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COMPARATIVE INVESTIGATIONS OF SOME FOREIGN AND DOMESTIC HYBRID VARIETIES OF VIRGINIA TOBACCO IN THE REGION OF PRILEP

Karolina Kocoska, Ilija Risteski

University"St.Kliment Ohridski"-Bitola, Scientific tobacco institute-Prilep, Republic of Macedonia, e-mail: karolina_kocoska@yahoo.com

ABSTRACT

Two-year investigations (2008 - 2009) were carried out in the field of Tobacco Institute-Prilep.

The trial included six varieties of domestic and foreign origin: K-326, Ø (USA), V-513 (Bulgaria), Virginia SKR (Zimbabwe) and V-78/07 CMS F_1, V-82/07 CMS F_1, V-63/04 CMS F_1 (created in Tobaccoo Institute-Prilep, Macedonia).

Investigations were performed using the standard methodologies. According to the obtained results, domestic hybrid CMS varieties have shown better quality characteristics compared to the foreign varieties. From all varieties investigated, we would especially emphasize the domestic male-sterile hybrid V-63/04 CMS F_1.

Key words: virginia, hybrid varieties, tobacco

РЕЗУЛТАТИ ОД КОМПАРАТИВНИ ИСПИТУВАЊА СО НЕКОИ СТРАНСКИ И ДОМАШНИ ВИРЏИНИСКИ ХИБРИДНИ СОРТИ ВО ПРИЛЕПСКИОТ РЕОН

Во двегодишните испитувања (2008-2009 год) беше изведен полски опит на површините од опитното поле во Научниот институт за тутун – Прилеп.

Во испитувањата беа вклучени 6 сорти од домашниот и странскиот сортимент и тоа : К-326 F_8 ø (САД), V-513 (Бугарија), Virginia SKR (Зимбабве), и V-78/07 ЦМС F_1, V -82/07 ЦМС F_1, V-63/04 ЦМС F_1, сите креации на НИТ – Прилеп.

Испитувањата се извршени по стандардни методологии. Според добиените резултати, домашните хибридни ЦМС сорти покажуваат подобри квалитативни својства во споредба со странските, при што посебно ја издвојуваме домашната новосоздадена хибридна машкостерилна сорта V-63/04 ЦМС F_1.

Клучни зборови: вирџинија, хибридни сорти, тутун

INTRODUCTION

China and USA are the world's largest producers of Virginia tobacco. This type originates from sub-tropic regions and is successfully grown in areas at 60^0 north and 40^0 south latitude (S.N. Hawks Jr., W.K. Collins, 1994). In the structure of world tobacco production, Virginia is represented with the highest percentage (63.12%) and its share in the content of blend cigarettes is over 60% (Filiposki K., Stojanoska S., 2000).

Production of Virginia tobacco in Macedonia started in 1970 in the region of Prilep, and afterwards it was spread in other regions with suitable soil and climate conditions.

According to Risteski I. (2000), about 1633 tons of Virginia were produced in the period 1976-1988 and 1475 tons in 1989-1997, but after 2002 this production was reduced to a minimum. Despite this reduction, new hybrid CMS varieties of Virginia tobacco with good quality and competitive with foreign varieties have been created in Tobacco Institute - Prilep.

The aim of this paper is to present the results of two-year investigations on the newly created Virginia varieties, for which we hope to find their place in the production of blend cigarettes.

MATERIAL AND METHODS

The trial was set up in the field of Tobacco Institute-Prilep with 4 replications in randomized blocks at 90 x 50 cm spacing. Six foreign and domestic varieties (K-326, Ø - USA, V-513 - Bulgaria, Virginia SKR (Zimbabwe) and V-78/07 CMS F_1, V-82/07 CMS F_1, V-63/04 CMS F_1 - created in Tobaccoo Institute-Prilep) were included in the investigation. The soil was pretreated and fertilized with 300 kg/ha NPK 8:22:20. Two hoeings and one nutrition with KAN (3-4 g/stalk) were made and irrigations were applied depending on climate conditions and plant requirements. During the vegetation period, several treatments were applied against diseases and pests.

After harvesting and stringing, tobacco was cured in barns specially designed for Virginia tobacco and the length of vegetation period was recorded (beginning of flowering, 50% of flowering and the end of flowering) for each variety in the trial. Weather conditions were registered in Meteorological Station of Tobacco Institute-Prilep and agro-ecological properties of soil were investigated in its Department of Agrotechnics, according to internationally recognized methods. Qualitative estimation of dry tobacco was made in accordance with the current Rules on tobacco quality. Yield per stalk and hectare was estimated by the method of Rimker, while evaluation of average price and gross income were based on valid price for Virginia tobacco.

RESULTS AND DISCUSSION

- Soil and climate conditions

Soil and climate conditions significantly affect tobacco yield and quality. For a more rapid growth, Virginia tobacco requires sandy or loam-sandy soils, with approximately 25-38 mm of rainfall every 7-10 days (S.N. Hawks Jr., W.K. Collins, 1994). Our trial was made in loamy soil with poor content of humus (1.27%), total N (0.059%) and low pH (6.48 and 5.69). The supplies of P_2O_5 and K_20 were extremely high (74.3 mg/100g and 28.6 mg/100g of soil, respectively). Fertilization with NPK and additional nutrition with KAN were applied prior to transplanting.

Table 1 Plot 26 - Seedbeds of Tobacco Institute - Prilep

Depth cm	Humus %	N %	pH		Available in mg/100g of soil		Soil classification after Wigner
			H_2O	KCl	P_2O_5	K_2O	
0 - 30	1,27	0,059	6,48	5,69	74,3	28,6	Light loam

Temperature, precipitations and relative air humidity are the factors which have essential impact on tobacco growth and its bio-morphological, technological and chemical characteristics.

Tobacco plant originates from regions with tropic climate and therefore requires higher temperatures compared to other crops. The optimum temperature range throughout the whole period of tobacco growth is 20 - 30^{0}C (Hawks & Collins 1994, Rubin B.A. 1971).

Data on climate conditions during vegetation period (May-September) in the two-year investigations are presented in Table 2.

Table 2. Meteorological data for the period May – September 2008/2009, Tobacco institute – Prilep

Meteorological data	Year	Months					X /Σ
		V	VI	VII	VIII	IX	
Mean monthly air temperature°C	2008	16,7	19,9	22,3	23,6	15,8	19,7
	2009	15,8	18,5	21,9	21,4	17,1	18,9
Mean monthly relative humidity of the air %	2008	60	53	49	50	71	57
	2009	58	57	42	50	54	52
Days with precipitation	2008	8	5	4	2	10	29
	2009	10	10	3	7	5	35
Total precipitations mm	2008	41,3	10,0	11,0	11,0	110,0	183,3
	2009	55,0	75,0	8,0	43,0	15	196,0

The lowest mean monthly air temperatures in both years were recorded in May (16.7 ^{0}C in 2008 and 15.8^{0}C in 2009), but they had no negative effects on tobacco growth and development. Optimum temperatures were recorded in July (22.3^{0}C in 2008 and 21.9^{0}C in 2009) and August (23.6^{0}C in 2008 and 21.4^{0}C in 2009).

The average air temperature in the period May-September reached 19.7^{0}C in 2008 and 18.9^{0}C in 2009 and is somewhat lower from the optimum.

Relative air humidity is closely related to precipitations, number and quantity of additional irrigations, air temperature, etc. The lowest values of relative humidity were recorded in June (49% in 2008 and 42% in 2009), and the highest in May and September (71% in September 2008 and 58% in May 2009). The average relative humidity for the whole vegetation period (May-Sept.) was 57% in 2008 and 52% in 2009.

Precipitation amounts in both years of investigation were the lowest in June and July (11.0 mm in 2008 and 8.0 mm in 2009) and the highest value was achieved in September 2008 (11.0 mm). Due to irregular precipitations, especially in 2008, additional waterings were applied, so that the total amount reached 183.3 mm in 2008 and 196.0 mm in 2009.

- Length of the vegetation period

The shortest period to the beginning of flowering stage (58 days) has the standard variety K-326. This period was the longest in varieties V-513 and V - 63/04 CMS F1 (68 days, i.e. 10 days later than the standard variety).

Table 3 - The length of the vegetation period

Variety	Crop	Days from transplanting to the beginning of flowering	Average 2008/2009	Difference from the average		Days to 50% of flowering	Average2008/2009	Difference from the average		Days to 100% of flowering	Average 2008/2009	Difference from the average	
				Absolute	Relative			Absolute	Relative			Absolute	Relative
K - 326	2008	50	58	/	100.00	67	66	/	100.00	72	70	/	100.00
Ø	2009	56				65				68			
V - 513	2008	70	68	+10	117.24	73	72	+6	109.09	78	77	+7	110.00
	2009	66				74				76			
Virginia	2008	66	64	+6	110.30	71	69	+3	104.54	76	74	+4	105.71
SKR	2009	62				67				72			
V-78/07	2008	67	66	+8	113.79	73	72	+6	109.09	79	77	+4	110.00
CMS F_1	2009	65				71				75			
V – 82/07	2008	68	66	+8	113.79	74	72	+6	109.09	78	78	+8	111.42
CMS F_1	2009	64				70				75			
V - 63/04	2008	70	68	+10	117.24	74	73	+7	110.60	80	79	+9	112.85
CMS F_1	2009	66				72				78			

Also the period to 50% of flowering was shortest in the standard variety K-326 (67 days) and longest in V-63/04 CMS F1 (73 days). The same regularity can be observed for the period 100% of flowering, which ranges from 70 days in the standard variety K-326 to 79 days in V-63/04 CMS F_1. The 10-days difference between the first-blossomed and last-blossomed varieties is not significant enough to seriously affect leaf maturation and collection of seed material. S.N. Hawks Jr. and W.K. Collins 1994) reported that For best maturation of plants, Virginia tobacco requires 60 days for flowering and 120 days without frost during its field growth. Our investigations revealed that the newly created varieties have adequate length of vegetation period which enables their complete development and leaf maturation.

- Corrected yield per stalk, g/stalk

The highest average yield of 135.75 g/stalk was achieved with the new domestic variety V-63/04 CMS F_1, with 11.18% higher relative difference compared to the check variety. In both investigating years this variety showed 5% statistically significant difference in relation to the check (Table 4).

Table 4. Yield per stalk, g/stalk

Variety	Crop	Average	Average 2008/2009	Difference from the average		Range
				Absolute	Relative	
K - 326 Ø	2008	127,7	122,10	/	100,00	4
	2009	115,5				
V - 513	2008	120,5	114,75	-7,35	93.98	5
	2009	109,0				
Virginia SKR	2008	99,0	94,40	-27,70	77,31	6
	2009	89,8				
V- 78/07 CMS F_1	2008	132,2	125,60	-3,5	102.87	2
	2009	119.0				
V – 82/07 CMS F_1	2008	130,7	123,85	-1,75	101.43	3
	2009	117,0				
V – 63/04 F_1	2008	142,5+	135,75	+13,65	111.18	1
	2009	129,0+				

2008	2009
LSD 5% = 12,42 g / stalk	5% = 10.60 g / stalk
LSD 1% = 17,21 g / stalk	1% = 14,67 g / stalk

The lowest average yield was observed in foreign varieties Virginia SKR (94.40 g/stalk) and V- 513 (114.75 g/stalk), which showed no statistically significant difference.

The other newly created varieties also showed higher average yield compared to the foreign varieties (V-78/07 CMS F1 - 125.60 g/ stalk ; V-82/07 CMS F1 - 123.85 g/stalk) .

- Corrected yield per hectare, kg/ha

The highest average yield (2998 kg/ ha) was achieved with the new domestic hybrid variety V-63/04 CMS F_1, with relative difference 11.70% higher than the check variety and 5% statistically significant difference in both years of investigation (Table 5).

Table 5 Yield per hectare, kg/ha

Variety	Crop	Average	Average 2008/2009	Difference from the average		Range
				Absolute	Relative	
K - 326 Ø	2008	2825	2684	/	100,00	4
	2009	2543				
V - 513	2008	2669	2536	-148	94,49	5
	2009	2403				
Virginia SKR	2008	2192	2083	-601	77,61	6
	2009	1973				
V- 78/07 CMS F_1	2008	2926	2780	+96	104,17	3
	2009	2634				
V – 82/07 CMS F_1	2008	2896	2896	+212	107,90	2
	2009	2896				
V – 63/04 CMS F_1	2008	3156+	2998	+314	111,70	1
	2009	2840+				

2008	2009
LSD 5% = 223,79 kg/ha	5% = 250,24 kg/ha
1% = 309,95 kg/ha	1% = 346,58 kg/ha

The lowest yield was observed in foreign varieties Virginia SKR (2083 kg/ha) and V- 513 (2536 kg/ha). The other newly created varieties showed higher average yield compared to the check (V-82/07 CMS F1 - 2896 kg/ha; V-78/07 CMS F1 - 2780 kg/ha).

-Gross monetary income (economic effect), den/ha

The highest economic effect (Table 6) was achieved with the new domestic hybrid variety V-63/04 CMS F_1 (169 712 den/ha), with relative difference 6.93% higher than the check variety. The lowest economic effect was observed in the foreign variety Virginia SKR, with relative difference 3073% lower than the check. Statistically important differences for this feature compared to the check have not been observed in both years of investigation.

Table 6 Gross monetary income (economic effect), den/ha

Variety	Crop	Average	Average 2008/2009	Difference from the average		Range
				Absolute	Relative	
K - 326 Ø	2008 2009	168 122 149 290	158 706	/	100,00	3
V - 513	2008 2009	138 742 124 258	131 500	- 27 206	82,86	5
Virginia SKR	2008 2009	116 549 103 315	109 932	-48 774	69,27	6
V-78/07 F_1	2008 2009	172 000 153 581	162 791	+4 085	102,57	2
V – 82/07 CMS F_1	2008 2009	169 922 153 925	161 924	+3 218	102,03	4
V - 63/04 CMS F_1	2008 2009	179 375 160 049	169 712	+11 006	106,93	1

	2008	2009
LSD	5% =25193,96 den/ha	5% = 19980.04 den/ha
	1% = 34893,04 den/ha	1% = 27671.88 den/ha

CONCLUSION

Based on the two-years investigation of qualitative characteristics of the foreign and some domestic newly created hybrid varieties of the type Virginia, the following can be concluded:

- Soil and climate conditions of R. Macedonia are not very favorable for production of the type Virginia, but with additional fertilization and irrigation it will be possible to obtain a good quality of this tobacco.

- The shortest period to the beginning of flowering (58 days) and 100% flowering stage (70 days) was observed in the variety K-326 . The longest period to the beginning of flowering was recorded in V-513 and V-63/04 CMS F_1 (68 days). The longest period to 100% of flowering was achieved in V-63/04 CMS F_1 (78 days) - 8 days later than the standard, but this difference has no negative impact on quality characteristics of this newly created variety.

- The highest average yield per stalk was observed in the new domestic variety V-63/04 CMS F_1 (135.75 g/stalk) and the lowest in foreign variety Virginia SKR (94.40 g/stalk).

- The average corrected yield per hectare was the highest in V-63/04 CMS F_1 (2998 kg/ha) and the lowest in foreign varieties Virginia SKR (2083 kg/ha) and V-513 (2536 kg/ha).

- The highest economic effect per hectare was achieved in domestic variety V-63/04 CMS F_1 (169 712 denars/ha), and the lowest in foreign variety Virginia SKR (109 932 den/ha).

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RATIO BETWEEN GREEN AND DRY MASS IN SOME TOBACCO VARIETIES OF THE TYPE PRILEP

Milan Mitreski

University"St.Kliment Ohridski"-Bitola, Scientific Tobacco Institute-Prilep
Kicevski pat bb, 7500 Prilep; e-mail: kirilfiliposki@yahoo.com

ABSTRACT

Investigations on the green/dry mass ratio were carried out in 2009 and 2010 in the trial field of Tobacco Institute-Prilep, with six varieties of the type Prilep: P-23, P 12-2/1, NS-72, 66-9/7, P-79-94 и Prilep Basma 82. The ratios for the two years ranged from 6.11:1 to 6.95:1, or 6,64:1 in average. Namely, the variety Prilep 66-9/7 had an average value of 6,11:1 and the Prilep Basma 82 variety achieved 6,58:1. The other varieties showed much higher average values compared to the check, in the following range: Prilep NS-72 - 6,77:1, Prilep P-79-94 - 6,86:1 and P 12-2/1 showed the highest average ratio - 6,95:1. The ratio between green and dry mass can be also expressed as plant productivity. In our investigations, the highest productivity among varieties was observed in P 66-9/7 and the lowest in Prilep P 12-2/1.

Key words: tobacco, Prilep, P-23, P-12-2/1, NS-72,P 66-9/7, P-79-94, Prilep Basma 82

СООДНОС НА КОЛИЧЕСТВО ЗЕЛЕН И СУВ ТУТУН КАЈ НЕКОИ СОРТИ ОД ТИПОТ ПРИЛЕП

Истражувањата на соодносот зелен/сув тутун се вршени во 2009 и 2010 година на опитното поле од Институтот за тутун Прилеп, кај шест сорти на тутун од типот прилеп и тоа: прилеп П-23, П 12-2/1, НС-72, 66-9/7, П-79-94 и прилеп басма 82. Просечните двегодишни вредности се движат во мал сооднос од 6,11:1 до 6,95:1, или просечно 6,64:1. Имено сортата прилеп П 66-9/7 има просечна вредност од 6,11:1, сортата прилеп басма 82 од 6,58:1. Останатите сорти на тутун покажале малку поголеми просечни вредности од контролата и тоа прилеп НС-72 од 6,77:1, потоа сортата прилеп П-79-94 со 6,86:1 и со најголем просечен сооднос се карактеризира сортата П 12-2/1 со вредност од 6,95:1. Соодносот на зелен и сув тутун може да се презентира и како продуктивност на растенијата. При што со најголема продуктивност од испитуваните сорти на тутун се одликува сортата прилеп П 66-9/7, а со најмала сортата прилеп П 12-2/1.

Клучни зборови: тутун**,** прилеп**,** П-23, П 12-2/1, НС-72, П 66-9/7, П-79-94, прилеп басма 82

INTRODUCTION

Water is specific chemical compound which is found in highest quantities in the composition of living plants. The quantity of water in young tobacco leaves and parts amounts from 80% to 94%. Uzunoski (1985) reported that in the leaves at technical maturity, the water amount reaches up to 75 - 90%, depending on the variety, conditions of growing and insertions. The quantity of water in cured leaves was 6 – 10% of the total weight of the leaf.

The amount of water declines from the lower towards the upper insertions. All biochemical processes taking place in tobacco leaves depend on water. It is a media in which all physiological processes of the living cell take place, i.e. formation of organic matter in the process of photosynthesis depends on the water content. The cell cytoplasm contains large amounts of water, which is not only a solvent of organic and inorganic substances but a highly important structural component.

According to Baylov D. and Popov M. (1965), tobacco leaves contain about 80% of water and 20% of dry matter. The highest percentage of water is found in the leaves from lower insertions (85-86%) , and the lowest in the leaves of upper insertions (62-63%).

Boceski D. (2003) reported that the ratio between green and dry tobacco leaves ranges from 6.1 to 4.1, so that dry tobacco accounts for 17 – 25% of the initial green mass quantity.

Karajankov (1995), in his three-year investigations, found that the average content of water in the leaves of Macedonian oriental tobaccos P 10-3/2, YK 7-4/2 and Djebel N-1 was 77,43%. In relation to the water content by types, the author reported the following data: in variety Prilep P 10-3/2 (type Prilep) it achieved 74,14%, in variety YK 7-4/2 (type Yaka) – 71.34% and in variety N-1 (type Djebel) - 80.22%.

Filiposki (1986) reported that tobacco fertilization affects the total content of water in tobacco leaves, increasing it from 5.54% to 5.83%. Soil humidity level also affects the water content in tobacco leaves.

According to Dimitrov C. (1964), by increasing the nitrogen rates, the content of water in green tobacco leaves also increases. In the process of tobacco curing, the higher water content results in a lower quality of tobacco, and vice-versa: the lower the water content is, the better quality of tobacco leaves is obtained. It should be emphasized that water retention ability of plant tissues is affected by proteins, which content is increasing with the increased nitrogen rates in the soil.

Atanasov D. (1972) reported that green/dry mass ratio in oriental tobacco variety Ustina ranged 5.5:1 - 6.7:1 in the lower insertions, 4.8:1 - 5.5:1 in the middle insertions and од 3.9:1 – 5.0:1 in the upper insertions. The author also recorded that the ratio between green and dry mass in Burley tobacco ranges from 6.7:1 to 8.3:1, and in Virginia tobacco from 5.5:1 to 6.7:1.

Patce L. and Georgievski K. (1987) referred to the data obtained by Baylov D. and Popov M. (1965), according to which the ratio between green and dry mass ranges from 3.68:1 to 9.77:1 (6.26:1 in average), i.e. 8,64:1, 5,56:1 and 4,29:1 in the lower, middle and upper insertions, respectively.

MATERIAL AND METHODS

The trial was set up in four replications, on a total area of 487 m^2, with 6 rows per plot and 42 stalks in a row. During the growing season, tobacco was irrigated with water. Several primings, at technical maturity of the leaves were made and curing was performed in traditional way, under transparent polyethylene. Green and dry tobacco mass was weighed on technical balance with 2 decimals precision. The green to dry mass ratio was assessed mathematically.

RESULTS AND DISCUSSION

Results of our investigations show that the average green/dry mass ratio in 2009 was 6.98:1. Compared to the check variety P-23, it achieved lower values in varieties Prilep P 66-9/7 (6.23:1), Prilep Basma 82 (7,01:1) and Prilep P-12-2/1 (7,05:1). Expressed in percentages, the decrease ranges from 13.35% in P 66-9/7 to 1,95% in Prilep P-12-2/1. Higher ratio was obtained in varieties Prilep NS-72 (7,27:1) Prilep P-79-94, which is an increase of 1.11% to 3.34% compared to the check. Out of these data, it can be stated that in 2009 the variety Prilep P-66-9/7 achieved the highest productivity and the lowest productivity was recorded in Prilep P-79-94.

