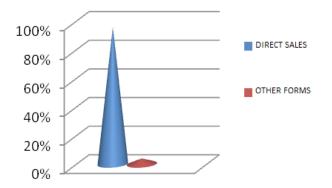




Research results on the development of sales channel go more in favor of its negative side, or underdevelopment of sales channels. The sales are mostly direct (96 %), while other types of sales (through trade fairs and the like) are used by 4 % of the respondents.

These results, however, should be viewed in light of the specific characteristics of the products (mainly agricultural products), because not all of them are suitable for sale through alternative channels. On the other hand, the analysis showed that a large part of the impossibility of developing alternative distribution channels and access to the market lies in the fact that these small financial companies are usually faced with liquidity problems, the inability to obtain quality information and a lack of skilled workers.

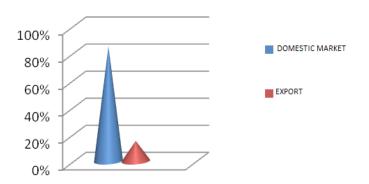
#### Fig 8. The development of sales channels



The main buyers of products of the enterprises surveyed are local agricultural cooperatives and local tobacco factory (85%), while the rest 15% refers to tobacco factories in the region (Macedonia, Serbia, Croatia...). With regard to products and

services, however, the existing customers complaints address the inability of production and delivery of large amounts (62 %), quality (20 %), price (16 %) and other similar reasons (2 %).

Fig 9. The main customers of the enterprises surveyed



From all previously mentioned, it can be concluded that the respondent companies generally have developed marketing and sales elements of the business, or they are not sufficiently developed to be able to seriously compete in the larger markets, where they encounter businesses that take a large portion of the budget allocated for marketing activities.

Basically, the most important problem for

the analyzed companies is the financial capacity and inability to seriously devote themselves and take advantage of the sales promotion.

It can be concluded that these problems can be overcome by effective cooperation and merging between the clusters. It is time to join the forces and to develop the sectors that are not able for independent promotion and implementation. On the other hand, the transfer of these functions to the cluster would give more time to pay more attention to the field that they are most capable of performing, and that is usually the production. This will increase productivity and avoid unnecessary costs in cases where they have to take care about them, but they do not have funds or qualified personnel.

# GENERAL MODEL FOR THE ESTABLISHMENT OF TOBACCO CLUSTER IN THE REPUBLIC OF SRPSKA

One of the most widely accepted model of the cluster is Porter's model of competition, where the competitive advantage of a nation determines its competitive positioning. To be successful, the industry has to offer to the market a higher value than its rivals, or the same value for less effort (value for efforts). According to Porter's approach, clusters represent a group of companies and other elements that make the industry competitive, including natural resources, infrastructure, equipment, service providers, other utility sectors, educational facilities and the capacity of training institutions, whose integrated and coordinated activities contribute to the improvement of business. "Porter's diamond" includes four groups of forces, which in their relations determine the competition: terms of demand, the

companies and their rivals, the inputs and sector support. Most competitive is the cluster that has the best conditions for the implementation of these elements. This is in line with Porter's representation of specialization, contrary to the traditional view of the economy where many planners promoted the strategy of a diversified economy.

With regard to the role of clusters in the "diamond" of competitiveness seen in the context of "value chain" in the tobacco sector, the tobacco cluster of Republic of Srpska can provide a focus that is required to enterprises, governments and institutions to align their efforts with specific competitiveness, value added and its preservation, as well as targeted export performances.

# CONCLUSION

The main goal of the tobacco cluster is to create a modern, developed, competitive industrial structure that will gradually fit into the economy of the global market. Such a development orientation is necessary since the economy of our region is based on the production of primary products and lower levels of processing, and that on these bases it cannot provide long-term growth of exports and overall economic development. It is necessary to reduce import dependence of the region and increase the export capacity of domestic industries with higher quality products and services.

Looking at the national level, through tobacco clusters the state can have a positive impact on building competitive export economy through its direct effect on the acquisition of new skills, create employment, regional development, increase living standards and other goals related to economic and social development.

Such arranged clusters could have promising initiatives for international networking on a global level. The international competitiveness of local enterprises in the global business environment is becoming increasingly dependent on the ability of mutual cooperation at the local level. Therefore, the competitiveness of a tobacco industry in future will be increasingly dependent on the efficiency of its clusters.

The Republic of Srpska Government, through the Ministry of Agriculture of the Republic of Srpska, other ministries and development agencies provide support to clusters of tobacco in the country, through promotion and creation of business environment and adoption of modern legislation that supports and facilitates the work of the cluster.

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