Table 1 Green to dry tobacco ratio in 2009

No.	Variety	Replications				Average	Index
		I	II	III	IV		
1	Prilep P-23 (Ø)	6,92 :1	7,64 :1	7,60 :1	6,61 :1	7,19 :1	100,00
2	Prilep P 12-2/1	7,09 :1	7,70 :1	7,09 :1	6,33 :1	7,05 :1	98,05
3	Prilep NS-72	8,01 :1	7,90 :1	6,87 :1	6,31 :1	7,27 :1	101,11
4	Prilep P 66-9/7	6,49 :1	6,82 :1	6,42 :1	5,21 :1	6,23 :1	86,65
5	Prilep P -79-94	7,80 :1	7,51 :1	7,70 :1	6,72 :1	7,43 :1	103,34
6	Prilep Basma 82	7,48 :1	7,27 :1	6,82 :1	6,46 :1	7,01 :1	97,50
	Average	7,27 :1	7,41 :1	7,05 :1	6,19 :1	6,98 :1	97,08

LSD

0,05 = 0,48
0,01 = 0,66
0,001 = 0,92

Table 2 Green to dry tobacco ratio in 2010

No.	Variety	Replications				Average	Index
		I	II	III	IV		
1	Prilep P-23 (Ø)	7,12 :1	6,18 :1	5,94 :1	6,06 :1	6,28 :1	100,00
2	Prilep P 12-2/1	7,17 :1	6,33 :1	7,01 :1	6,88 :1	6,85 :1	109,08
3	Prilep NS-72	7,26 :1	5,94 :1	6,42 :1	5,63 :1	6,28 :1	100,00
4	Prilep P 66-9/7	6,47 :1	6,08 :1	6,14 :1	5,33 :1	5,99 :1	95,38
5	Prilep P -79-94	6,18 :1	6,43 :1	6,59 :1	6,02 :1	6,29 :1	100,16
6	Prilep Basma 82	6,29 :1	6,31 :1	6,19 :1	5,89 :1	6,16 :1	98,09
	Average	6,75 :1	6,21 :1	6,38 :1	5,97 :1	6,31 :1	100,48

LSD

0,05 = n.s.

Table 3 The average green to dry mass ratio

No.	Variety	Year		Average	Index
		2009	2010		
1	Prilep P-23 (Ø)	7,19 :1	6,28 :1	6,73 :1	100,00
2	Prilep P 12-2/1	7,05 :1	6,85 :1	6,95 :1	103,27
3	Prilep NS-72	7,27 :1	6,28 :1	6,77 :1	100,59
4	Prilep P 66-9/7	6,23 :1	5,99 :1	6,11 :1	90,79
5	Prilep P -79-94	7,43 :1	6,29 :1	6,86 :1	101,93
6	Prilep Basma 82	7,01 :1	6,16 :1	6,58 :1	97,77
	Average	6,98 :1	6,31 :1	6,64 :1	98,66

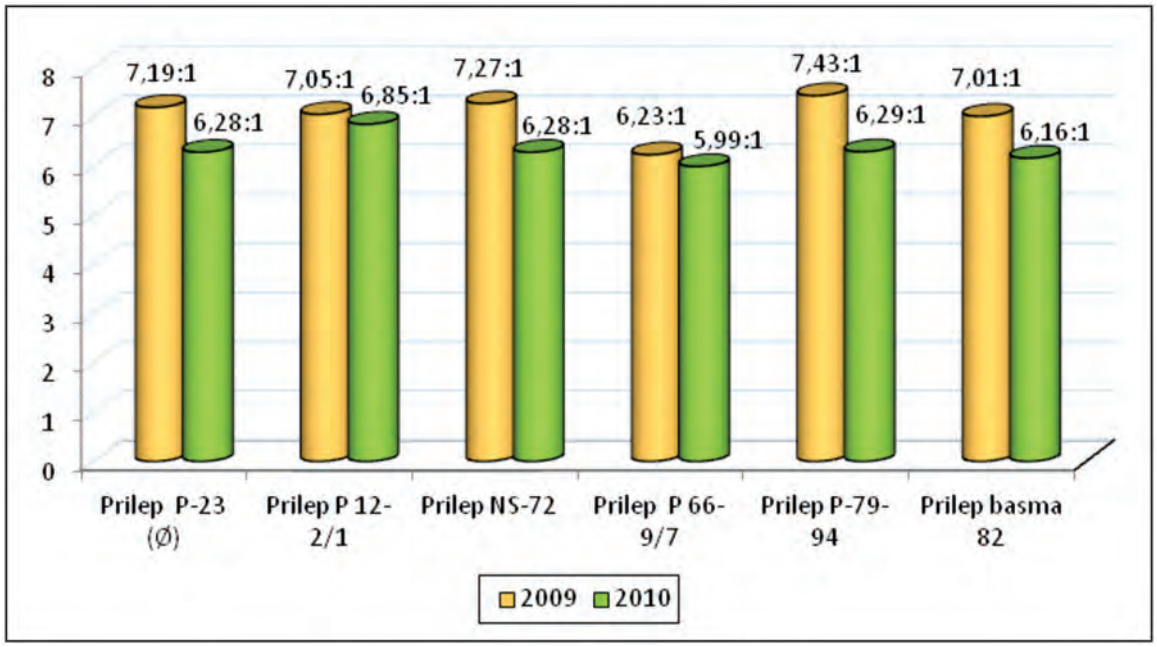

Figure 1 Graphical presentation of the green to dry mass ratio

In 2010 again, the lowest green to dry tobacco ratio was calculated in the variety Prilep P 66-9/7 (5.99:1), followed by Prilep Basma 82 (6.16:1). The same value as that of the check variety was achieved by Prilep NS-72, while in Prilep P-79-94 the achieved value was insignificantly higher than the check (6.29:1), and the highest ratio for this year was calculated in Prilep P 12-2/1 (6.85:1).

The two-year values of the green to dry tobacco ratio range in close limits from 6.11:1 to 6.95:1 and the average ratio for all tobacco varieties is 6.64:1. Namely, Prilep P 66-9/7 had an average ratio of 6.11:1, Prilep Basma 82 – 6.58:1, and the ratios of the other varieties were somewhat higher than the check, increasing from 6.77:1 in Prielp NS-72 to 6.86:1 in Prilep P-79-94 and up to 6.95:1 in P 12-2/1.

Expressed in relative figures, the average values for the green/dry mass ratio in investigated tobacco varieties show little variation. The largest decrease of the ratio in relation to the check variety was calculated in Prilep P 66-9/7 variety – 9.21%, followed by Prilep Basma 82 with 2.23%. The values for this ratio were somewhat higher in the varieties Prilep NS-72 (0.59%) and P-79-94 (1.93%), and the highest value was achieved in Prilep P 12-2/1 (3.27%).

Our data are in accordance with references data at the beginning of this paper. In literature data, the ratio between green and dry mass of tobacco is presented as plant productivity.

CONCLUSIONS

From the data obtained in our investigations, it can be stated that the green/dry mass ratio is a variety characteristic of tobacco.

The green/dry mass ratio ranges from 6.11:1 to 6.95:1 in tobacco varieties Prilep P 66-9/7 and Prilep P 12-2/1, respectively, which is an average of 6.64:1.

It can be also stated that the highest productivity among the investigated varieties was achieved in Prilep P 66-9/7 and the lowest in Prilep P 12-2/1.

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THE CONTENTS OF Pb, Cd, Ni and Cr IN SOILS AND TOBACCO IN VARIOUS REGIONS OF R. MACEDONIA

Valentina Pelivanoska, Kiril Filiposki, Biljana Jordanoska

University "St.Kliment Ohridski"-Bitola, Scientific Tobacco Institute-Prilep, Republic of Macedonia e-mail: vpelivanoska@yahoo.com; www.tip.edu.mk

ABSTRACT

Tobacco is one of the most important crops grown in the Republic of Macedonia. This crop can easily accumulate certain metals in its leaves. High concentration of these metals affects the quality of tobacco products and can have a harmful effect on consumers' health. The soil study was conducted on family farms in different parts of Macedonia. Analyses were made on pH, humus, total content of Pb, Cd, Ni and Cr in soils, as well as concentrations of these elements in the three primings of tobacco. Correlation coefficients between the total metal concentration in investigated parameters in tobacco and soils indicated the existence of strong relationship between the lower, middle and upper primings for each metal.

Statistically significant dependence was found between Pb contents of the plant material (all three primings) and humus, clay and Cd, Cu, Zn and Ni content in the soil. All other obtained results showed that the Pb, Cd, Ni and Cr contents in soil had no influence upon their accumulation in tobacco leaves. Metal concentrations in tobacco plants and respective soils were below the permissible limits.

Key Words: soil, heavy metals, tobacco

СОДРЖИНА НА Pb, Cd, Ni И Cr ВО ПОЧВАТА И ТУТУНОТ ВО НЕКОИ РЕГИОНИ ВО Р. МАКЕДОНИЈА

Тутунот е еден од најважните култури што се одгледува во Република Македонија. Познато е дека тутунот е култура која има способност да акумулира поголема количина на одредени метали во своите вегетативни органи. Високата концентрација на овие метали може да има штетно влијание врз квалитетот на тутунската суровина, а со тоа и врз здравствената состојба на консуматорите. Тргнувајки од суштината на изнесеното ги поставивме целите на оваа студија. Истражувањата беа спроведени на почви од семејни земјоделски стопанства од повеќе позначајни локации низ републиката. Беа спроведени анализи на pH, хумус, вкупната содржина на Pb, Cd, Ni и Cr во почвата, како и концентрацијата на овие елементи во три бербени појаси на тутунскиот страк. Корелационите коефициенти меѓу вкупната концентрација на испитуваните метали во тутунот и почвите покажуваат дека постои силна врска меѓу концентрациите на бербениот појас (прв, втор и трет) со секој испитуван метал. Статистички значајна зависност беше пронајдена помеѓу содржината на Pb во растителениот материјал (во трите берби) и хумус, глина, како и содржина на кадмиум, цинк, никел и бакар во почвата. Сите други резултати покажуваат дека содржината на Pb, Cd, Ni и Cr во почвата немаат влијание врз нивната акумулација во тутунските листови.

Добиените концентрации на испитуваните метали се во согласнст со стандардите и се под дозволените граници.

Клучни зборови: почва, Pb, Cd, Ni, Cr, тутун

INTRODUCTION

During the past decades, there has been a growing concern for environmental pollution. This is due to the high concentrations of heavy metals in the environment, since they can be easily absorbed from the soil. The specific climatic conditions and soil properties contribute to high quality production of Oriental tobacco in Macedonia. About 90% of the total tobacco production is exported and this culture is a strategic product viewed from economic, trading, fiscal, social and demographic aspects. Heavy metals from soil can be easily absorbed by the plants and humans have the greatest influence upon the levels of these elements. The heavy metals content in plants depends on several factors: bio-ecological characteristics of plant species, concentration, chemical form of these elements in the soil and eco-pedological conditions which determine their mobility and availability (Knezević, 2006).

Because of the wide use of tobacco in cigarette industry, the monitoring of heavy metals accumulation in tobacco is of crucial importance for human health (Adamu et al., 1989; Bell et al., 1992).

The group of potentially toxic elements investigated in this paper includes Pb, Cd, Ni and Cr. Tobacco is well-known accumulator of heavy metals and is characterized as a high-leaf, high-root Cd accumulator (Matsi, 2002). The aim of this work was to analyze the heavy metal contents (Cd, Cr, Ni and Pb) in agricultural soils and in Oriental tobaccos collected from different locations in Macedonia. These results will provide an insight into possible heavy metal contamination that may be occurring in the study area.

MATERIALS AND METHODS

50 composite soil samples were collected from pedological profiles at fixed depths of 0 - 10 cm, 10 – 20 and 20-30 cm. Two samples from each locality were taken during November, 2010. In addition to soil samples, two dry tobacco samples were taken each from the lower, middle and upper primings. The lower primings included sand and bottom leaves, the middle primings - the first, second and third middle leaves and the upper primings - lower top and top leaves. The samples were collected from family farms in the well-known tobacco growing regions in the central part of Macedonia (Prilep, Krivogashtani, Mogila, Novaci, Bitola, Demir Hisar, Krushevo, Dolneni, Veles, Cashka, Studenicani), as well as some regions in Eastern Macedonia (Strumica, Vasilevo, Bosilevo, Novo Selo, Radovish and Konce). Soil samples were provided in accordance with ISO 11464:2006. First, they were air-dried and after that crushed and sieved through a 2-mm sieve. Determination was made of their physical properties, such as: clay content (Korunović & S.V. Stojanović, 1989), pH (10390:2005), Total nitrogen (modified Kjeldahl method-ISO 11361:1995), humus (Standard method developed by I.V. Tjurin, modified by Simakov), potassium and phosphorus (Al-method, validated at the Scientific Tobacco Institute - Prilep, Macedonia, 2009). The total concentration of metals was determined using the Aqua Regia (HCl-HNO_3, 3:1) extraction method (ISO 11047:1998) after digestion at 180ºC for 2 h. All reagents were of analytical grade (Merck, Germany). Appropriate blanks were included in all extractions. The data were statistically analyzed using correlation analysis (Pearson correlation, two-tailed). Results from two replicates were averaged prior to statistical analyses. Statistical analyses were performed using SPSS 9.0 software. Correlation analysis was used to establish relationships between physical and chemical characteristics of the soil samples and between these characteristics and the heavy metal content of Oriental tobacco leaf samples from three positions of the plant.

RESULTS AND DISCUSSION

Descriptive statistics for heavy metal content in soils and tobacco leaves from the study area are shown in Table 1. In Oriental tobacco leaves, the mean concentrations of Cd and Pb (as presented in Table 1) had higher values in the first primings and the lowest values in the third primings. Ni had higher concentrations in the second primings, whereas Cr in the third primings. The leaves of the lower primings seemed to accumulate high amounts of metals, but in all cases the concentrations were lower than those reported by other investigators (Murty et al., 1986; Clarke and Brennan, 1989; Bell et al., 1992; Gondola and Kadar, 1994; Ruso et al., 2001; McNeill et al., 2006).

Table 1. Content of Cd, Pb, Cr and Ni in tobacco and soil (n=50)

Elements	Statistical index	Lower primings	Middle primings	Upper primings	Depth (cm)		
					0-10	10-20	20-30
Cd mg/kg	Mean	0.6	0.5	0.4	0.3	0.3	0.3
	Minimum	0.20	0.10	0.05	0.10	0.07	0.13
	Maximum	2.1	1.6	1.2	0.5	0.6	0.6
	CV, %	67.6	66.0	65.5	29.8	30.2	30.5
Pb mg/kg	Mean	1.3	1.3	1.3	8.65	9.34	8.64
	Minimum	0.7	0.7	0.2	0.00	1.00	1.5
	Maximum	2.2	2.3	2.4	31.0	41.00	30.5
	CV, %	31.3	30.8	37.1	85.6	87.5	81.4
Cr mg/kg	Mean	2.9	2.9	3.3	57.6	57.1	55.9
	Minimum	1.0	1.1	1.3	3.2	12.8	5.5
	Maximum	4.9	5.0	8.2	222.7	240.9	125.5
	CV, %	35.6	35.9	42.3	58.4	59.1	46.2
Ni mg/kg	Mean	4.9	5.2	5.1	42.7	47.4	43.9
	Minimum	2.3	2.0	1.8	15.6	15.3	14.1
	Maximum	9.0	11.0	9.7	104.0	126.4	91.5
	CV, %	37.4	36.9	37.6	43.7	50.2	40.7

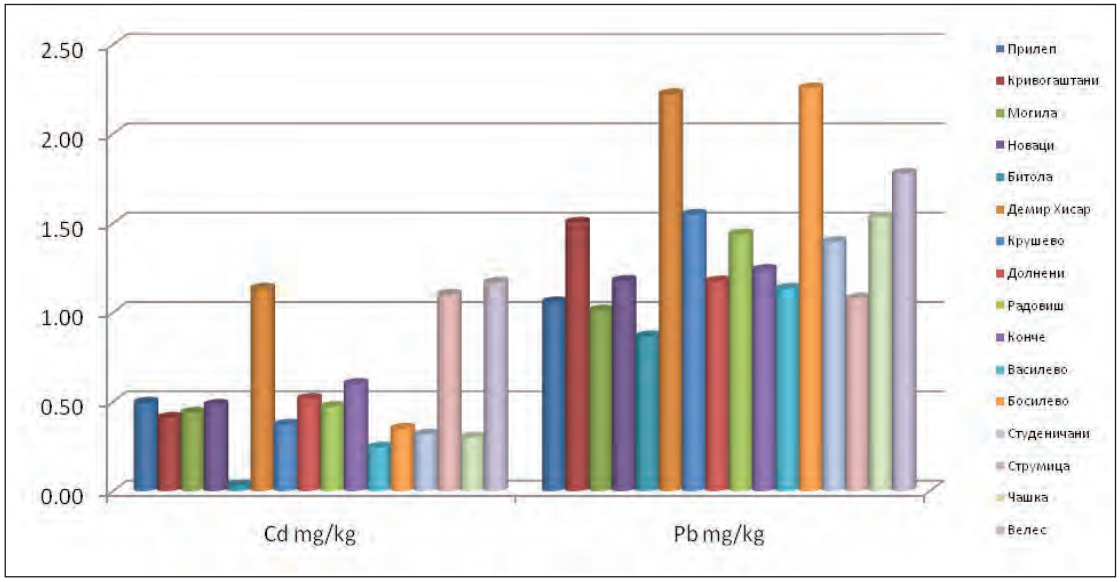

Figure 1. Cd and Pb average concentrations in tobacco samples

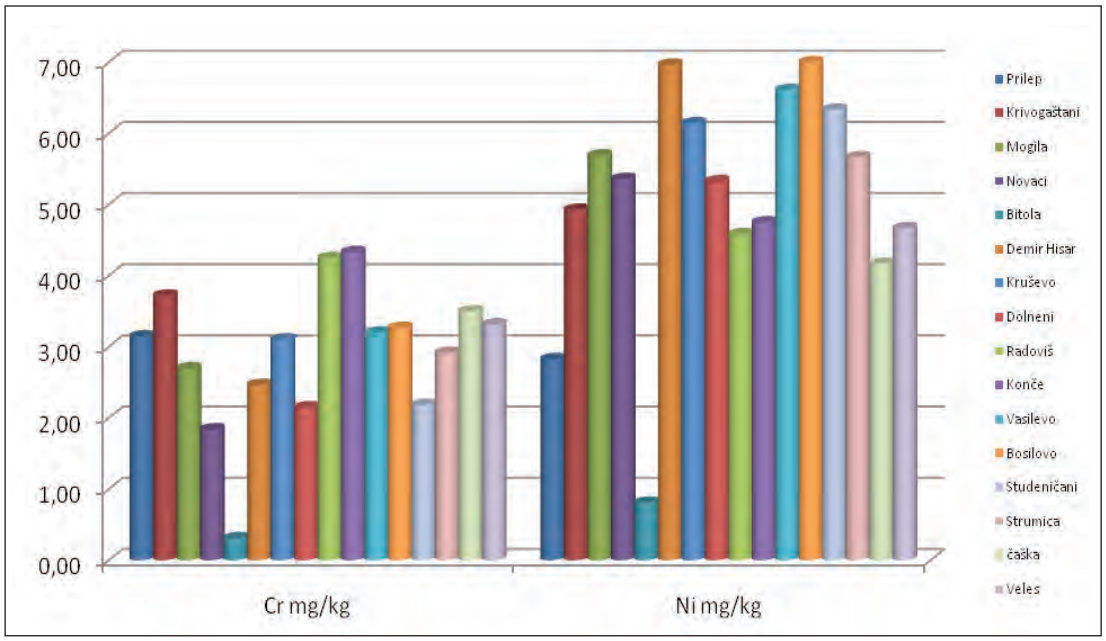

Figure 2. Cr and Ni average concentrations in tobacco samples

Figure 1 presents concentration of Cd and Pb in the tobacco samples from different sampling spots. Highest average Cd concentrations were detected in Demir Hisar, Strumica and Veles, with 1.1 mg/kg, 1.1 mg/kg and 1.2 mg/kg respectively. Pb concentrations varied from 0.2 to 2.4 mg/kg. Highest average values for Pb were recorded in Demir Hisar, while lowest in Bitola with an average of 0.2 mg/kg. Cr и Ni concentrations are given in Figure 2. The lowest Cr concentrations were measured in Radoviš and Konce, and the lowest Ni concentrations were recorded in the areas of Demir Hisar and Bosilevo.

Mean soil concentrations in the soil for Cd, Pb. Ni and Cr were: 0.3 mg/kg; 1.3 mg/kg; 43 mg/kg and 56 mg/kg respectively (Table 1). The average metal concentrations recorded in soil samples are as follows: Cd – 0.3 mg/kg; Pb - 8.9 mg/kg, Ni - 43 mg/kg and Cr - 56 mg/kg (Table 1). Figure 3 represents the average Cd and Pb concentrations in soils. The highest average concentrations were measured in Prilep, with an average of 16.2 mg/kg, while the lowest concentrations were measured in Bitola. Figure 4 represents Cr and Ni in soils in all sampling locations. Highest concentration of 122 mg/kg for Cr was measured in Novaci, Ni average concentrations varied from 15 to 48 mg/kg. The total Pb had the highest coefficient of variance. The mean values of all metals were similar in the three soil depths.

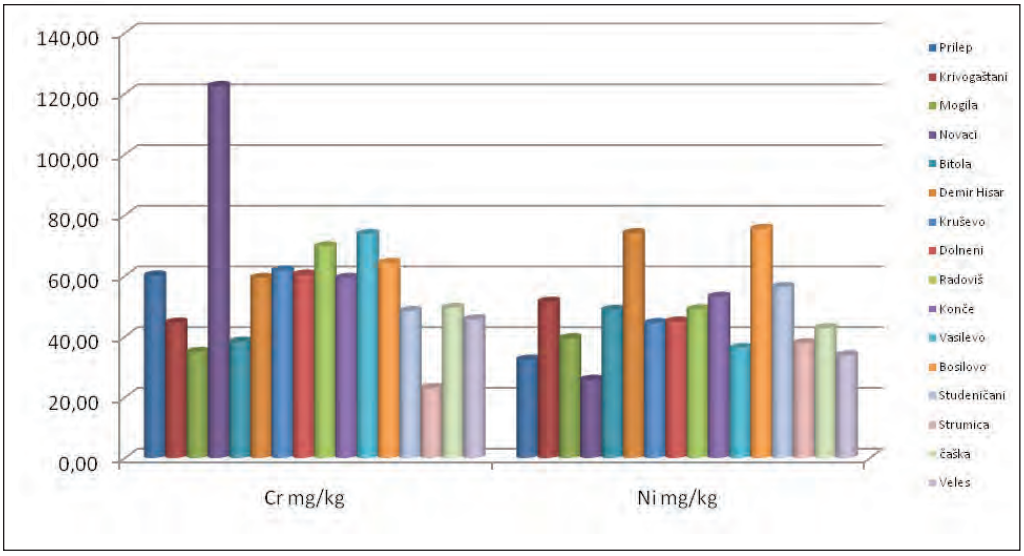

Figure 3. Cr and Ni average concentrations in soils in some tobacco-growing regions in R. Macedonia

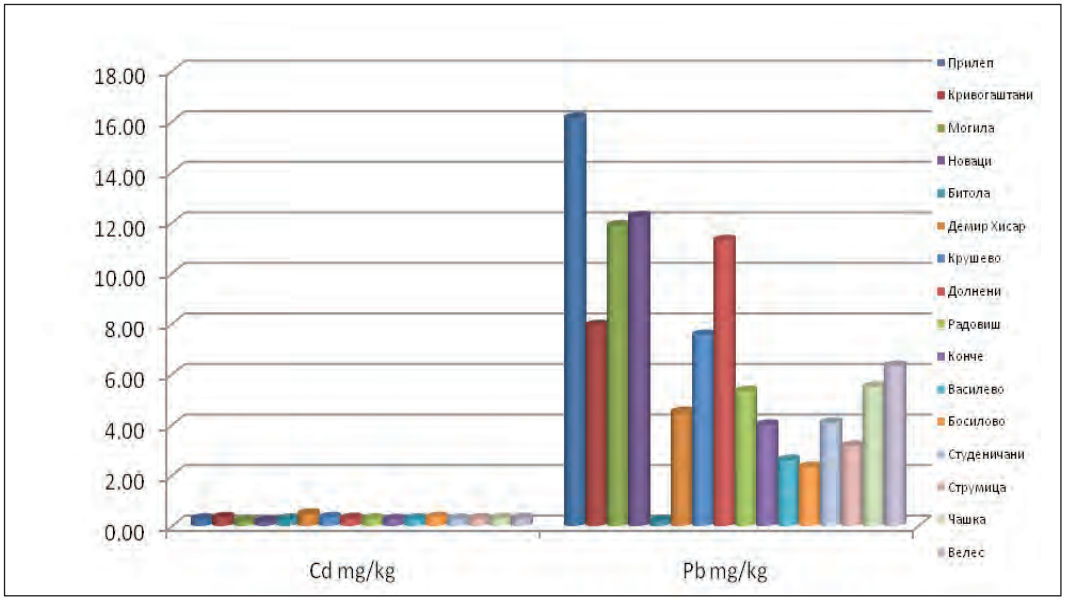

Figure 4. Average Cd and Pb concentrations in soils in some tobacco-growing regions in R. Macedonia

Correlation coefficients (Table 2), showed a strong relationship between the concentrations of each tobacco priming (first, second and third) for almost every studied metal at 0.01 level. Comparing the obtained results of heavy metal analysis to those of plant material (tobacco leafs), no significant statistical correlation was determined between the soil parameters and Cd, Ni and Cr content of the plants. That was not case with Pb content of all three priming of the oriental tobacco where: clay, humus as well as well as Cd, Cu, Zn and Ni content in the soil had significant coorelation. Significant coorelation was found and for Pb content of the soil and Ni concentration in second and third primings of tobacco plants.

Table 2. Correlation between soil parameters and concentration of metals in oriental tobacco leaves (n = 50)

Parameter	Cd 1st	Cd 2nd	Cd 3d	Pb 1st	Pb 2nd	Pb 3d	Cr 1st	Cr 2nd	Cr 3d	Ni 1st	Ni 2nd
humus	0,024	0,072	0,090	0,399**	0,429**	0,190	-0,081	-0,116	-0,024	0,013	-0,068
pH	0,186	-0,113	-0,165	0,146	0,129	-0,028	0,091	0,118	0,014	-0,103	-0,088
Clay %	0,179	0,040	0,162	0,645**	0,603**	0,318*	0,244	0,102	0,308*	0,098	0,019
Cd soil	-0,031	0,076	0,008	0,399**	0,396**	0,292*	-0,138	0,003	-0,069	0,315*	0,306*
Pb soil	-0,015	0,146	0,243	-0,225	-0,217	-0,280*	0,050	-0,157	0,019	-0,457**	-0,489**
Mn soil	0,116	0,237	0,266	0,052	0,078	-0,068	-0,273	-0,140	-0,048	-0,062	0,102
Fe soil	-0,114	0,030	-0,046	0,184	0,223	0,027	0,392**	0,253	0,349*	-0,165	-0,152
Cu soil	-0,160	0,059	0,160	0,456**	0,389**	0,314*	0,195	0,111	0,434**	0,276	0,420**
Zn soil	0,038	-0,006	0,205	0,279*	0,287*	0,125	0,026	0,176	0,270	0,258	0,246
Cr soil	-0,238	-0,160	-0,164	-0,007	-0,013	0,047	-0,070	-0,104	0,059	-0,067	-0,032
Ni soil	0,145	0,075	0,161	0,641**	0,691**	0,458**	-0,042	0,106	0,256	0,213	0,320*
Cd 1st		0,316*	0,317*	0,133	0,106	0,096	-0,067	0,041	-0,115	0,029	-0,031
Cd 2nd			0,638**	0,295*	0,320*	0,183	-0,096	0,050	0,166	-0,069	0,028
Cd 3d				0,307*	0,335*	0,247	0,023	0,029	0,056	-0,144	0,040
Pb 1st					0,955**	0,701**	0,075	0,158	0,289*	0,312*	0,314*
Pb 2nd						0,699**	0,009	0,136	0,259	0,237	0,247
Pb 3d							0,107	0,294	0,180	0,287	0,263
Cr 1st								0,580**	0,471**	-0,164	-0,230
Cr 2nd									0,535**	-0,091	-0,075
Cr 3d										-0,172	-0,134
Ni 1st											0,716**
Ni 2nd											

1st - Lower primings
2nd - Middle primings
3d - Upper primings
*.Correlation is significant at the 0.05 level (2-tailed).
**.Correlation is significant at the 0.01 level (2-tailed)

CONCLUSION

Clay and pH had no influence on the concentrations of Cd, Cr and Ni in all three primings of the Oriental tobacco, as stated by Adamu et al., 1989; Golia et al. 2007. Clay, humus and Cd, Cu, Zn and Ni content of the soil had a significant correlation with the concentration of Pb in all three primings and Cr content in the upper primings of tobacco plants. According to the results, a conclusion can be drawn that most of the tested soils are ideal for producing a high quality oriental tobacco. Considering the fact that heavy metal concentrations in investigated tobacco leaves and soils are below permissible limits, it can be stated that tobacco-growing regions of R. Macedonia are not contaminated with heavy metals. Comparing the results obtained in soil to those in tobacco leaf, it can be concluded that metal content in soil has a negligible or no influence upon the heavy metal content in tobacco leaf.

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Original scientific paper

ANTIOXIDANT ACTIVITY OF TOBACCO (*Nicotiana tabacum* L.) IN INTOXICATION WITH HEAVY METALS

Lenka Cvetanovska[1], Gordana Dimeska[1], Marija Srbinoska[2], Ivana Klincarska-Jovanovska [1], Svetlana Bozinovska [3], Ana Cvetanovska [1]

[1]Institute of Biology, Faculty of Natural Sciences and Mathematics, 1000 Skopje, Republic of Macedonia
[2]University"St.Kliment Ohridski"-Bitola, Scientific tobacco institute-Prilep
[3]Postgraduate student of Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje

ABSTRACT

For determination of bio-physiological parameters, fresh leaf material of two tobacco varieties (Prilep P-156/1 and Yaka Yv-125/3) was used. Soil cultures were grown in controlled experimental conditions, in a glasshouse of the Institute of Biology (Faculty of Natural Sciences and Mathematics-Skopje). For each treatment ten plants were taken and were treated with four concentrations of heavy metals: $CuSO_4$ x $5H_2O$ (0.25 mg/kg soil, 0.5 mg/kg soil, 1mg/kg soil, 5 mg/kg soil), $CdSO_4$ x $8H_20$ (0.1 mg/kg soil, 0.2 mg/kg soil, 0.4 mg/kg soil and 0.8 mg/kg soil), $Pb(NO_3)_2$ (50 mg/kg soil, 100 mg/kg soil, 200 mg/kg soil, 400 mg/kg soil) and the excess concentration (unallowable for global use) of *Antracol WP-70* (0.2 mg/kg soil, 0.4 mg/kg soil, 0.8 mg/kg soil and 1.6 mg/kg soil). *Antracol WP-70* is fungicide of the dithiocarbamate group, with zinc as its basic constituent. In the same time, a trial with control group of ten tobacco plants was set up. Of ten fully matured leaves from lower, middle and upper leaf belts was collected for analyses. All analysis was performed in triplicate. For the aim of this paper, leaves from the upper belt (III insertion) were used. The accent was put on the analyses of chloroplast pigments content (spectrophotometric method of Holm & Wettstein, 1958) catalase activity (titrimetric method of Bach & Oparin) and anthocyanins content (spectrophotometry at 510 nm).

Key words: intoxication, tobacco, heavy metals, fungicide, chloroplast pigments

АНТИОКСИДАТИВНА АКТИВНОСТ КАЈ ТУТУНОТ (*Nicotiana tabacum* L.) ПРИ ИНТОКСИКАЦИЈА СО ТЕШКИ МЕТАЛИ

Контаминацијата со тешки метали претставува приоритетен проблем во современото општество кој иницира низа малформации, посебно кај растенијата, во услови на силна интоксикација со истите. Целта на оваа студија е одредување на биохемиско-физиолошките параметри на тутунот (*Nicotiana tabacum L*) од реколтата 2008 кај две ориенталски сорти (*Прилеп П-156/1* и *Јака Јв-125/3)*. Растенијата се одгледувани во контролирани, експериментални услови во стакленик на Ботаничката градина при Институтот за биологија (Природно-математички факултет, Скопје). За секој третман земени се по десет тутунските растенија кои беа третирани со тешки метали (бакар, кадмиум и олово) и фунгицид (*Antracol WP-70*) во четири различни концентрации. Истовремено е поставена контролна група од десет тутунски растенија. За анализа се земани по десет целосно зрели листови од појаси на долен, среден и горен лист.

Сите испитувања се направени во три повторувања. За овој труд анализиран е материјал од горниот лист, или трета инсерција од вегетацискиот период на растенијата. Во постапките за анализа акцентот беше ставен на испитувањето на содржината на хлоропласни пигменти (спектрофотометриски метод по Holm и Wettstein, 1958), активноста на каталазата (титриметриски метод по Бах и Опарин), како и содржината на антоцијани (спектрофотометрирање на 510nm). Ефектите од интоксикацијата се одредени преку споредба на третираните садови со контролната група на тутунски растенија, со цел да се потврдат сознанијата за влијанието на тешките метали врз карактеристиките на тутунот како комерцијалн.

Клучни зборови: интоксикација, тутун, тешки метали, фунгициди, хлоропласни пигменти

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) was cultivated thousands of years ago. At first it served as a medicinal plant and later it started to be used as commercial raw and agricultural crop. In the last few decades its application has been broadened and it became widely used as a model-system in plant cells cultures and in genetic engineering researches. Due to its huge economic importance, it became an object of many investigations both of its origin and evolution and of its genetic and structural organization.

Three main types of tobacco grown in this area are Prilep, Yaka and Basmak.

Heavy metals present a strong stress factor to plant metabolism. Their content in plants depends on genetic specificity of the species, their selective abilities for metals uptake and the level of environment pollution. According to William (1981), heavy metals can affect the activity of some enzymes. They function as antimetabolites, create resistant precipitations or chelates in constituent parts of the cells, act as catalysts in decomposition of plant metabolites, change the permeability of cell membrane or replace the major structural chemical elements in the cell. Cadmium, nickel and thallium show high mobility and can be easily removed from the plant, while other elements (mercury, vanadium, lead and zinc) have an ability to accumulate in plant organs.

Cadmium provokes oxidation, but unlike other heavy metals, e.g. Cu, it does not directly influence the production of reactive types of oxygen. Activation and slowing of the antioxidative enzymes depends not only on intensity of the effect and its duration but also on the type of tissue and plant age. Lead can provoke inhibition of plant growth and development, prolongation of root, germination and seed development, division of cells, photosynthesis, transpiration, chlorophyll synthesis, growth of ethioplates and lamellar organization of the chloroplasts (Wozny & Jerczynska, 1991).

Copper uptake by plants is in small amounts, mostly as Cu-ions in chelate form. With increased concentration of copper in the environment, however, the plants ability for absorption of Cu also increases. Cu-ions are absorbed not only by plant roots, but also by its above-ground parts. Plant sensitivity to copper insufficiency differs, and oat, wheat, barley, tobacco and spinach belong to the group of very sensible. Fungicides, insecticides and pesticides can be potential toxicants.

Uniformity and constancy of biological-morphological and technological-industrial properties are of special importance for tobacco quality. For improvement of quality, especially of tobacco types Prilep (P-156/1) and Yaka (Yv-125/3), the effect of heavy metals (Cu, Cd, Pb) and fungicide *Antracol WP-70* was investigated through analysis of the chloroplasts and content and catalase activity. The effects of intoxication were determined through comparison of treated crops with the control group of tobacco plants.

MATERIAL AND METHODS

For determination of bio-physiological parameters, fresh leaf material of two tobacco varieties (Prilep P-156/1 and Yaka Yv-125/3) was used. Soil cultures were grown in controlled experimental conditions, in a glasshouse of the Institute of Biology (Faculty of Natural Sciences and Mathematics-Skopje). Tobacco plants were treated with four concentrations of heavy metals: $CuSO_4$ x $5H_2O$ (0.25 mg/kg soil, 0.5 mg/kg soil, 1mg/kg soil, 5 mg/kg soil), $CdSO_4$ x $8H_20$ (0.1 mg/kg soil, 0.2 mg/kg soil, 0.4 mg/kg soil and 0.8 mg/kg soil), $Pb(NO_3)_2$ (50 mg/kg soil, 100 mg/kg soil, 200 mg/kg soil, 400 mg/kg soil) and the excess concentration (unallowable for global use) of *Antracol WP-70* (0.2 mg/kg soil, 0.4 mg/kg soil, 0.8 mg/kg soil and 1.6 mg/kg soil). *Antracol WP-70* is fungicide of the dithiocarbamate group, with zinc as its basic constituent. In the same time, a trial with control group of tobacco plants was set up. Material from lower, middle and upper leaf belts was collected for analyses. For the aim of this paper, leaves from the upper belt (III insertion) were used. The accent was put on the analyses of chloroplast pigments content (spectrophotometric method of Holm & Wettstein, 1958) catalase activity (titrimetric method of Bach & Oparin) and anthocyanins content (spectrophotometry at 510 nm).

RESULTS AND DISCUSSION

In addition to heavy metals intoxication, it was determined that tobacco itself is a source of high amounts of Cu and Zn, in concentrations of 12.90 mg/g and 55.62 mg/g dry mass weight, respectively (Massadeh et al., 2003).

Results of the bio-physiological analyses are presented in tables and figures.

Table 1.Catalase activity in dry tobacco leaves of varieties Prilep P-156/1 and Yaka Yv-125/3, upper belt (III incertion)

Treatment	Catalase activity Prilep P-156/1 (mg H_2O_2)	Catalase activity Yaka Yv-125/3 (mg H_2O_2)
Control	1.35	1.09
Cu I	1.02	1.47
Cu II	0.26	0.93
Cu III	0.56	0.34
Cu IV	1.02	0.69
Pb I	0.63	1.96
Pb II	0.29	0.46
Pb III	0.24	0.66
Pb IV	0.22	0.64
Cd I	0.49	0.46
Cd II	0.9	0.43
Cd III	0.25	0.51
Cd IV	0.49	0.17
Fungicide I	1.21	0.45
Fungicide II	0.64	0.85
Fungicide III	1.06	0.21
Fungicide IV	1.96	1.08

In relation to its structure, catalase is a proteide which role is to decompose the highly toxic H_2O_2. Its translocation and negative impact on plant metabolism is difficult to follow (Willekens et al., 1997). Catalase activity is particularly important in the case of intoxication with heavy metals, when the production of hydrogen peroxide is increased.

Catalase activity reflects the ability of organism to supply strong protective mechanism against unfavorable environmental conditions. In this context the photosynthetic activity of plants should be mentioned (Zeltich, 1992). Investigations of cadmium, copper, iron and zinc effects on the activity of antioxidant enzymes in *N. tabacum* (Sokolnik et al., 2007) revealed that these elements stimulated the activity of dismutase but reduce the catalase activity. It can be noted that catalase activity is reduced when tobacco is treated with Cu, Cd and Pb, but it is also negatively affected by the application of fungicides. The catalase activity shows higher values in the following cases: the fourth concentration of fungicide for variety P-156/1 (1.96 mg dissolved H_2O_2), the first concentration of Cu for Yv-125/3 (1.47 mg dissolved H_2O_2) and the first concentration of lead (1.96 mg dissolved H_2O_2). In all other treatments of tobacco plants with metals, the level of catalase activity is lower compared to the control group.

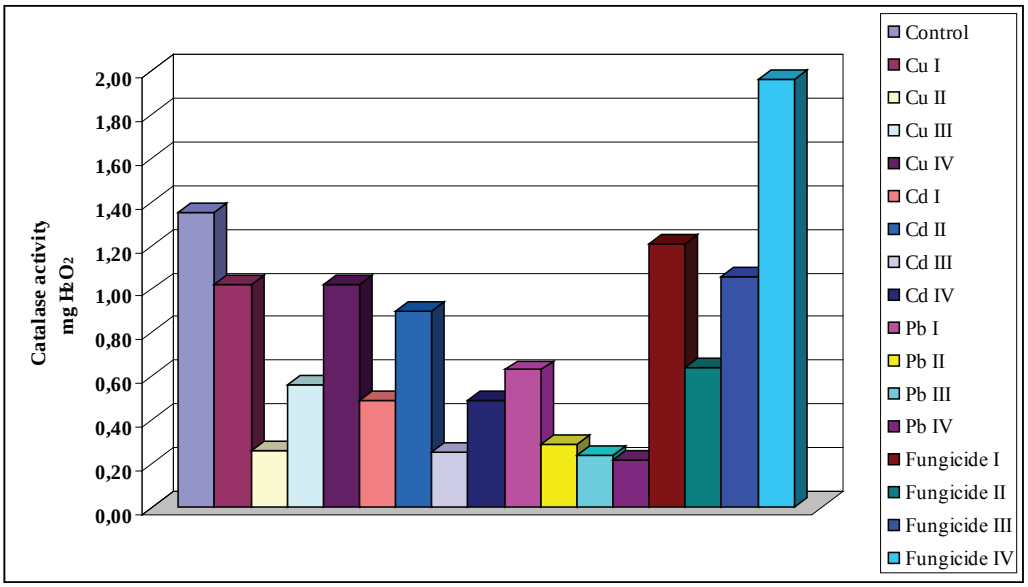

Figure 1. Catalase activity in dry tobacco leaves of variety Prilep P-156/1, upper belt (III incertion)

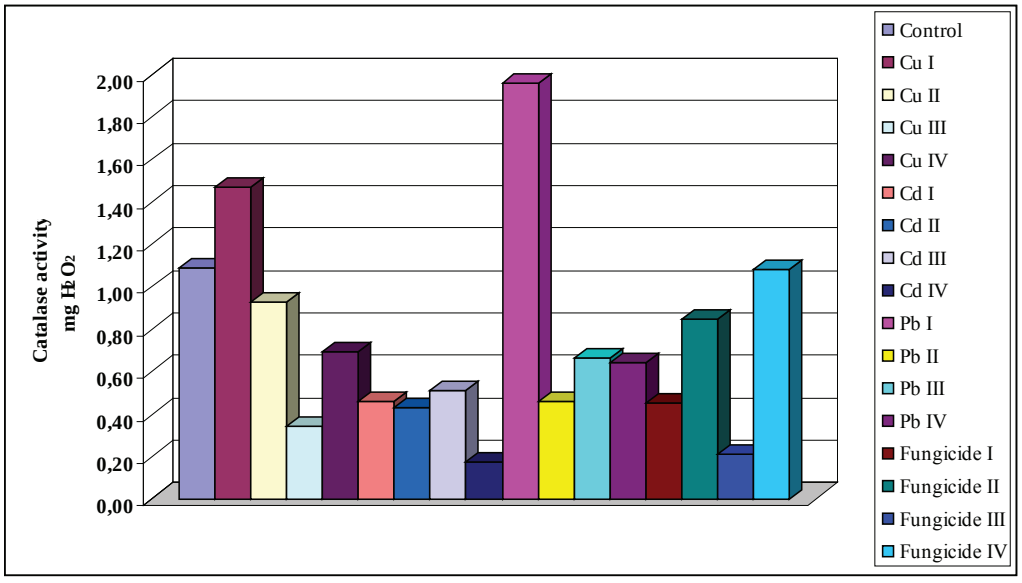

Figure 2. Catalase activity in dry tobacco leaves of variety Yaka Yv-125/3, upper belt (III incertion)

Table 2. Content of chloroplast pigments (mg/g) in fresh material of tobacco leaves of variety Prilep P-156/1, upper belt (III incertion)

Treatment	Chl-a (mg/g)	Chl-b (mg/g)	Chl-a+b (mg/g)	Carotenoids (mg/g)
Control	0.68	0.67	1.34	0.20
Cu I	0.30	0.35	0.65	0.13
Cu II	0.90	0.41	1.32	0.14
Cu III	0.19	0.30	0.50	0.10
Cu IV	0.12	0.36	0.48	0.11
Pb I	0.34	0.44	0.79	0.16
Pb II	0.54	0.56	1.10	0.19
Pb III	0.05	0.49	0.80	0.16
Pb IV	0.22	0.26	0.49	0.10
Cd I	0.26	0.27	0.53	0.14
Cd II	0.33	0.34	0.67	0.16
Cd III	0.27	0.36	0.63	0.15
Cd IV	0.16	0.16	0.32	0.10
Fungicide I	0.20	0.18	0.38	0.11
Fungicide II	0.16	0.16	0.32	0.10
Fungicide III	0.10	0.07	0.17	0.06
Fungicide IV	0.68	0.67	1.34	0.20

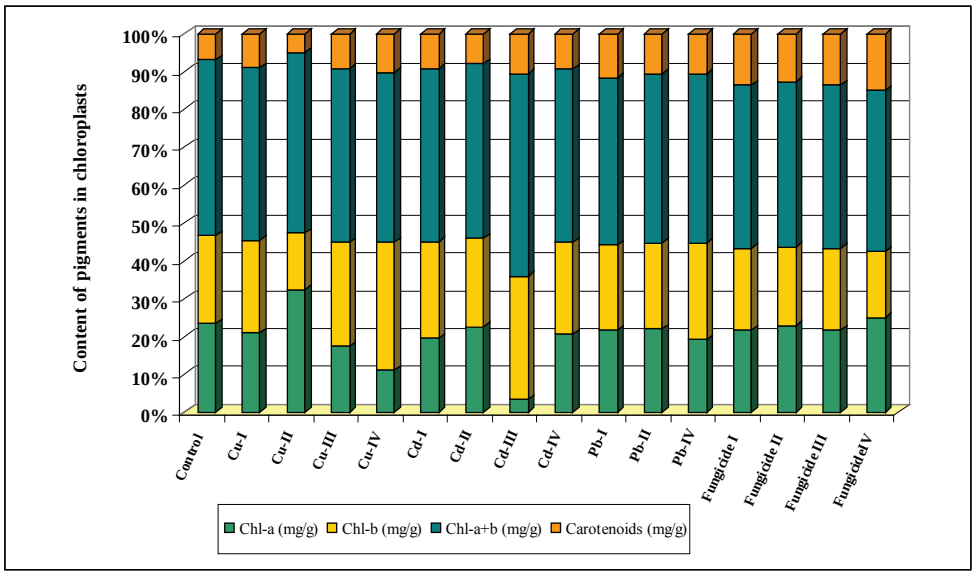

Figure 3. Content of pigments in chloroplasts in fresh material of tobacco leaves of variety Prilep P-156/1,upper belt (III incertion)

According to a number of literature references from internationally recognized laboratories, the process of photosynthesis can detect external contaminants. Chlorophyll assimilation, for example, can be used as highly relevant bio-indicator of toxic effects of many external contaminants.

Table 3. Content of chloroplast pigments (mg/g) in fresh material of tobacco leaves of variety Yaka Yv-125/3, upper belt (III incertion)

Treatment	Chl-a (mg/g)	Chl-b (mg/g)	Chl-a+b (mg/g)	Carotenoids (mg/g)
Control	0.33			0.14
Cu I	0.43	0.49	0.93	0.80
Cu II	0.38	0.30	0.68	0.14
Cu III	0.40	0.46	0.87	0.17
Cu IV	0.46	0.35	0.83	0.08
Pb I	0.44	0.38	0.83	0.08
Pb II	0.40	0.33	0.73	0.14
Pb III	0.31	0.32	0.63	0.11
Pb IV	0.22	0.20	0.42	0.09
Cd I	0.47	0.46	0.93	0.17
Cd II	0.38	0.45	0.83	0.15
Cd III	0.37	0.35	0.72	0.11
Cd IV	0.33	0.30	0.64	0.23
Fungicide I	0.28	0.28	0.55	0.11
Fungicide II	0.43	0.43	0.86	0.16
Fungicide III	0.22	0.23	0.45	0.09
Fungicide IV	0.40	0.48	0.89	0.17

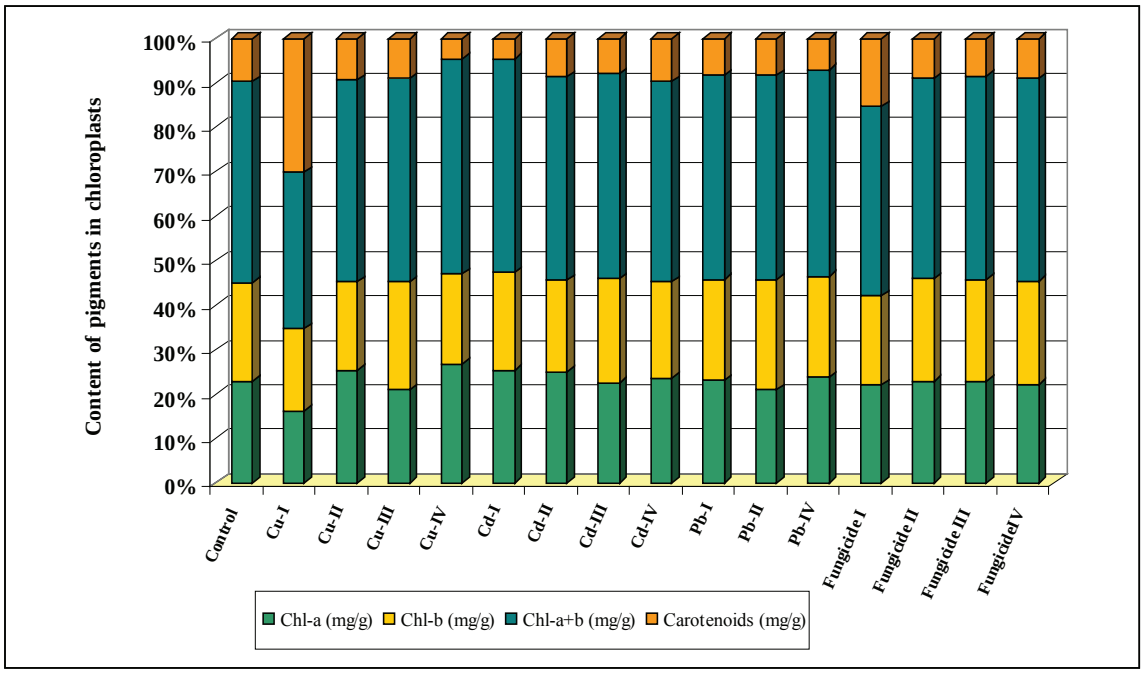

Figure 4. Content of pigments in chloroplasts in fresh material of tobacco leaves of variety Yaka Yv-125/3, upper belt (III incertion)

It can be stated from the results that the applied concentrations of heavy metals reduce the content of chloroplast pigments as a result of the chlorophyll destruction, but on the other side, the concentration of carotenoids is increased. High cadmium concentrations provoke disbalance and damages of the whole pigment system, which is reflected in reduced biosynthesis and organic production, In Prilep tobacco, chl-a decreases from 0.68 mg/g (control) to 0.05 mg/g (Cd

III concentration) and in Yaka tobacco, chl-b decreases from 0.32 mg/g (control) to 0.20 mg/g (Cd IV concentration). Copper is constituent part of the enzymes and thereby affects the life processes in plants. In plants treated with Cu, the chloroplast pigments content is slightly increased due to the general physiological effects of Cu accumulation in strengthening the structure of chloroplasts and in formation of organic matter. Chl-a concentration increases in both investigated types, ranging from 0.68 mg/g (control) to 0.90 mg/g (Cu II concentration) in Prilep and from 0.33 mg/g to 0.46 mg/g (IV concentration) in Yaka. Known by their constancy and protective function in chlorophyll stabilization, carotenoides are not significantly affected by application of heavy metals. The highest impact on their level was noticed after Pb application in soil (0.14 mg/g - 0.23 mg/g IV Pb).

According to investigations on poppy (Spasenoski M, Gadzovska S. 2000-2001), various concentrations of cadmium and lead affect the content of chloroplast pigments. Thus, the increased cadmium concentration reduces the content of these pigments. The results are in accordance with Skorzynska-Polit and Baszynski (1995), who report that cadmium causes significant changes of thylakoid membrane, which is in direct correlation with ultrastructural changes of the photosynthetic apparatus. Heavy metals and the fungicide are considered to have a destructive effect on biosynthetic apparatus, leading to disorder of the electron transport in one of the stages and in the process of formation of pigment system. As it is well known, tobacco (*N. tabacum*) can easily accumulate certain metals and particularly cadmium in leaves. Cadmium is indispensable, potentially toxic, widely spread contaminant, accumulated by tobacco and transferred through the smoke to humans.

Phosphate fertilizers applied in tobacco cultivation contain high concentration of heavy metals. A number of factors affect the amounts of heavy metals in tobacco: soil type, pH genotype, stalk position, soil and leaf residues resulting from metal-containing pesticides (Golia et co., 2001) and from soil amendments with fertilizers and municipal sludge.

Anthocyanins are powerful antioxidants which signalize the toxic effects of environment on plants. Together with certain enzymes, they are one of the first biomolecules which reveal the level of plant intoxication by changing their concentrations. The increase in heavy metals concentration in treated plants is a stress situation, but plants are attempting to normalize that situation (Table 4).

Table 4. Content of anthocyanins in dry tobacco leaves of varieties Prilep P-156/1 and Yaka Yv-125/3, upper belt (III incertion)

Treatment	Content of anthocyanins Prilep P-156/1 (mg/100 g)	Content of anthocyanins Yaka Yv-125/3 (mg/100 g)
Control	1.35	1.09
Cu I	1.02	1.47
Cu II	0.26	0.93
Cu III	0.56	0.34
Cu IV	1.02	0.69
Pb I	0.63	1.96
Pb II	0.29	0.46
Pb III	0.24	0.66
Pb IV	0.22	0.64
Cd I	0.49	0.46
Cd II	0.9	0.43
Cd III	0.25	0.51
Cd IV	0.49	0.17
Fungicide I	1.21	0.45
Fungicide II	0.64	0.85
Fungicide III	1.06	0.21
Fungicide IV	1.96	1.08

Although the two tobacco varieties investigated have similar physiological parameters, they differ by their resistance ability. Cadmium is potentially toxic and when its concentration is increased, it is included in the anthocyanins synthesis in treated plants. In our investigations, P-156/1 showed the highest anthocyanin contents with application of 1st, 2nd and 4th concentration of fungicide (0.2 mg/kg, 0.4 mg/kg and 1.6 mg/kg). In Yv-125/3 its highest value was recorded with application of 1st and 4th concentration of cadmium (0.1 mg/kg and 0.8 mg/kg), and 1st and 4th concentration of fungicide (0.2 mg/kg and 1.6 mg/kg). Direct fungicide application on tobacco leaves resulted in immediate antioxidant response, which leads to increased concentration of anthocyanins. Herefrom it can be concluded that Yaka tobacco has higher affinity to take protective measures in unfavorable conditions.

CONCLUSIONS

The study confirmed our expectations for the negative effects of heavy metals and disorder they make in the production of organic biomolecules. The following conclusions can be drawn from the results obtained:

1. Tobacco treatment with heavy metals Cu, Cd and Pb reduces the catalase activity.

2. The antioxidant enzyme increases its activity with the fourth concentration of fungicide in variety P-156/1 (1.96 mg dissolved H_2O_2), while in Yv-125/3 with the first concentration of copper (1.47 mg dissolved H_2O_2) and first concentration of lead (1.96 mg dissolved H_2O_2). In all other tobaccos treated with heavy metals, the levels of catalase activity were lower compared to the control variant.

3. High concentrations of cadmium cause disbalance and damage the whole pigment system, which is reflected in reduced biosynthesis. In Prilep tobacco, chl-a decreases from 0.68 mg/g (control variant) to 0.05 mg/g (Cd - III concentration). In Yaka tobacco, chl-b decreases from 0.32 mg/g (control variant) to 0.20 mg/g (Cd - IV concentration).

4. Copper increases the concentration of chloroplast pigments because it can be involved in strengthening the chloroplast structure.

5. Increased levels of anthocyanins in treated plants present a stress situation, but plants are attempting to normalize the stress.

6. Stress situation in P-156/1was noticed with first, second and fourth concentration of fungicide (0.2 mg/kg, 0.4 mg/kg and 1.6 mg/kg). In Yv-125/3 it was recorded with first and fourth concentration of cadmium (0.1 mg/kg and 0.8 mg/kg) and first and fourth concentration of fungicide (0.2 mg/kg and 1.6 mg/kg), which resulted in increased anthocyanins concentration.

7. Direct fungicide application on tobacco leaves resulted in an immediate antioxidant response, resulting in increasing the anthocyanins concentration.

8. Herefrom it follows that Yaka tobacco is more capable of taking protecting measures in unfavorable conditions.

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FAUNISTIC AND QUANTITATIVE ANALYSIS OF SPECIES OF THE GENUS PARAGUS

Vesna Krsteska

University"St.Kliment Ohridski"-Bitola, Scientific tobacco institute-Prilep
Kicevska bb, 7500 Prilep. Republic of Macedonia. e-mail: vkrsteska@yahoo.com

ABSTRACT

Larvae of the aphidophagous species of hoverflies play a significant role in regulation of the number of aphids on tobacco leaf.

During the three-year investigations, we determined the following species of the genus Paragus: *P. quadrifasciatus, P. tibialis and P. testaceus*.

The abundance of predatory species of the genus Paragus primarily depends on the abundance of leaf aphids, but since they are xerophytic and thermophilic organisms, climate factors play an important role in their development.

Key words: aphids, aphidophagous hoverflies, *P. quadrifasciatus, P. tibialis, P. testaceus*.

ФАУНИСТИЧКА И КВАНТИТАТИВНА АНАЛИЗА НА ВИДОВИТЕ ОД РОДОТ *PARAGUS*

Ларвите на афидофагните видови на осилки муви имаат големо значење за регулирање на бројноста на лисните вошки на тутунот.

При тригодишните испитувања ги утврдивме следните видови од родот *Paragus: P. quadrifasciatus, P. tibialis* і *P. testaceus.*

Бројноста на предаторски видови од родот Paragus е во зависност од бројноста на лисните вошки, но како жители на ксерофилни и термофилни услови, значајна улога за нивниот развој имаат и климатските фактори.

Клучни зборови: вошки, афидофагни осилики муви, *P. quadrifasciatus, P. tibialis, P. testaceus*

INTRODUCTION

Faunistic investigations are used in many scientific disciplines with a final aim to conceive the basic ecological, bio-geographical and evolutional principles.

Hoverflies are important predatory species. In their adult stage they are useful as pollinators and in larval stage as natural enemies of pests, especially of aphids.

Genus Paragus is a common and widely spread predator species.

MATERIAL AND METHOD

Investigations were carried out during 2003-2005 and included the following methods of catching: check of 20 tobacco stalks; check of 100 tobacco leaves (Davies method); yellow water vessels and mowing with catcher.

Material was sampled during the season of tobacco growing, from transplanting to the last harvest, in a period of 10 days.

During investigations, detailed quantitative analysis of the genus Paragus was made in the region of Prilep.

Based on the selected material, faunistic investigations were made using the following parameters: active dominance, active abundance, constancy or frequency and population dynamics. For estimation of the number of male and female individuals, Si gender indices were determined.

RESULTS AND DISCUSSIONS

We determined three hoverflies species of the genus Paragus in tobacco agrobiocenosis, belonging to:

Tribus: **Paragini**

Genus: **Paragus Latreille, 1804**

Species: 1. ***Paragus quadrifasciatus* Meigen, 1822**

2. ***Paragus testaceus (bicolor)* Meigen, 1822**

3. ***Pandasyophthalmus (Paragus) tibialis* Fallen, 1817**

Species of this genus are the smallest predator hoverflies. Their larvae are aphidophagous and imagos are adapted to thermophillic and xserophitic conditions, which can be related to their Mediterranean origin. According to Pek (1971), only the species of this genus prefer the plants growing in dry areas.

Paragus is mostly distributed in Mediterranean region, where the number of aphids and aphidophagous hoverflies is small, because most of them dwell in colder and more humid conditions.

Glumac (1965, cit. by Vujic, 1987) reported that conditions of the Mediterranean region are most favorable for hoverflies with small dimensions, which feed on small number of aphids, like the species of this genus.

Identification of Paragus should be made with special carefulness, because of the absence of key which will include all European species.

Stuckenberg (1954) created a subgenus Pandasyophthalamus to which *P. tibialis* belongs (cit. Dusek Láska, 1967).

Vujic (1987) reported that from 20 species of this genus, 10 were found in former Yugoslavia and in Bulgaria, with high population densities.

1. *Paragus quadrifasciatus* Meigen, 1822

P. quadrifasciatus is one of the smallest hoverflies. Its larvae are well known predators of leaf aphids.

According to Simic (1987) and Glumac (1955), this species is spread throughout the Central and South Europe, whereas Vujic, Glumac (1994) report about its spreading in Palearctic area.

On the Balkans the species was recorded in Serbia, Dalmatia, Bosnia and Herzegovina, Montenegro and Bulgaria (Simic 1987, Glumac 1955, Vujic, Glumac 1994). All specimens are typical for the variety *quadrifasciatus* Meig.

In Macedonia, Janusevska (2001) reported *P. quadrifasciatus* as predator of *Myzus persicae* Sulzer on tobacco in the region of Prilep.

Krpac (2006) recorded this species in the regions of Otesevo and Veles.

Photo. 1 Male and female of *P. quadrifsaciatus*

Distribution of *P. quadrifasciatus* in the region of Prilep was determined by the method of mowing with catcher (2003 - 2005). The species was recorded in tobacco fields hedges, near meadows, in vegetable gardens and in cereal crops.

P. quadrifasciatus density is higher in multiculture than in monoculture fields (Gao, 1996). It can be found in high abundance in soybean fields and near water resources (cit. Wu et al.,

In our investigations, imagos of *P. quadrifasciatus* in individual samples were recorded from 1 August to 1 September. Simic (1987) and Glumac (1955) recorded the imagos from April until August. Gao (1991) reported that imagos appear by the end of April to the beginning of May. From late August to early September *P. quadrifasciatus* reappears among the aphids in wheat fields, vegetable gardens or in dense grass.

2004). It was also reported that *P. quadrifasciatus* is more abundant in weeds than in cultivated crops.

Adults feed on flowers of the weed species Apiaceae, Euphorbiaceae, Brassicaceae. etc. They prefer flowers with simple anatomy where pollen and nectar are easily accessible. They are particularly attracted by white and yellow flowers.

P. quadrifasciatus is an obligate aphidophagous species, which means that it develops normally only when fed on leaf aphids.

In our investigations, this species was identified as predator of *M. persicae* on tobacco.

2. *Paragus testaceus* Meigen, 1822 (var. *bicolor* Fabricus, 1794)

P. testaceus is aphidophagous species and belongs to the group of the smallest hoverflies.

Glumac (1955) reported that this species is distributed in Europe, North America and Africa.

According to Simic (1987) and Vujic, Glumac (1994), the species is spread in the Holarctic area.

On the Balkan Peninsula, *P. testaceus* was recorded in all parts of former Yugoslavia, as well as in Bulgaria and Greece (Simic 1987, Vujic, Glumac 1994, Glumac 1955).

According to Krpac (2006), there are very few data on the occurrence of this species in Macedonia. Simic (1987) and Glumac (1994) noted that the flying period of imagos is from April to September.

The species has two generations per year, the first one appearing from mid-May to mid-June or somewhat later and the second in August or later (Metcalf, 1911).

Distribution of *P. testaceus* in the region of Prilep was determined by the method of mowing with catcher (2003 - 2005). The species was recorded near tobacco and vegetable plots.

According to a great number of authors, the imagos are usually found in meadows, parks, along rivers, roads, etc.

Láska (1978) noted that *P. bicolor* (var. *testaceus*) can be recorded in fields and steppes.

Adults feed on flowers of the weed families Apiaceae, Euphorbiaceae, Brassicaceae etc.

Pek (1971) reported that favorite plants of *P. testaceus* are various species of Umbelliferae family in the forests. Imagos prefer the flowers with simple anatomy, where pollen and nectar are easily accessible. They are particularly attracted by white and yellow flowers (Adashkevich, 1975).

Photo. 2 Male of *P. testaceus*

Photo. 3 Female of *P. testaceus*

P. testaceus is an obligate aphidophagous species, i.e. it develops normally only when fed on leaf aphids.

A number of authors reported it as polyphagous species aphids of various plant species.

P. testaceus larvae were recorded feeding on aphids in vegetable crops, on *Myzus sp*. in *Rumex crispus* L., on various aphids of *Rumex obtusifolius* L., *Arctiumminus Schk*., *Carduus* sp. (Metcalf, 1991).

3. *Pandasyophthalamus (Paragus) tibialis* Fallén, 1817

P. tibialis is one of the smallest hoverflies and a very active predator of aphids.

The species is abundant in Palearctic and Nearctic North American region (Pek, 1981).

According to Simic (1987) it is distributed in the Holarctic region, while Vujic and Glumac (1994) reported Holarctic-Oriental region as its area of distibution.

On the Balkan Peninsula, *P. tibialis* was recorded in all parts of former Yugoslavia, in Bulgaria and Hungary (Simic 1987, Vujic, Glumac 1994, Glumac 1955).

In Macedonia, the occurence of this species was reported in Otesevo Krpac (2006).

The flying period of imagos is from April (March) to September (Simic, 1987; Glumac, 1955; Daminova, 1975).

Photo. 4 Male of *P. tibialis*

Photo. 5 Females of P. *tibialis*

According to Peck (1981), the imagos appear in June and reach the maximum in July-August, visiting several flowers species. The massive flight of the late-summer species *P. tibialis* is in the second decade of August (Mutin, 1983, b), in May-June (Daminova, 1975) and during the summer (Bugg, www.sarep.ucdavis.edu).

Distribution of *P. tibialis* in the region of Prilep was determined by the method of mowing with catcher (2003 - 2005). The species was spread through the whole region, on various biotopes, weeds, meadows, cereal fields, vegetable crops etc.

Many authors reported that *P. tibialis* is most frequently found in meadows, deciduous forests, cereal fields, soybean fields, vegetable cropsand other cultivated and weed species.

The adults feed on pollen and nectar from flowers of weed families Apiaceae, Euphorbiaceae, Brassicaceae etc. Favorite plants to imagos are various plants of Umbelliferae family in the forests. The imagos feed on pollen and nectar from plants like *Scenecio sp., Euphorbia cyparissias, Ranunculus sp., Mentha aquatilis, Sambicus ebulus, Euphorbia sp., Lepidium latifolium, Prunus fruticosa, Eriogonum sp., Polygonum aviculare,* plum, hibiscus etc. (Peck, 1981; B u g g -sarep.ucdavis.edu, etc).

In gardens, the imagos live on flowers of apples, cherries, peaches, pears etc., but in hilly regions they are mainly concentrated on flowers in meadows and bushes.

P. tibialis is an obligate aphidophagous species, which develops normally only when fed on leaf aphids.

According to Daminova (1975), this species feeds on wide spectrum of leaf aphids on many different plants. During its growth period, the larvae can consume up to 1000 aphids.

Photo 6. Paragus sp.

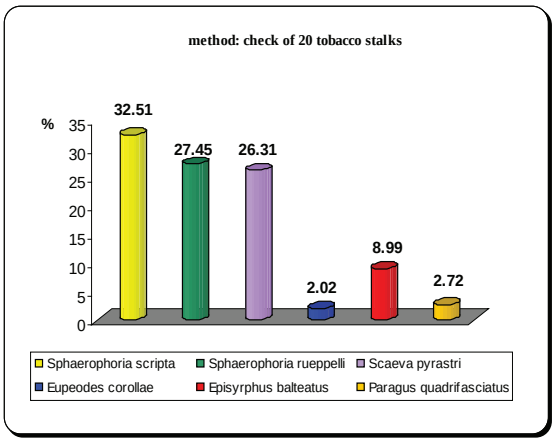
In the region of Strumica *Paragus sp*. have been recorded and we hope it will be a subject of our further investigation (Photo 6).

4. Quantitative analysis of species of the genus Paragus

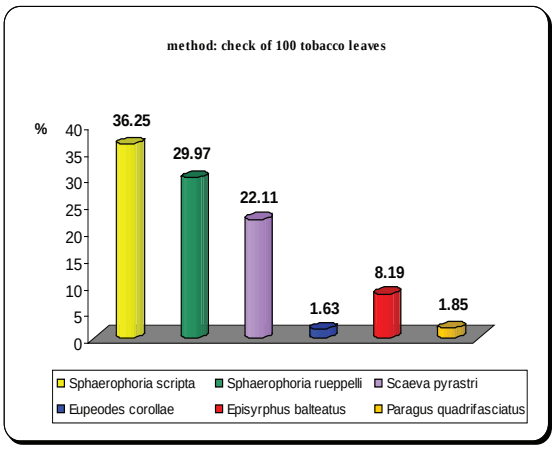
The analysis of quantitative composition of the fauna of Syrphidae family on tobacco in the region of Prilep (2003 - 2005) revealed various levels of hoverflies population, depending on the year.

- By the method of survey of 20 tobacco stalks, 147 individuals of *P. quadrifasciatus* species of the genus Paragus were determined.

In 2003, this species was recorded in August and early September in lower abundance - 6 eggs, 44 larvae, 11 pupae and 1 imago. No parasitised pupae were observed. The maximum number of larvae was recorded on 20 August. In 2004 the abundance of *P. quadrifasciatus* was even lower and no parasitised pupae and imagos were determined. In 2005 the species was recorded only in small number in August - 6 eggs, 46 larvae and 15 pupae. No parasitised pupae or imagos were determined. The share of hoverflies in the total percentage of hoverflies is 2.72% (Graph. 1).



Graph. 1 Total percentage of hoverflies

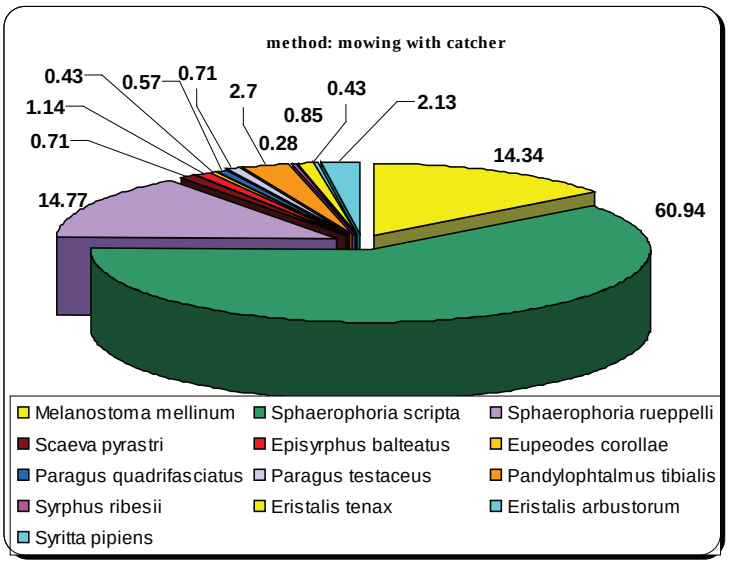


Graph. 2 Total percentage of hoverflies

• By the method of Davies (survey of 100 tobacco stalks), only *P. quadrifasciatus* species of the genus Paragus was determined, with low abundance of larvae and pupae. Imagos were not recorded because only some of tobacco leaves were detached. *P. quadrifasciatus* was represented with 1.85% (Graph. 2).

• No species of this genus were determined by the method of yellow water vessels.

• By the method of mowing with catcher, the following Paragus species were determined in the region of Prilep: *P. tibialis*, with 19 imagos, *P. testaceus* with 5 imagos and *P. quadrifasciatus* with 4 adults. In the three-years investigation, *P. tibialis* was represented with 2.70%, while *P. testaceus* and *P. quadrifasciatus* bellow 1% (Graph. 3).



Graph. 3 Total percentage of hoverflies

5. Faunistic analysis of species of the genus Paragus

P. quadrifasciatus is an obligate aphidophagous species. It is present in tobacco biocenosis from 1 July to 1 September.

Table 1. Total representation of hoverflies according to the applied methods and level of dominance

Methods	*P. quadrifasciatus*			*P. testaceus*			*P. tibialis*		
	total individuals		active dominance	total individuals		active dominance	total individuals		active dominance
	number	%	%	number	%	%	number	%	%
check of 20 stalks	147	79,89							
check of 100 leaves	33	17,94							
sweep net catcher	4	2,17		5	100,00		19	100,00	
Total	184	100,00	2,29	5	100,00	0,06	19	100,00	0,24

Quantitaive analysis was based on 184 individuals of *P. quadrifasciatus*, which accounts for 2.29% of the total number of Diptera Syrphidae individuals on tobacco. *P. quadrifasciatus* appeared as subdominant species in 2003 and 2005, a recedent species in the fauna of Diptera Syrphidae in 2004 and accidental species in tobacco entomocenosis in the region of Prilep (Table 1, 2; Fig.1).

Table 2. Quantitative data of hoverflies population

Year	*P. quadrifasciatus*			*P. testaceus*			*P. tibialis*		
	active dominance	active abundance	constancy	active dominance	active abundance	constancy	active dominance	active abundance	constancy
	%	%	%	%	%	%	%	%	%
2003	4,11	1,46	16,07	0,10	0,04	3,57	0,25	0,09	5,36
2004	0,71	0,43	12,50	0,09	0,45	3,57	0,18	0,11	8,93
2005	2,94	1,39	16,07	-	-	-	0,30	0,14	8,93

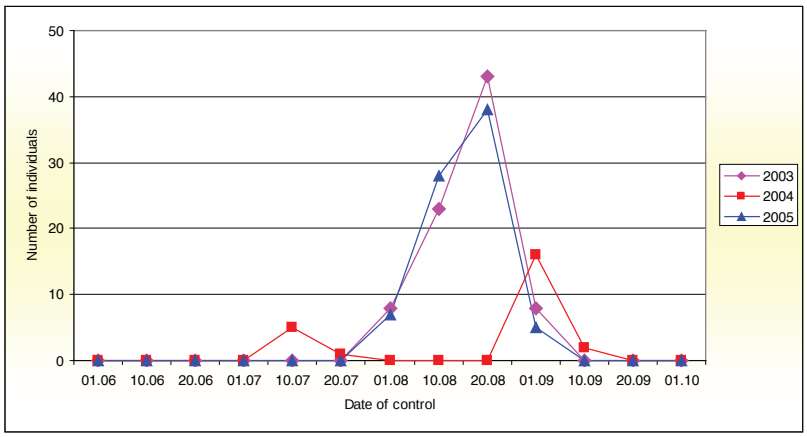


Fig. 1. Dynamics of population of *Paragus quadrifasciatus*, 2003-2005

P. testaceus is an obligate aphidophagous species. In tobacco biocenosis it can be observed in small number and inconstant presence by the middle of July. In that period small colonies of aphids can be found in tobacco fields. In 2005 the species was not recorded (Table 1, 2; Fig.2).

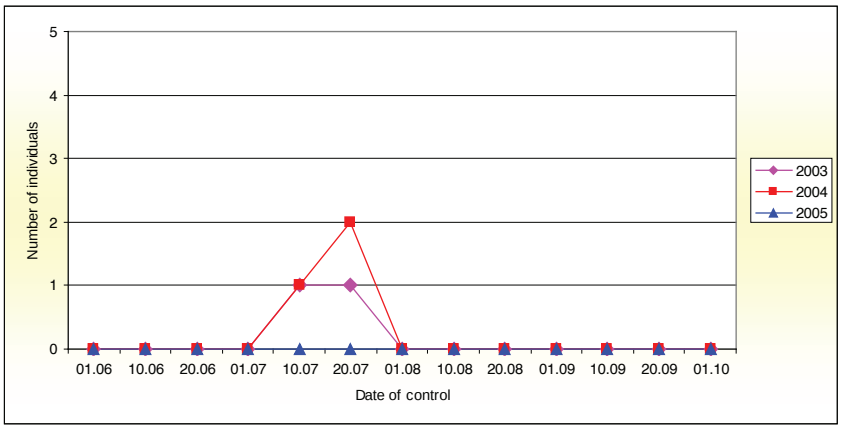


Fig. 2. Dynamics of population of *Paragus testaceus*, 2003-2005

Quantitative analysis is based on 5 individuals of *P. testaceus*, which account for 0.06% Of the total number of Diptera Syrphidae individuals on tobacco. *P. testaceus* has a low level of dominance and belongs to the species accidentally present in tobacco entomocenosis in the region of Prilep

P. tibialis is an obligate aphidophagous species. In tobacco biocenosis it could be observed in small number and inconstantly from 10 June to the end of September in 2003 and 2004. In 2003 it only appeared in a short time period, from 20.08 to 10.09. Quantitative analysis is based on 19 individuals of *P. tibialis*, which account for 0.24% of the total number of Diptera Syrphidae individuals on tobacco. *P. tibialis* appeared as subrecedent species in the fauna of Diptera Syrphidae and accidental species in tobacco entomocenosis in the region of Prilep (Table 1, 2; Fig.3)

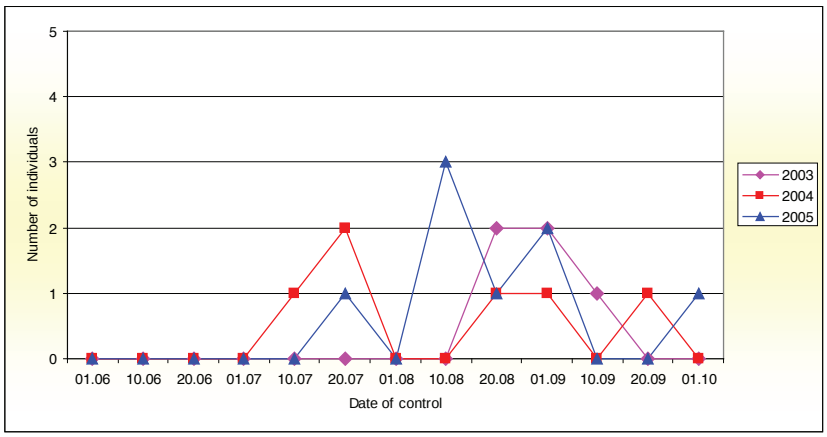


Fig. 3. Dynamics of population of *Pandylophtalmus tibialis*, 2003-2005

CONCLUSION

Species of the genus Paragus are the smallest of all hoverflies**.**

P. quadrifasciatus, P. tibialis and *P. testaceus* are obligate aphidophagous species, i.e. they normally develop only when larvae are fed on leaf aphids.

Since they xerophilic and termophilic species, the climatic factors have an important impact on their development.

P. quadrifasciatus is present in tobacco biocenosis from 1 July to 1 September, in tobacco fields hedges, near meadows, in vegetable gardens and in cereal crops.

P. testaceus was recorded on tobacco in the middle of July, along tobacco plots and vegetable crops.

P. tibialis can be observed in tobacco biocenosis from 10 June to the end of September. This species is spread throughout the whole region of Prilep, in biotops where various crops are grown, in weeds, meadows, cereal fields, vegetable crops, etc.

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BIOLOGICAL CHARACTERISTICS OF *ALTERNARIA ALTERNATA* IN TOBACCO

Biljana Gveroska[1], Petar Jovancev[2]

[1] University"St.Kliment Ohridski"-Bitola, Scientific Tobacco Institute-Prilep
Kicevski pat bb, 7500 Prilep; e-mail: gveroska@t-home.mk
[2]Faculty of Agricultural sciences and food - Skopje

ABSTRACT

Brown spot disease causes severe economic losses on tobacco in R. Macedonia, mainly because of the impairment of quality of tobacco raw. Investigations must be made on pathogenic fungus *Alternaria alternata*, the causing agent of this disease, in order to find solutions to the problems.

Due to the wide range of host-plants and the important role of ecological factors on occurrence and distribution of this disease, our aim was to study their influence on biology of the pathogen.

Estimations were made on nutrient medium, temperature and light effects on percentage of germinated conidia and on the way of conidia germination. It can be stated that germination rate was higher in media with better supply of nutrient matters. The highest rate of conidia germination was noticed in tobacco juice and the highest speed of germination in tomato juice.

Temperature increase had a positive effect on conidia germination and the optimal temperature was 28^0C. We also found that germination was higher in dark conditions than in light.

We believe that these investigations will contribute to understand the pathology of brown spot disease and to take preventive measures for its control.

Key words: *Alternaria alternata*, tobacco

БИОЛОШКИ ОСОБИНИ НА *ALTERNARIA ALTERNATA* КАЈ ТУТУНОТ

Болеста кафена дамкавост на тутунот во Р. Македонија предизвикува штетни економски ефекти главно поради нарушување на квалитетот на тутунската суровина. Поради тоа, за решавање на проблемите поврзани со оваа болест во наши услови, неопходно е проучување на предизвикувачот - патогената габа *A. alternata.*

Поради широкиот круг на домаќини и големата улога на еколошките фактори врз појавата и ширењето на болеста, со овие истражувања сакавме да испитаме какво е влијанието на истите врз биологијата на овој патоген.

Проучувано е влијанието на хранливата средина, температурата и светлината врз процентот на из'ртени конидии и начинот на 'ртење на конидиите. Притоа беше констатирано дека тие 'ртат подобро во средините побогати со хранливи материи. Конидиите из'ртеле во најголем процент во сок од тутун, а најбрзо во сок од домат.

Зголемувањето на температурата позитивно влијае врз 'ртењето на конидиите, а оптималната температура изнесува 28^{o}C. Исто така, конидиите ртат подобро во темни услови, отколку на светлина.

Бидејќи проучувањето на одредена болест се заснова врз првичниот пристап кон патогенот и, поконкретно, неговите биолошки особини, сметаме дека овие истражувања се од особен интерес за проучување на патологијата на болеста кафена дамкавост и дека ќе придонесат за преземање на превентивни мерки за заштита од неа.

Клучни зборови: *Alternaria alternata*, тутун

INTRODUCTION

The occurrence of plant diseases is greatly affected not only by he specific plant-pathogen relations, but also by ecological factors. They have a direct impact on biological properties of the pathogen, making possibilities for development of further stages in disease pathogenesis.

Sometimes even the microclimate conditions can enable the occurrence of disease and its further development.

Environmental factors are so influential that some investigations point out to differences of their effect in certain stages of pathogen's biology.

In the case of brown spot disease on tobacco, conidia germination of its causing agent - the pathogenic fungus *A. alternata* highly depends on environmental factors, i.e. nutrition media, temperature and light.

Conidia germination occurs in media rich with nutrient elements, especially with sugars (Stavely and Main, 1970). They stimulate conidia germination and spread of infections (Pearson and Hall, 1975).

According to Rotem (1994), the rich inoculum is of particular importance for necrotrophic parasites as *Alternaria sp*.

Waggoner and Parlange (1975), Rotem (1994) and Jovancev (1997) determined the optimal temperature for conidia germination and its effect on germination duration. According to Norse (1973, cit. by Rotem 1994), conidia germination and growth of germ tubes are differently affected by temperature, especially in suboptimal conditions. Germination occurs in considerably wider temperature range than germ tubes growth.

The effect of light was investigated from the aspect of activity spectra, especially the ultraviolet and infrared light. It was reported that the lifespan of conidia is 6 to 30 times shorter when exposed to direct sunlight than in laboratory conditions. The long wave UV radiation is the principal factor for spores mortality (Rotem et al., 1985).

Simmons (1992) reported that exposure of cultures to UV light induces changes in pigmentation and structure of conidia walls, which makes them different from those grown in natural conditions.

Leach (1975) reported that a number of fungi, including *A. tenuis*, intensively release spores when relative humidity is reduced from saturation state in the presence of IR light.

Rotem (1994) explains the effect of light and activity spectra on genus *Alternaria*.

The aim of our investigations was to study biological characteristics of pathogenic fungus *Alternaria alternata* in various environmental conditions. Results obtained in our investigations should contribute to tobacco protection from brown spot disease.

MATERIAL AND METHODS

Conidia germination was investigated by application of hanging drop method in Van Tieghem cell.

Percentage of germinated conidia was estimated by the method of Ko et al. (1973).

Germ tube length was determined with an ocular micrometer. The effect of liquid medium on germination and germ tube length was investigated on conidia from naturally infested material, with application of tap water, distilled water, solution of 1% glucose, tobacco juice and ripe tomato juice. The conidia suspensions

prepared in adequate media were incubated in a thermostat at 28^{0}C.

Monitoring of germination was made 30-40 min after the start of incubation up to 6 hours, at 1-hour intervals. Experiments for each liquid medium were made in three replications, monitoring 5 preparations for each interval.

Measurements of germ tube length started two hours after incubation. At least 200 samples of each liquid medium, randomly chosen in the preparations, were measured. Mean value of the measurements was used for size determination.

Investigations on the effects of temperature and light were made in conidia from pure fungus culture, obtained from isolates of several tobacco varieties and localities.

To study the temperature effect on germination, conidia suspensions in distilled water were placed in Van Tieghem cells and incubated at 5, 10, 15, 20, 25, 28, 30 and 32^0 C. The experiment was made in three replications, monitoring 5 microscopic preparations for each temperature. Percentage of germinated conidia was determined 4-5 hours after the start of incubation.

The effect of light on conidia germination was investigated in laboratory conditions, in two variants: in light and in dark. Van Tieghem cells with prepared suspensions in distilled water were placed in light and in dark place. The experiment was replicated three times, with 3 microscopic preparations for each time interval and each variant. Control of conidia germination was made at 3-40 minutes up to 5 hours.

RESULTS AND DISCUSSION

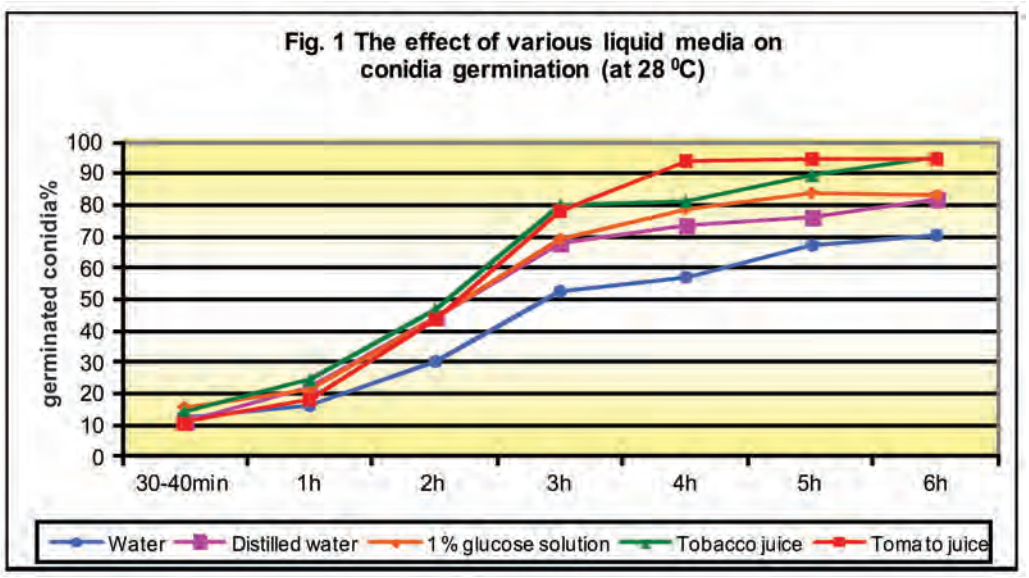
The highest rate of conidia germinate in the interval of 3-4 hours, with over 50% of germinated conidia in all investigated media (Table 1).

The highest percentage of germinated conidia in the initial intervals was recorded in the solution of glucose and tobacco juice. But in the interval of 5 hours, this percentage was the highest in tomato juice, where maximum germination of 94.47% was achieved.

In the last interval (6 hours after incubation), germination percentage ranged from 70.09% in water to 95.06% in tobacco juice. Accordingly, best results of germination are achieved in tobacco and tomato juices. Although in tomato juice germination is not markedly expressed in the initial interval, the maximum value of germinated conidia in this medium is achieved two hours earlier than in tobacco juice.

Table 1. The effect of various liquid media on conidia germination (at 28^{0}C)

Liquid media	Germinated conidia %						
	Incubation period						
	30-40 ‘	1h	2h	3h	4h	5h	6h
Water	12,50	16,29	30,30	52,51	57,01	67,41	70,09
Distilled water	11,09	21,56	44,00	67,55	73,40	76,02	81,76
1% glucose solution	15,80	21,18	44,05	69,20	78,71	83,82	83.04
Tobacco juice	14,28	24,52	46,59	79,67	81,06	89,28	**95,06**
Tomato juice	10,71	17,91	43,44	78,00	**93,66**	94,47	**94,52**



The lowest rate of germinated conidia from the start to the end of incubation was recorded in tap water (Table 1, Fig. 1).

Accordingly, higher rate of germination was recorded on nutrient-rich media (tomato and tobacco juice, glucose solution). Jovancev (1997) also reported highest germination of conidia in the juice of ripe or green tomato.

The above results were comparable to those of Stavely and Main (1970), who reported that addition of sugars increases conidia germination. According to Pearson and Hall (1975), glucose and fructose solution in exudates of ripe tomatoes stimulate the conidia germination and thereby favor the spread of infections. Rotem (1994) found that in necrotrophic parasites like *Alternaria sp*. the addition of sugar strengthens the inoculum and accelerates its penetration in physiologically weakened tissue.

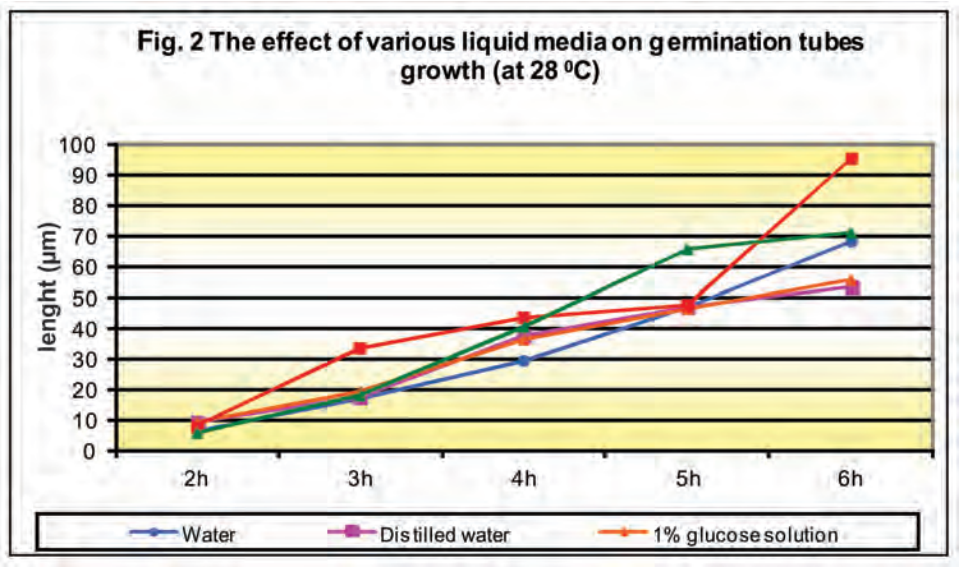
After conidia germination, germ tubes continue to grow (Photo 1, 2). In the starting interval (2 hours), their length ranges from 5.91 µm in tomato juice to 9.46 i.e. 9.23 µm in 1% solution of glucose and distilled water, respectively. The highest length is achieved in tomato juice in the 5-hours interval, when in all other media they have almost equal lengths (Table 2).

At the end of investigation, the length of germ tubes was 53.33 µm in distilled water, 71.02 µm in tomato juice and 95.06 µm in tobacco juice.

Table 2. The effect of various liquid media on germ tubes growth (at 28^{0}C)

Liquid media	Germ tubes length (µm)				
	Incubation period				
	2h	3h	4h	5h	6h
Water	6,29	16,92	29,52	47,08	68,25
Distilled water	9,23	17,46	37,77	47,02	53,33
1% glucose solution	9,46	19,38	36,24	46,59	55,67
Tobacco juice	7,80	33,52	43,33	47,50	**95,06**
Tomato juice	5,91	18,14	40,43	66,60	71,02

According to Table 2 and Graph 2, the length of germ tubes in the investigated time intervals was increasing and the highest rates were achieved in the 5- and 6-hour intervals in tomato and tobacco juices respectively.



From the aspect of nutrient media, the highest length of germ-tubes was recorded in tobacco juice and in distilled water.

The results of investigation reveal that nutrient rich media enable faster conidia germination and germ tubes growth.

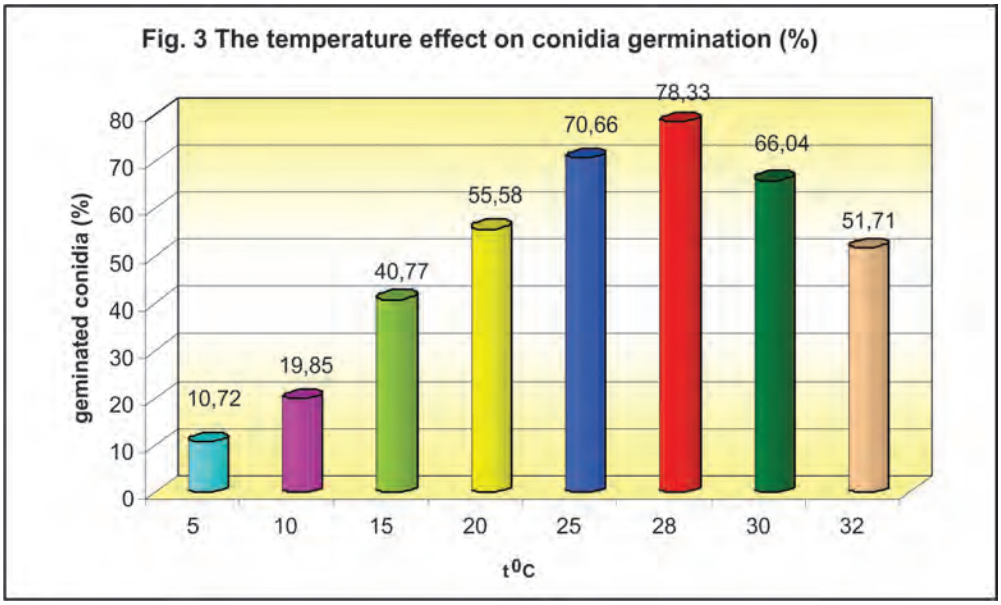
Photo 1. *A. alternata* – conidia

Photo 2. *A. alternata* - Germ tubes growth

At 5^{0}C, the range of germinated conidia is from 8.88% to 12.08% in the isolates MB 179 and IP 163 respectively. At higher temperatures, however, the percentage of germinated conidia increases. Thus, at 15^{0}C, the percentage of germinated conidia is almost doubled, raising from 26.74% to56.35%, and also at 20^{0}C (from 42.75% to 63.44%). (Table 3)

Table 3. The temperature effect on conidia germination (%)

Isolate \ t ºC	5	10	15	20	25	28	30	32
MB 179	8,88	21,89	41,24	55,42	72,73	75,75	64,10	48,81
MB 157	10,52	13,98	56,35	60,73	74,83	75,88	59,35	47,20
KP 166	11,39	19,11	26,74	42,75	62,89	82,33	68,44	51,54
IP 163	12,08	24,45	38,74	63,44	72,18	79,35	72,26	59,28
Average	10,72	19,85	40,77	55,58	70,66	78,33	66,04	51,71



The highest percentages of germinated conidia among the isolates were obtained at 28^{0}C, and the differences between them were smaller than at other temperatures investigated (ranging from 75.75% for the lowest and 82.33% for the highest). (Table 3).

The average results for all investigated isolates reveal that the lowest percentage of germinated conidia was obtained at 5^{0}C and the highest at 28^{0}C, which indicates that the optimal temperature for germination is 28^{0}C Table 3, Fig. 3).

Other research workers also reported 25^{0}C - 30^{0}C as optimal temperature range for conidia germination, which is in accordance with our results.

Rotem (1994), investigating several species of *Alternaria* found that the optimal germination temperature was 25^{0}C, and the maximum about35^{0}C. For *A. alternata* the minimum temperature is below 5^{0}C, the optimum is 22.5 - 29^{0}C and the maximum 35-40^{0}C. Investigations of Jovancev (1997) also confirmed that the highest conidia germination is achieved at 20 - 28^{0}C.

With temperature increase, conidia germination in all isolates decreases. Percentage of germinated conidia at 32^{0}C is almost one-third lower compared to those at 28^{0}C (Table 3). The above results are confirmed by Waggoner and Parlange (1974), who investigated the dependence of germination and temperatures. They reported that germination increases linearly at temperatures up to 30^{0}C, while at temperatures above 30-40 ^{0}C the germination decreases. They also found that higher temperatures lead to increased time of germination.

The results on conidia germination in investigated isolates show that at almost all temperatures the highest percentage of germinated conidia was achieved in the isolate IP 163. In isolates where lowest percentage of germinated conidia was obtained at lower temperatures (MB 157 and MB 179), the values were significantly higher at optimal temperatures. When temperature increased above the optimum, however, conidia germination was lower comoared to the other isolates.

Accordingly, the isolate IP 163 shows better adaptability to temperature changes, while the other isolates required higher and optimal temperatures for conidia germination.

Germination of *A. alternata* conidia is highly affected by light (Table 4).

In the starting intervals of incubation, the rate of conidia germinated in light was only 5.26 - 13.63%. An hour later it increased from 8.10% in IP 163 isolate to 24.61% in MB 179. At the end of incubation, the percentage of germinated conidia ranged from 58.46% to 85.71%.

In dark conditions, all isolates even in the starting intervals showed higher conidia germination than in light, ranging from 54.41% to 88.33% (Table 4).

Table 4. The light effect on conidia germination (%)

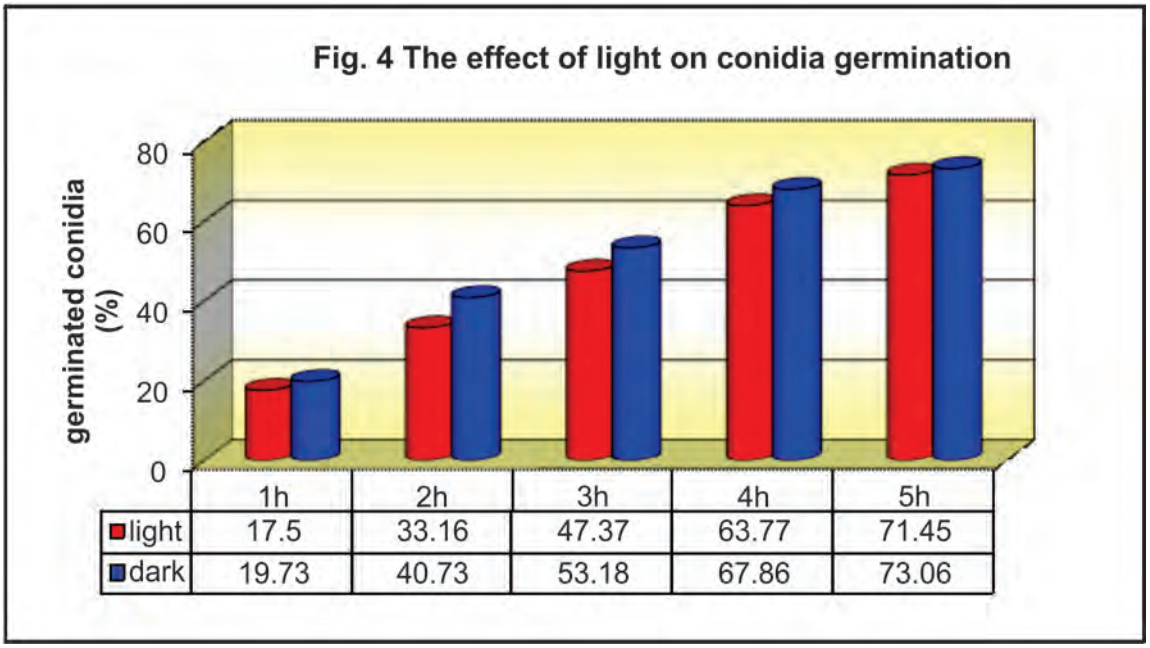
Isolate	LIGHT						DARK					
	incubation period						incubation period					
	30 ’	1 h	2 h	3 h	4 h	5 h	30 ’	1 h	2 h	3 h	4 h	5 h
MB 157	9,47	19,78	42,40	70,86	80,85	85,71	18,86	22,87	54,31	76,65	82,38	88,33
MB 179	13,63	24,61	27,07	34,96	64,63	70,17	10,71	27,58	35,78	44,14	71,87	76,43
IP 163	5,26	8,10	30,00	36,19	45,83	58,46	6,66	8,75	32,11	38,76	49,32	54,41
Average	9,45	17,50	33,16	47,37	63,77	71,45	12,08	19,73	40,73	53,18	67,86	73,06

Significant increase of germinated conidia in light conditions was noticed 3 hours after the start of incubation in MB 157 and 4 hours after in IP 163 and MB 179 isolates.

In dark, the highest percentage of germinated conidia in MB 157 was noticed after 3 hours (just like in light), indicating that this isolate has better conidia germination compared to all others. In dark conditions, too, MB 179 the percentage of germinated conidia increased after 4 hours, while in IP 163 the increase was gradual.

It can be stated that environmental factors have a great impact on biological properties of the pathogen, but they also depend on the isolate itself.

Lacey (1992) recommended alteration of light and dark periods. However, investigations of the three isolates reveal higher average rate of germinated conidia in dark compared to light conditions. This difference was more noticeable up to 3 hours after the start of incubation, and then it became smaller. At the end of incubation, the rate of germinated conidia was 73.06% in dark and 71.45% in light (Table 4, Fig.4).



CONCLUSIONS

- Various liquid media are suitable for conidia germination.
- The highest percentage of conidia (more than 50%) is germinating in the interval of 3-4 hours.
- Conidia germination percentage is the highest in tobacco and tomato juices. The maximum percentage of germinated conidia in tomato juice is achieved two hours earlier than in tobacco juice.
- The highest length of germination tubes is achieved also in the above media (tobacco and tomato).
- The temperature affects the conidia germination. The highest percentage of germinated conidia was observed at 28^{0}C, which is denoted as an optimal temperature for this process.

The percentage of germinated conidia is higher in dark conditions than in light.

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Original scientific paper

IMPORTANT FEATURES OF TOBACCO PRODUCTION IN THE REPUBLIC OF MACEDONIA

Mile Pesevski, Blaze Filiposki, Dragic Zivkovic, Snezana Stojanoska

This scientific paper is a part of the master thesis presented by Blaze Filiposki on 28th May 2010 at the Scientific Tobacco Institute, Prilep, R. Macedonia

Mile Pesevski, Ph.D., Full Professor, Faculty of Agricultural Science and Food, Skoje, R. Macedonia, blvd. Aleksandar Makedonski bb, 1000 Skopje, e-mail: milepesevski@yahoo.com
Blaze Filiposki, MA, Scientific Tobacco Institute, Prilep, R. Macedonia
Dragic Zivkovic, Ph.D., Full Professor, Faculty of Agriculture, Belgrade, R. Serbia
Snezana Stojanoska, Ph.D., Associate Professor, Scientific Tobacco Institute, Prilep, R. Macedonia.

ABSTRACT

According to the statistical data, the authors determined that Macedonian tobacco was grown on area of 18,500 ha between 2001-2010, which is 3 % of the arable land or 75% of the industrial crops.

The average yield of dry tobacco in the investigated period ranged between 1.000 and 1,500 kg/ha. This is an opportunity for about 17 to 30 thousand tons of Macedonian tobacco to be found on the market of oriental tobacco. Almost 92 % of them is exported, while the rest is used in the country. However, Macedonia is also importer of tobacco, mainly broadleaf tobacco.

The analysis of export, import and purchase prices showed that the price of tobacco export and tobacco products has the greatest stability. The price was increasing with the same intensity from year to year, following the growth of the U.S. dollar. In contrast, the greatest fluctuation was noticed in purchasing price of tobacco .

Key words: tobacco, production, export, import, price

ПОВАЖНИ ОБЕЛЕЖЈА НА ТУТУНОПРОИЗВОДСТВОТО ВО РЕПУБЛИКА МАКЕДОНИЈА[1)]

Врз основа на статистички податоци за периодот 2001-2010 година, авторите утврдиле дека тутунот во Република Македонија се одгледува на површина од 18,500 ха, што е 3% од обработлива површина или 75% од индустриските култури.

Просечните приноси на сув тутун, по години од истражуваниот период, се движат помеѓу 1,000 и 1,500 kg/ha. Тоа овозможува на пазарот на ориенталски тутуни да се стават на располагање помеѓу 17 и 30 илјади тони македонски тутун. Од тоа, речиси 92% се извезува, а остатокот се користи во земјата. Но, Македонија и увезува тутун, главно крупнолисни тутуни.

Анализата на цените (откупни, извозни и увозни) покажала дека најголема стабилност има кај цената при извозот на тутун и преработки од тутун. Имено, таа расте од година во година со ист интензитет, при што, речиси, го следи порастот на курсот на американскиот долар. Наспроти тоа, пак, најголема флуктуација имаат цените при откупот на тутунот.

Клучни зборови: тутун, производство, извоз, увоз, цена.

INTRODUCTION

The oriental tobacco was brought to Macedonia (Vardar region) by the Turkish settlers in 1574. The first warehouse for tobacco buyout and export preparation was built in 1771 in Kavala by a French company (Lape Lj., 1974). The area and production of tobacco historically have large variations. For instance; within the past hundreds of years, the smallest area was recorded in 1995 (only 10,891 ha), while the greatest drop in production took place in 1939 (only 1,840 tons). In contrast, the largest amount of area was recorded in 1966 (34,342 ha), and the greatest production in 1986 when it was produced 35,020 tons of dry tobacco (Filiposki K., Snezana Stojanoska, 2000). In recent times, tobacco covers approximately 3.5% of the arable land and around 75% of the industrial crops. About 5.6% of the total number of families grows tobacco in the country (Pesevski M., et al., 2010). The extent of involved area and tobacco production is in correlation (with rare exceptions) with the number of households engaged in primary production of oriental tobacco. For example; according to the data by the Ministry of Agriculture, Forestry and Water Economy, during

2000, 33,906 households were cultivating oriental tobacco. Each household had averagely 0. 67 ha and produced 654kg dry tobacco. After ten years (2008), the number of households dropped at 29,771 or it was decreased by 12.2%. The area per household also went down at 0.57ha, which is 15% less. At the same time, the production fell down at 574kg dry tobacco or decreased by 12.2%. The Government of the Republic of Macedonia has been subsidizing the production since 2001 in order to encourage the production, increase market competitiveness and the budget of family holdings. For the same purpose, in 2006, value added tax was reduced from 19 to 5% for certain inputs in agricultural production (Pesevski M., and so on., 2008). However, the production of tobacco in Macedonia is focused on two statistical regions. Particularly, the region of Pelagonia covers approximately 50%, while the Southeast 37% of the total area under tobacco in Macedonia (Pesevski M., et al., 2010). These studies showed that the best economic effects (highest gross margin) had the producers of Polog and the Southeast region, and the worst in the Southeast, Southwest and Vardar region. Due to the natural conditions and tradition of tobacco production, Macedonia plays an important role (not only in exporting sitnolisen, but also importing broadleaf tobacco) on World's market of oriental tobacco. The Republic of Macedonia exports aromatic tobacco to around thirty countries over the world. 79.6% of the tobacco is exported to EU countries (Greece 39.2%, Belgium 18.3%, Bulgaria 10.6%, etc.). In the total exports of oriental tobacco dominates the type Prilep (62.5%), followed by Yaka (25.5%) and Basmak (10.4%) (Pesevski M., et al., 2010).

The results of many investigations in the world and in our country confirm that tobacco plant has other forms of use other than smoking. For example, it can be used in food industry because of its content (over 10%) of citric acid (Srbinoska A. Marija, 2005). The pectines, carotinoides, enzyme pectinase, solanesol (Coenzyme Q10) etc. are separated from tobacco (Tso T. C., 1977). It is interesting to be mentioned that beside lignin, tobacco contains relatively high quantity of cellulose. According to Veselinov (1964), the highest cellulose content is found in tobacco stems (35-40% of dry matter). Uzunoski (1985) reported that the main rib of the leaf of some Bulgarian tobacco varieties contains 10-15% of cellulose. Tobacco stems and middle leaf ribs can be used for production of paper and cardboard packaging (Agrupis S. C., et al., 1977; Hepworth D. G., et al., 1998). Dry tobacco stems are ligneous and middle leaf ribs are big and have a pulp structure. The cell walls of tobacco stem xylem contain 40-45% lignin, which is in accordance with the quantity of lignin in the trees, with one difference - the density of tobacco stem is lower (Hepworth D. G., et al., 1998). Today, tobacco stems and ribs are used as media for mushroom production. Tobacco residues (small parts, tobacco dust, ribs etc.) are used in production of reconstituted tobacco (Nuneski I., 2000), and because of the relatively high content of K and N they can be used as substitute for mineral fertilizers (Tso T.C., 1977). Tobacco seed contains high percentage of oil (30-40%), which can be used in nutrition, in production of biodiesel fuel (Filiposki K., et al., 2008), as well as in manufacture of dyes and varnishes because of its content

of linoleum oils (Srbinoska A. M., 2005). The seed cake obtained after extraction of oil from tobacco seed can be used as additional animal feed because of its high protein content (Filiposki K., et al., 2008). Because of high content of lignin (40-45%) and cellulose (28-40%), tobacco stems can be used for production of energetic briquettes where with tobacco obtains new use value (Peševski M., et al., 2010).

The main goal of our research is to give a full description of some important features in tobacco production in the Republic of Macedonia.

MATERIAL AND METHODS

The statistical yearbooks of the State Statistical Office of the Republic of Macedonia from 2002 to 2011 were used as a basic material for our research. From this source, we used the data for areas, average yields, total production, and purchasing prices of tobacco. For export and import analysis as well as for export and import prices of tobacco, there were used data provided by the statistical review for international trade.

The research is mainly based on secondary date, using a method of indices, comparative method, inductive-deductive methods and other methods applied in agro-economy.

RESULTS AND DISCUSSION

- **Areas under tobacco**

In the Republic of Macedonia, the areas under tobacco cover between 17,064 ha (2008) and 20,538 ha (2002), which is averagely 18,502 ha. Analysis of data for "planted and harvested" area (Table 1) shows that there was no great difference, i.e. the tobacco production was quite successful. Analysis of the areas within ten-year period of research (2001-2010) indicated that there was instability. The reasons for this situation were of different character: market, technological, social, demographic, political and suchlike reasons. Our opinion is that the last above-mentioned reason had the greatest impact on. When the Government of the Republic of Macedonia subsidized the tobacco production through the Ministry of Agriculture, Forestry and Water Management with 15.00 MKD/kg in 2001 (Table 1), the Macedonian tobacco producers increased the amount of area by 1.1%. However, when they realized that the government ceased to support it in 2002, in 2003, the tobacco producers reduced the amount of area by 2,437 ha, or by 11.9%. The reduction of area continued even in 2004. The same year, the Government through the Program for Stimulating Agriculture announced that the tobacco production would be subsidized 15.00 MKD/kg in 2005, and consequently, the farmers increased the amount of area by 4, 4%.

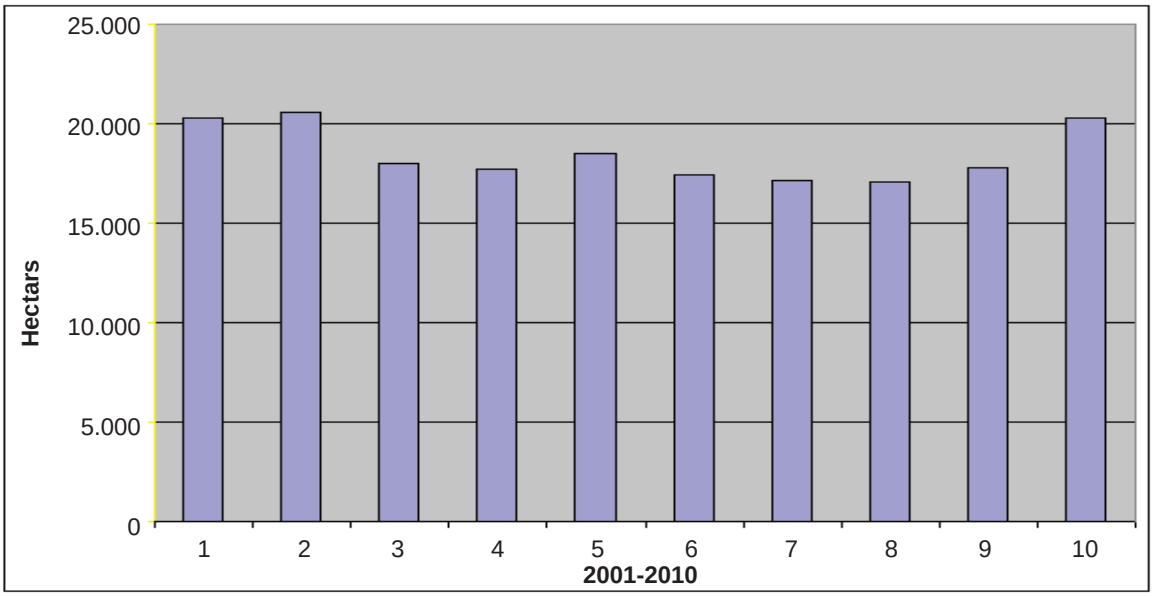
Furthermore, although the subsidization continued, the areas were on decrease until 2008. It appeared as a consequence of reduced purchasing prices for tobacco. In the next two years, the increase of area continued due to the increase of financial support from 45.00 to 60.00 MKD/kg or above 33.3%.

Table 1 – Dynamics of the area under tobacco and subsidies

Year	Hectares		Subsidies (MKD/kg)
	Planted area	Harvested area	
2001	20,310	20,274	15.00
2002	20,538	20,538	/
2003	18,101	18,008	/
2004	17,717	17,716	/
2005	18,490	18,488	15.00
2006	17,507	17,438	15.00
2007	17,183	17,132	30.00
2008	17,064	17,064	45.00
2009	17,809	17,800	60.00
2010	20,300	20,300	60.00
Average	18,502	18,476	/

Source: Statistical Yearbook of the R. Macedonia, 2002 – 2011, and date MAFWE

The general impression is that the decrease is continuous and changes are minimal.



Picture 1 – Harvested area of tobacco

- **Production of tobacco**

It is known that the total tobacco production is a function of the amount of area and gained average yields. In the study period, the total production of tobacco ranged from 17,081,064 kg (2008) to 20,287,600 kg (2010) or, with difference of 13, 2 thousand tons of dry tobacco (Table 2). In 2008, it was measured the lowest average yield per unit area of 1,001kg/ ha due to the poor climatic conditions. In contrast to this, highest average yields were achieved at the time when the tobacco production was subsidized. It is worth mentioning that the subsidization was given per 1kg of production, regardless of its quality. In this case, producers increased the degree of intensification in order to achieve higher yields.

Table 2 – Dynamics of total production and yields

Year	Total production (t)	Yield (kg/ha)
2001	23,226	1,157
2002	22,920	1,116
2003	23,987	1,332
2004	21,631	1,221
2005	23,695	1,498
2006	25,041	1,436
2007	22,049	1,287
2008	17,081	1,001
2009	24,122	1,355
2010	30,288	1,492
Average	23,404	1,290

Source: Statistical Yearbook of the R. Macedonia, 2002 - 2011

Comparison of data shows that the total production is not in parallel with the gained yields (Figure 2). In general, the total production depends mostly on the "harvested" area, whose average yields are important as well. In most cases, these parameters are closely connected.

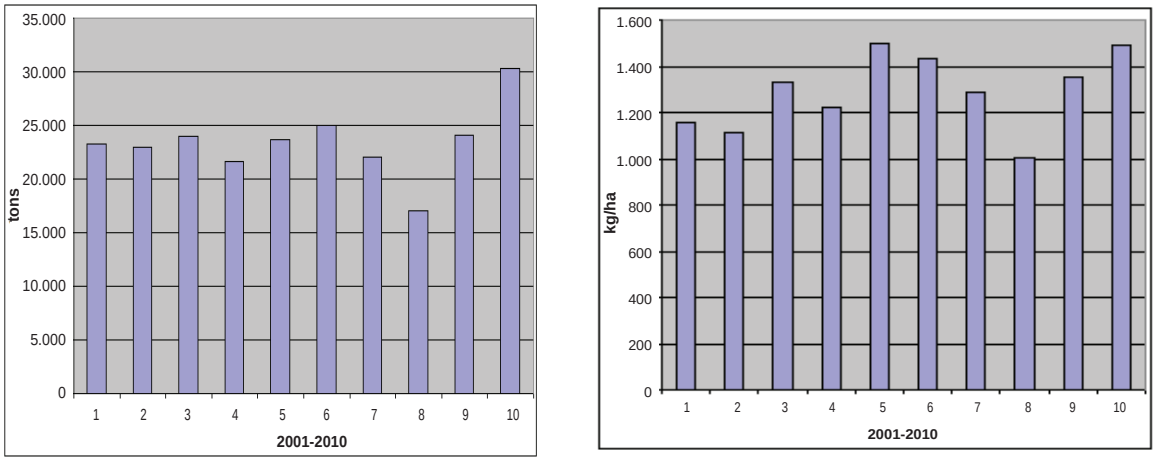


Figure 2 – Dynamics of tobacco production and its yields

- **Value of production**

The total value of tobacco depends on the purchasing prices which are dictated by internal and mostly external (international) market conditions. The quality of tobacco and the business culture of the purchasing companies in Macedonia have a great impact. However, Macedonian tobacco producers have an average annual production of tobacco with value of around 52 million EUR (Table 3).

Table 3 – Value of produced tobacco

Year	Indicators			
	Tobacco production (t)	Purchase price (MKD/kg)	Total value (000) MKD	EUR
2001	23,226	115.22	2,676,056	44,023
2002	22,920	123.18	2,823,336	46,314
2003	23,987	81.31	1,950,355	31,935
2004	21,631	123.60	2,673,621	43,620
2005	23,695	138.67	3,285,789	53,610
2006	25,041	125.36	3,139,136	51,312
2007	22,049	115.52	2,547,087	41,637
2008	17,081	150.78	2,575,481	42,107
2009	24,122	186.94	4,509,367	73,598
2010	30,288	179.43	5,434,504	88,351
Average	23,404	134.00	3,161,473	51,651

Source: Statistical Yearbook of the R. Macedonia, 2002 - 2011

The graph shows that there was a high fluctuation in the value of tobacco from year to year during the research period.

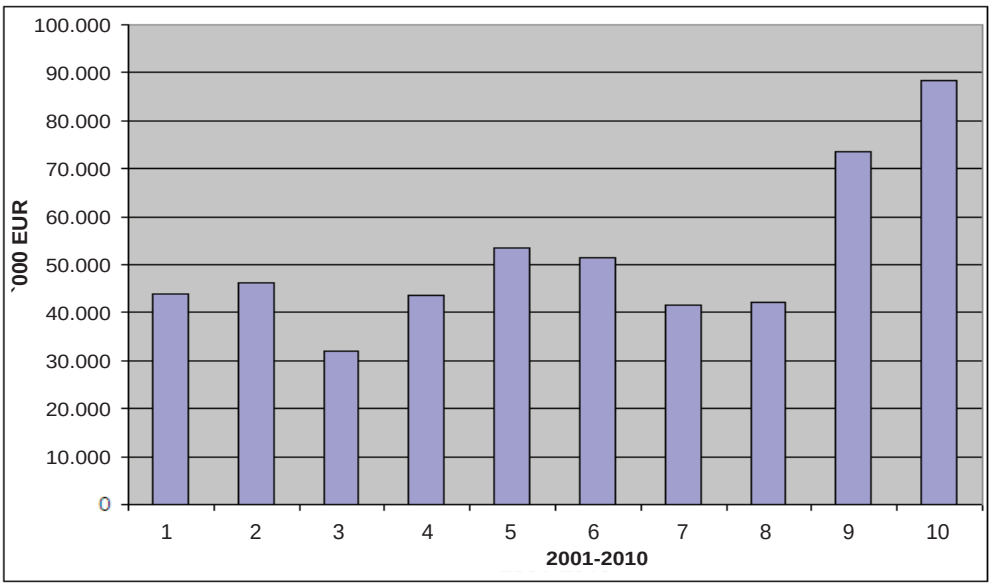


Figure 3 – Dynamics of produced tobacco value

- **Tobacco export**

Macedonia is a traditional exporter of tobacco and tobacco products. In the period of our research (2001-2010), the quantity of exported tobacco ranged between 15.4 and 32 thousand tons (Table 4). It consisted of Macedonian tobacco, some imported tobacco and tobacco products which were part of the Macedonian products (mainly cigarettes) and exported thereafter. The comparative analyses of the previous presented tables show that the quantity of exports does not overlap with the amount of produced tobacco in the Macedonian fields.

The asymmetry of the data is due to loss of section (up to 15%) of the organic matter and weight in the process of fermentation and processing of tobacco. Since tobacco is not exported in the same year of production, companies leave an amount of it for "better times", and keep another amount for commodity stocks.

Table 4 – Quantity of exported tobacco

Year										
2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Average
Quantity (t)										
15,399	17,260	22,455	16,353	22,764	32,031	26,241	23,372	18,748	20,688	21,531

Source: Statistical Review: Foreign Trade-2001-2010

During the research period (2001-2010), the export of tobacco and tobacco products reached its peak in 2006 when 32 thousand tons of tobacco was exported (Figure 4). In the structure of the total export, not stemmed tobacco was the most dominant (about 87%) (Filiposki, B., 2010)

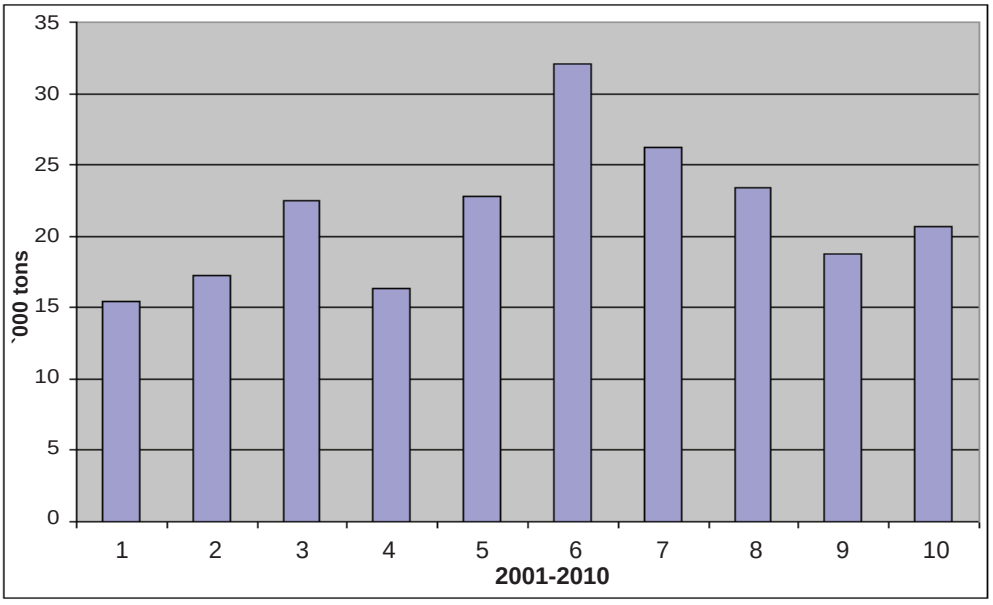


Figure 4 – Dynamics of the amount of exported tobacco

- **Tobacco imports**

Although The Republic of Macedonia has a relatively large amount of tobacco in warehouses, the country also imports tobacco and tobacco products. This tobacco is imported by two cigarette factories. In the structure of imports, Virginia and semi-oriental tobacco are the most dominant. In Macedonia, such raw material has not been produced for a long time due to various reasons.

In the structure of imports, the most dominant are wholly or partly stemmed tobacco (almost 60%) (Filiposki, B., 2010). It is followed by tobacco waste (above 20%), imports of cigarettes containing tobacco and so on. However, the amount and structure of imports is dictated by the needs of cigarette factories. In the 10 year researched period, the average annual amount was 3,825 tons of tobacco and tobacco products (Table 5), which was nearly 18% of the total exports.

Table 5 – Quantity of imported tobacco

Year										
2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Average
Quantity (t)										
2,660	2,785	2,774	4,457	4,490	3,673	3,556	4,860	3,652	5,341	3,825

Source: Statistical Review: Foreign Trade-2001-2010

The data analysis shows that the quantity of exports had relatively large variations from year to year during the research period (Figure 5).

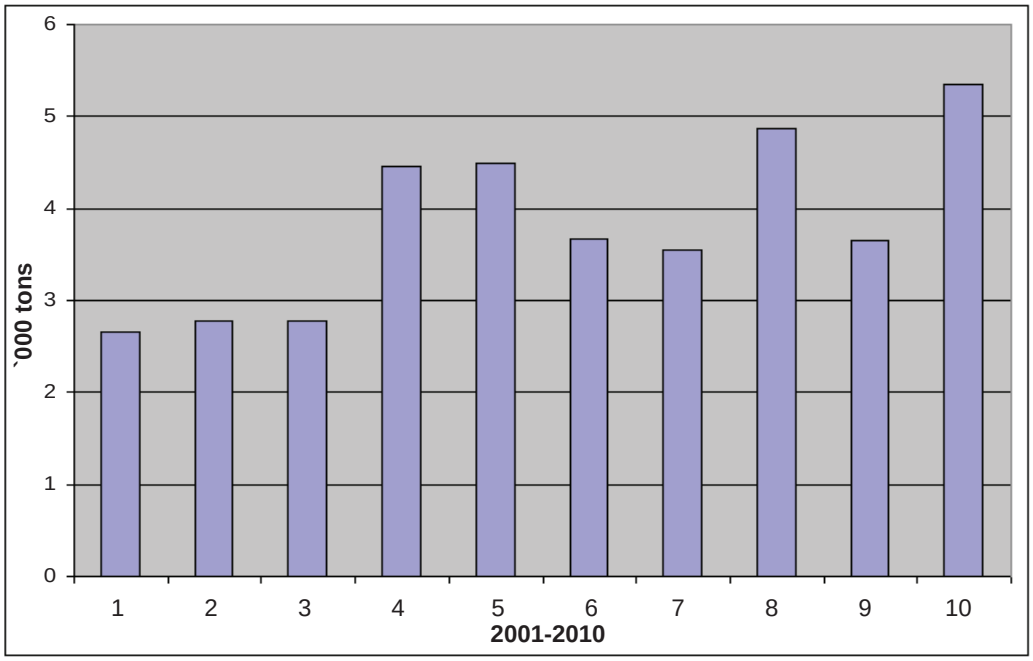


Figure 5 – Dynamics of the quantity of imported tobacco

- **Tobacco prices**

It is known that the value of tobacco depends on the purchase price to a great extent. On the other hand, the purchase price depends on the quality of tobacco and asked quantities of raw tobacco in the domestic market. During the research period, the average purchase price was 129.45 MKD/kg. At the time of raw tobacco buying out, the bottom price was (81, 32 MKD/kg) in 2003, while the highest price was (189, 94 MKD/kg) in 2009 (Table 6). According to the presented data, a dramatic fall in purchase prices was recorded in 2003, which was decreased by 34 % compared to the previous year. This was mainly due to poor quality of tobacco in the previous year of production (2002). The poor quality was a result of rainfall exceeding during the period of tobacco drying. Consequently, it led tobacco to be classified in lower classes (IIIa, IIIB and IV) which have lower purchase prices.

Table. 6 – Dynamics of tobacco prices

Indicators	Year										Average
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	
	Price (MKD/kg)										
Purchase	115.22	123.18	81.32	123.60	138.62	125.36	115.52	150.78	186.94	134.00	129.45
Exports	205.78	231.16	181.70	181.99	201.40	182.84	189.50	265.72	258.46	277.90	217.65
Import	194.02	191.26	194.51	144.62	155.54	145.77	165.07	153.52	217.63	214.17	177.61

Sources: Statistical Yearbook of the R. Macedonia, 2002 - 2011, and Statistical Review: Foreign Trade, 2001-2010

The analysis of the purchase prices shows that they are almost in parallel with export prices. Such parallelism is due to the fact that not only the companies buy tobacco, but they also export it. The price of imported tobacco rages between the prices of buying out and exports.

CONCLUSIONS

We can draw several conclusions based on the analysis of some key elements which characterized the tobacco production in Macedonia.

- Areas under tobacco are steadily decreasing, and thus, appropriate measures should be undertaken for stability to be brought.
- In R. Macedonia, there are real possibilities for sustainability in the total tobacco production from 23,000 to 26,000 tons.
- The better quality of tobacco will contribute to higher export price of tobacco and greater competitiveness on the world market.
- The analysis of tobacco export quantity determines a cyclical character which is due to the cyclical demand for tobacco in the world market.

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A NEW AND MORE PRODUCTIVE VARIETY OF PRILEP TOBACCO

Miroslav Dimitrieski, Gordana Miceska

University"St.Kliment Ohridski"-Bitola, Scientific Tobacco Institute-Prilep, Republic of Macedonia, e-mail: dimitrieskimiroslav@yahoo.com; miceskag@mail.net.mk

ABSTRACT

The need to create and introduce new and more productive oriental varieties of tobacco with better quality than the existing ones permanently increases.

As a result of scientific and research work on this problem during the last two decades, the Department of genetics and breeding in Tobacco Institute-Prilep created a great number new lines of Prilep tobacco with significantly better quality compared to the standard. Some of them were recognized as varieties, among which Prilep 66-9/7 should be especially emphasized for its productivity.

Key words: tobacco, variety, lines, P 66-9/7

НОВА И ПОПРОДУКТИВНА СОРТА ОД ТУТУНОТ ТИП ПРИЛЕП

Потребата од создавање и воведување на нови попродуктивни ориенталски сорти тутун, со подобри квалитетни својства во споредба со постојните стандардни сорти, перманентно расте, поради што и истражувањата од ваков вид имаат континуиран карактер. Како резултат на повеќегодишната научноистражувачка работа насочена во овој правец, во одделението за Генетика и селекција при Научниот институт за тутун- Прилеп, во последниве две децении се создадени голем број на линии тутун од типот прилеп, со мошне поквалитетни својства во однос на стандардот. Некои од нив се признати и како сорти, од кои по својата продуктивност во последниве неколку години посебно се истакнува новосоздадената сорта Прилеп 66-9/7.

Клучни зборови: тутун, тип, линија , сорта П 66-9/7

INTRODUCTION

Along with commercialized aromatic tobaccos, quantitative increase of high-yielding varieties has been observed in the primary production of oriental tobacco of the type Prilep in R. Macedonia. Such heterogeneous assortment, however, dramatically affects the quality of Prilep tobacco and threatens to destroy its high reputation on the foreign market. These alarming predictions can be avoided only by creation and introduction of new varieties with higher productivity and better quality. With their biological potential and quality characteristics, these varieties should correspond not only to the interests of the primary production but also to the requirements of the market and manufacturers (2,3,8). Therefore, in breeding of new Prilep varieties, our aim was to obtain optimum yields and to improve the quality of tobacco raw. One of the most perspective new varieties created in Tobacco Institute was Prilep 66-9/7. It was officially recognized in 2004 and in 2010 crop it is expected to account for 70-80% of the primary production of the type Prilep.

It has higher productivity than the existing commercialized varieties and is suitable for obtaining an authentic, standard and high-quality tobacco raw.

The aim of the paper is to present the most important characteristics of this newly created aromatic variety of the type Prilep.

MATERIAL AND METHODS

Investigations related with creation of the new variety Prilep 66-9/7 were performed in the period 1992 - 2002. It was obtained by intervariety hybridization.

The breeding process started in 1992, by crossing the oriental line Pb 66-9, used as mother component, with oriental variety Prilep 7, used as father component. Selection of parents was based on previous investigations. Line Pb 66-9 has higher yield and more intensive aroma, and Prilep 7 is distinguished by its good quality and percentage of highest classes (I, II, IIIa), fine leaf tissue and strong aroma. In 1993 hybrid progeny from F1 generation was obtained (150 plants), and in 1994 several lines were selected from F2 progeny (400 plants). Up to 1999, selection and consolidation of selected lines from hybrid progenies were made according to their morpho-biological characters (leaf number, plant habitus and height, vegetation period, etc). Among all consolidated lines included in comparative investigations performed in 1998 and 1999, line P 66-9/7 was especially emphasized. In the following period (2000, 2001, 2002), investigations continued in the scope of micro-trials performed by the State Commission of the Ministry of Agriculture, Forestry and Water Economy of the Republic of Macedonia, and this line was recognized as variety under the same name (Prilep 66-9/7). Tobacco of the parental varieties, hybrid progenies and consolidated lines was transplanted at 45 cm spacing between rows and 15 cm within the row. Morphological measurements were made on a representative sample of 20 individuals from one plot. Traditional agrotechnical measures were applied during the period of vegetation. Chemical and tasting analyses were made by application of standard methods in the laboratories of Tobacco Institute-Prilep. Limit values for certain parameters of the new variety Prilep 66-9/7 were based on the results obtained. from several year-investigations carried out in the Experimental field of the Institute and in primary production.

RESULTS AND DISCUSSION

The Prilep 66-9/7 variety was included in the list of newly recognized domestic agricultural crops in 2004 (Official Gazette of R. Macedonia, 70/2004).

Plants are ellipsoid-conic in shape, with height average of 65 - 75 cm, depending on conditions of growing and applied cultural practices. The stalk is relatively thin and strong. Average leaf number of leaves is 52 and they are uniformly distributed on the stem. Average

size is in the limits of 18 - 22 cm for the largest leaf, 16-18 cm for the middle and 8 - 10 for the top leaves. Inflorescence is relatively small, moderately to tightly condensed, semi-oval in shape (Photo 1 and 2).

Prilep 66-9/7 is suitable for growing at loose, light and drained soils, with poor supply of nutrient elements, showing especially good results under irrigation conditions. The variety also achieves good yields and quality in soils with medium supply of nutrients, where no possibilities for irrigation exist, yielding small-leaf aromatic and substantial tobacco typical for the type Prilep. Intensive raining during the growing period can lead to some increase of lower middle leaves, but it doesn't affect the quality of tobacco. Fertilization is made with 250 - 330 kg/ha NPK (8 : 22 : 20), depending on soil and previous culture. Transplanting is made at 40 - 45cm between rows and 12 - 15 cm between plants (in the row). Optimum period for transplanting is 10 - 30 May.

Length of the growing season from planting to the beginning of flowering is 70 - 75 days. The growth in the first 12 to 15 days is slower, but in this period a well branched root system is developed, which makes the variety adaptable and persistent in dry conditions. Leaves mature consecutively and overmaturation is not observed. The first leaves ripe about 40 days after transplanting, and the total period to the end of maturation of top leaves is 115 - 120 days. Majority of leaves are ripening simultaneously. Tobacco is primed in 6 to 7 hands, picking 5-8 leaves together. The variety is resistant to blue mold and bassara disease, and tolerant to viruses. The Dry tobacco yield averages 2000 - 3600 kg/ha, depending on conditions of growing and applied cultural practices (1, 4). Pelivanoska (7) reported that dry tobacco yield of Prilep 66-9/7 varied from 1794 kg/ha in the check variant (unfertilized, unirrigated) to 3988 kg/ha in variant fertilized and irrigated with $N_{40}P_{80}K_{100}$ + 55% FWC (field water capacity).

Prilep 66-9/7 belongs to the group of small-leaf aromatic tobaccos with uniform raw typical for the type Prilep. It has fine, soft and substantial dry leaf tissue, with yellow-orange color of the middle leaves and orange to light red of the upper. It is distinguished by high percentage of high-graded tobacco.

Chemical composition of this tobacco is variable and depends greatly on conditions of growing and applied agrotechnical measures (5). The average values of major chemical compounds range within the following limits: nicotine 1.0 % (irrigated) - 2.30 % (non-irrigated), proteins 5 % - 8%, soluble sugars 18.50% - 29.00% and Shmuk's quality index 2.5 - 5.0.

During smoking, this tobacco is medium in strength, with full and sweetish taste and strongly expressed, intensive aroma (6).

Photo 1. - P 66-9/7

Photo 2 - Seed plot of P66-9/7

CONCLUSIONS

Based on the data obtained during the several-year selection work, the following statements can be drawn:

1. The creation of genotype Prilep 66-9/7 enriched the structure of tobacco type Prilep with a new oriental variety.
2. Prilep 66-9/7 achieves higher productivity than the existing commercialized varieties and it is suitable for obtaining a standard, authentic and high-quality raw of tobacco type Prilep, which was the aim of our investigation
3. Due of its adaptibility to various conditions of growing, the new variety has raised a great interest in primary production. The obtained tobacco raw satisfies the requirements of the market, which is confirmed by the fact that it accounts for 80% of Prilep tobacco production in R. Macedonia for 2010, as well as by the great interest for introduction of this variety shown by the neighboring countries.

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SOME CHARACTERISTICS OF PELAGONEC - THE NEWLY CREATED VARIETY OF BURLEY TOBACCO

Ilija Risteski

University "St.Kliment Ohridski"-Bitola, Scientific Tobacco Institute-Prilep, Kicevska bb 7500 Prilep, Republic of Macedonia, e-mail: ilija.r@t-home.mk

ABSTRACT

Burley variety Pelagonec CMS F1 was approved by the State Commission of the Ministry of Agriculture in 2010. Its stable and high yields and typical Burley characteristics make this variety attractive both for the farmers and for manufacturers. In the present conditions, it can play a role of initial variety for restarting the production of Burley tobacco in R. Macedonia, but it can be also interesting for our neighboring countries and wider.

Key words: burley tobacco, production of burley

НЕКОИ КАРАКТЕРИСТИКИ НА НОВОСОЗДАДЕНАТА БЕРЛЕЈСКА СОРТА ПЕЛАГОНЕЦ

Берлејската сорта Пелагонец ЦМС F_1 е призната од страна на Државната сортна комисија во 2010 год. Стабилните и добри приноси, проследени со типични берлејски својства на суровината, ја прават оваа сорта интересна како за производителите така и за фабрикантите. Во услови на мирување на производството на типот берлеј во Р. Македонија, таа може да одигра улога на иницијална сорта со која би се рестартирало производството, со можности да биде интересна за нашето опкружување и пошироко.

Клучни зборови: тутун берлеј, производство на берлеј

INTRODUCTION

As a result of the intensive selection activity, a great number of varieties from the types Prilep, Yaka, Djebel, Otlia, Virginia and Burley have been created in Tobacco Institute-Prilep. Up to this moment four Burley varieties in CMS form (B-96/85, Burley 1, B-2/93 and Pelagonec) and one in fertile form (Burley Pel BB295) have been created and recognized. Each of them has its own specifications and corresponds to the taste and requirements of manufacturers and consumers in the time of its creation.

Male-sterile variety Pelagonec was created by intervariety hybridization after two-year investigations and in 2010 it was approved by the State Commission of the Ministry of Agriculture and registered in the List of Macedonian newly created agricultural plants (Official Gazette of R. Macedonia, July 16, 2010).

MATERIAL AND METHODS

In preparation of this paper we used materials obtained from comparative and productional trials, application forms and Decision for approval of the variety. Physical and chemical analysis were made in the accredited laboratories of Tobacco Institute - Prilep and tasting properties were determined by the Tasting Panel of Tobacco Combine - Prilep.

RESULTS AND DISCUSSION

Results and discussions will be presented through some productional-morphological, bio-technological, chemical and tasting characteristics of the variety.

Seedling production- Having in mind the climate conditions of this area, primarily the temperature, the best period for sowing this variety is between 10 and 15 March. Depending on seed quality (total germination and energy), the rate of sowing should be 2-3 g/10 m^2, if seedlings are produced under polyethylene. When seedbeds are not covered with polyethylene, sowing should start about 25 March, but the amount of seed should be increased for about 50%. In the case of application of pelleted seed and float system, the amount of seed needed for transplanting is dramatically reduced. Depending on temperatures in the period of seedling production and applied cultural practices (nutrition, weeding, irrigation, protection), seedlings covered with polyethylene are ready for transplanting in 45-65 days. In uncovered seedbeds, this period is prolonged for 10-15 days.

Time of transplanting and spacing - In our agro-ecological conditions, transplanting should start about 10-15 May and should be finished in the shortest possible time, in order to avoid differences in stalk size during tobacco growth and development in field, which could later cause problems in harvest (uneven ripening of leaves from the same belt).

So far, in our comparative investigations, transplanting was made with 90 x 50 cm spacing, i.e. each stalk obtained 0.45 m^2 nutrient area. In this way, about 22 000 stalks were needed for 1 ha. Having in mind the genetic potential of the variety, however, and the increased yield due to higher leaf size and number, the recommended spacing can be even higher, in order to achieve easier soil treatment between rows, better protection and harvest and to increase the competition among stalks in utilization of nutrients, water and light.

Morphological properties - The hybrid male-sterile variety Pelagonec has a conical habitus and well developed root system. Depending on soil fertility and applied cultural practices (fertilization, number of irrigations etc.), stalk height achieves 180 - 220 cm and more. The number of leaves averages about 34. Depending on their position on the stalk, leaves can be 48 - 75 cm long and 30 - 40 cm wide. In the stage of technical maturity, leaf color is green-yellowish and the midrib and nerves are whitish. The stem is considerably firm and resists to stronger winds, due to its thickness of 5 cm at

the soil level and 4 cm in the middle. Depending on its age, the color of the stem varies from yellow-greenish to whitish.

The flower is 4-5 cm long, without anthers, with pale pink lobes and it does not yield seed. Flowering stage begins in about 65 and ends up in 85 days after transplanting.

Biological properties - For successful production of the Pelagonec variety, the following requirements should be met:

Soils should be fertile, deep, well aerated, fertilized with NPK in amounts determined according to the previous analyses of agrochemical composition of the soil. Additional nutrition with KAN is well accepted by this variety.

Photo.1 - Pelagonec CMS F1

Photo 2 - Leaves from the middle belt

Photo 3 - Flowers of Pelagonec CMS F1

Temperature of 24-27^{0}C, followed by a higher relative air humidity (over 70%), has a favorable effect on tobacco growth in field. In dry periods, especially when yield and quality of tobacco are formed (July and August), additional and abundant irrigations are needed, prefferably after each priming.

This variety shows certain tolerance to some economically important diseases (PTA, TMV, PVY etc.).

Harvest and curing - Harvest can be performed by insertions (leaf by leaf) and whole-plant-picking (reaping of the stalks) and the period from transplanting to the end of priming of top leaves is 115-120 days (moderately long growth period)

In both ways of harvest, curing is performed in curing barns specially designed for

Burley tobacco (air-curing), and the technology of curing is as usual for all Burley varieties.

Yield and quality - In tobacco producing region of Prilep, the Pelagonec variety yields 3800 kg to 4350 kg. In a more favorable regions where suitable cultural practices are applied, the yields will be certainly higher. Of the total yield, the percentage of high classes is over 70% (45% - I grade, 20% - II grade, 8% - III grade).

Technological properties - In conditions of proper harvest and curing, the color of dry leaves in lower primings is lighter, and in all other primings it is brown. In average, the main nerve content in the middle belt leaf is 29% to 31.3 %. Lamina thickness in middle belts is about 83 μm, and substantiality about 36 g/m^2.

Photo 4-Pelagonec CMS F, lower primings

Photo 5 - Pelagonec CMS F1, middle primings

Photo 6 - Pelagonec CMS F1, upper primings

Chemical properties - Depending on soil and climate conditions during the growth period, cultural practices applied and the methods of harvest and curing, chemical composition of the middle belt leaves in this variety is as follows: nicotine 2.30% and over, soluble sugars below 1%, proteins 7-8%, Total N 3.0-3.40% and ashes about 18%.

Tasting properties- According to the Report of the Tasting Panel of AD Tutunski Kombinat - Prilep dated 4.06.2008, all characteristics of the variety Pelagonec produced in Tobacco Institute-Prilep in 2007 are typical for Burley tobacco.

CONCLUSIONS

- According to its productional-morphological, bio-technological, chemical and tasting characteristics, variety Pelagonec CMS F1 offers a good guaranty for successful restart of Burley tobacco production in the Republic of Macedonia.

- This variety can be also attractive and interesting for tobacco producers from our neighboring countries and wider.

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WEEDS AS HOST PLANTS TO TOBACCO DISEASES AND PESTS

Vera Dimeska, Spiridon Stojkov, Vesna Krsteska, Biljana Gveroska

University"St.Kliment Ohridski"-Bitola, Scientific tobacco institute-7500 Prilep Republic of Macedonia, e-mail: veramilka@yahoo.co.uk, vkrsteska@yahoo.com

ABSTRACT

Weeds compete with tobacco for water, soil space, light and nutrient elements. They serve as hosts to pathogenic agents which contribute to development of various diseases. They also harbor a great number of harmful insects - vectors that transmit diseases from weeds to tobacco. For this reason, implementation of effective and timely measures in the control of weeds is of crucial importance for obtaining a healthy and high yield raw material.

Key words: Weeds, tobacco, Myzus persicae Sulz., Portulaca oleracea L., Chenopodium album L, Potato virus Y

ПЛЕВЕЛИТЕ КАКО ДОМАЌИНИ НА БОЛЕСТИ И ШТЕТНИЦИ КАЈ ТУТУНОТ

Плевелите се конкуренти на тутунот за вода, животен простор, светлина и хранливи елементи. Тие служат и како домаќини на патогените агенси и придонесуваат за развојот на разни болести. Исто така, тие претставуваат засолниште на голем број штетни инсекти - вектори кои ги пренесуваат болестите од плевелите на тутунот. Поради тоа, воведувањето на ефикасни и навремени мерки за контрола на плевелните растенија е од суштинско значење за добивање на здрава и квалитетна тутунска суровина.

Клучни зборови: плевели, тутун, лисни вошки, тучница, лобода, компиров Y вирус

INTRODUCTION

Weeds can cause economically important loss of tobacco, because they compete with this crop for living space and nutrients. They usually have a strongly developed root system and their water intake is 2 to 3 times greater than that of their tobacco host. They are also more capable of using the available mineral matters and organic fertilizers than the tobacco (1,3,4,7).

Weeds themselves appear as hosts to various pathogens - disease causing agents (6,8). They might also harbor a number of harmful insects which often move to tobacco fields, causing significant damage on tobacco leaf and transmitting dangerous viruses from diseased to healthy plants (2, 5).

MATERIAL AND METHODS

Investigations of the most frequently found weeds on tobacco fields were made in 2008 and 2009. Tobacco of the type Prilep (P 23) was transplanted on 10.6.2008 and 11.6. 2009.

The results about diseases and pests were recorded over a period of several years. The intensity of disease attacks was estimated by the methods of CORESTA.

RESULTS AND DISCUSION

The most frequently represented weeds in the investigation period were Amaranthus retroflexus L (redroot peegweed), Chenopodium album L (lamb's quarters), Digitaria sanguinalis L (Scop.) (hairy crabgrass), Solanum nigrum (black nightshade), Hyosciamus niger L (black henbane) and Portulaca oleracea L (little hogweed).

Amaranthus retroflexus L (redroot peegweed), family Amaranthaceae, genus Amaranthus. Annual terrophytic plant with woody stem, which can grow up to 1 m in height. The leaves are broad, ovate, with pointed tips. The inflorescence is dense cluster consisting of thick uninterrupted ears and the fruit is an ellipsoid capsule. Redroot pigweed can reproduce by seed (Photo 1).

Chenopodium album L (Lamb's quarters), family Chenopodiaceae, genus Chenopodium. Annual plant that grows in late spring and summer, distinguished by its cosmopolitanism and great potential of adaptability. The stem is erected and usually grows up to 1m in height. The leaves are elongated-ovoid, spirally arranged on the stem. The lower leaves are often toothed, not much longer than wide. The flowers are clustered in a brush-shaped inflorescence. The fruit has a shape of radiant black soybean. It reproduces by seed (Photo 2).

Photo 1. Amaranthus retroflexus L.

Photo 2. Chenopodium album L.

Digitaria sanguinalis L (Scop.) (hairy crabgrass), family Poaceae, genus Digitaria. Annual weed that grows in late spring. It has many stems, creeping or erected, high about 30 cm and over. The leaves are long, linear to lanceolate, covered with short hairs. The

inflorescence is ear-like brush consisted of 4-10 fingers, about 12 cm long. The seed is poorly transparent, ovoid and elongated. The weed reproduces by seed (Photo 3).

Photo 3. Digitaria sanguinalis L. (Scop.)

Solanum nigrum (black nightshade), family Solanaceae, genus Solanum.Annual, herbaceous weed that grows in late spring. The stem is erected and branched, 10-15 cm high. The leaves are with irregular shape, rhomboid or ovate, with long petioles. The flowers are usually white, forming a shield-like sparse inflorescence. The fruit is black berry, and the seed is flat, kidney-like, with uneven surface. They reproduce by seed (Photo 4).

Photo 4. Solanum nigrum L.

Hyosciamus niger L (black henbane), family Solanaceae, genus Hyosciamus. Annual plant, grows in late spring. The stalk is erected, up to 1 m high. The leaves are broad and irregularly toothed. The flowers are large, formed in the axilla of the upper leaves. They are yellowish, with pink nervature. The fruit is a many seed capsule, and the seed is irregular and ovate. Reproduction is made by seed (Photo 5).

Photo 5. Hyosciamus niger L.

Portulaca oleracea L (little hogweed), family Portulaceae, genus Portulaca. Annual succulent weed, occurring in late spring, prostrate, smooth and branched from the bottom. The stem is 10-30 cm in length and often forms adventive roots. The leaves are shoveled to ovate, succulent and sessile. The flowers are small, yellow, single or 2-3 together, formed in the leaf axilla or at the top of the stem. It reproduces from the seed, which is kidney-like, tiny, flat and dark-brown (Photo 6).

Photo 6. Portulaca oleracea L.

At present, tobacco weed is controlled by application of selective non-phytotoxic herbicides.

Due to their exceptional viability, many weeds start to develop in spring and later, prior to tobacco transplanting. They are hosts to various harmful insects, like green tobacco aphid (Muzus persicae Sulz) and tobacco flea beetle (Epithrix hirtipennis Melsh).

Green tobacco aphid has a worldwide distribution. It appears as a pest on tobacco every year. The main host to this aphid is peach, but it also appears on tobacco, potato, tomato, peppers etc. It has a high reproductive ability and has several generations in one year. It damages tobacco directly, by sucking the sap from young leaves, and indirectly - by contamination of leaves with "honeydew" and with shed skins of numerous generations (Photo 10).

This aphid is economically most dangerous as a vector of **PVY** (**Potato virus Y**) - the causing agent of tobacco vein necrosis. The aphid is hosted by vegetable crops of Solanaceae family and by almost all cultivated varieties of tobacco. The PVY symptoms vary depending on the host plant and virus race. Tobacco varieties which are attacked by the less aggressive races usually develop a mild mosaic on the leaves, without any deformations. In the beginning, discoloration among veins of leaf lamina is noticed, with dark green zones remaining on each side of the vein. 2-3 weeks later the appearance of mosaic and deformation of leaves is noted. The aggressive race of the virus shows strong symptoms on the veins, which obtain a dark brown or black color. Sometimes the infection spreads towards the conduction system of the stem. Leaves become yellow before maturation, necrosis spreads along the stem and the plant dies (Photo 9).

Another economically important pest is **tobacco flea beetle** (Photo 11). It is a small insect which imagos hibernate in the trash around plant beds or in weed plants. The beetle attacks both seedlings and transplanted tobacco in a form of larva, imago and virus vector. It makes small rounded holes on tobacco leaves which give them a sieve-like appearance. When the attack is stronger, i.e. when pest population is higher, the rounded holes on seedlings and transplanted tobacco plants merge and only the veins remain on the leaf.

Leaves affected by the above mentioned pests or by other mechanical damages can be easily infected by tobacco mosaic virus and brown spot disease.

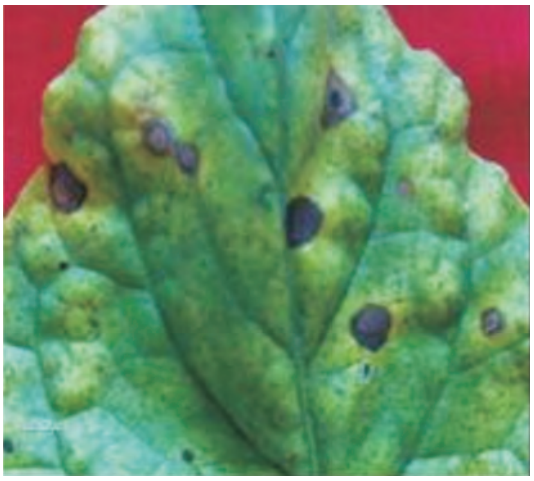

Photo 7. Alternaria sp.

Photo 8. TMV

Tobacco mosaic virus (TMV) is one of the oldest viral diseases on tobacco. It can be hosted by all tobacco types, vegetable plants of the Solanaceae family (tomato, pepper, potato), woody plants (pear, apricot, plum), the weeds of the Convolvulaceae, Chenopodiaceae, Labiateae and Asteraceae families, etc. The mosaic symptoms can be stronger or weaker, depending on the tobacco growth stage, weather conditions and aggressiveness of TMV race. Characteristic symptom of virosis is the occurrence of mosaic pattern with lighter and darker zones on the leaf,

Photo 9. PVY

by which the disease was named. The virus attacks almost all parts of tobacco plant. The infected plants have stunted growth and deformations and they lose their quality characteristics (Photo 8).

Brown spot is mycosis caused by the fungus Alternaria sp. (Photo 7). Its host range includes oriental, semi-oriental and large-leaf tobaccos.

Symptoms appear in a form of small circular spots colored from light green to yellow. With spreading of disease, the spots increase and occupy a larger part of leaf area. As spots enlarge, their central parts become necrotic and turn brown in color. In dry conditions, sharp lines divide the infected from the healthy tissue. In lower insertions the brown spots develop concentric circles. The disease can attack aphids, stems, flowers and seed capsules. In cases when topping is performed, the disease also attacks the late-appearing suckers. As disease spreads, the infected leaves age prematurely, lose their quality and die.

Protection of tobacco involves application of systemic insecticides in control of viral vectors and systemic fungicides in control of the brown spot disease.

Photo 10. Myzus persicae Sulz.

Photo 11. Epitrix hirtipennis Melsh.

CONCLUSION

Weeds compete with tobacco for water, soil space, light and nutrient elements. They serve as hosts to pathogenic agents which contribute to development of various diseases. They also harbor a great number of harmful insects - vectors that transmit diseases from weeds to tobacco. For this reason, implementation of effective and timely measures in the control of weeds is of crucial importance for obtaining a healthy and high yield raw material.

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