UDC 633.71 ISSN 0494-3244
Тутун/Tobacco, Vol.60, N° 7-12, 71-78, 2010
University "St. Kliment Ohridski" - Bitola UDC: 633.71-152.61(497.775)"2008/09"
Scientific Tobacco Institute – Prilep, R. Macedonia Original scientific paper

MORPHOLOGICAL PROPERTIES OF SOME DOMESTIC AND INTRODUCED BURLEY TOBACCO VARIETIES (LINES) IN AGROECOLOGICAL CONDITIONS OF PRILEP

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ABSTRACT

Two-year investigations (2008-2009) were carried out in the region of Prilep with six domestic and introduced varieties (lines) of Burley tobacco. Investigations were prompted by the needs of Macedonian fabrication for this type of raw, which has been fully supplied from imports. Although the region of Prilep is considered less suitable for production of Burley, results have shown that domestic varieties Pelagonec CMS F1 and B-98/N CMS F8 can be grown with great success. According to the number and size of the 5th, 10th and 15th leaf, these varieties can achieve high yields and quality typical for Burley tobacco.

Key words: burley tobacco, production of burley

МОРФОЛОШКИ СВОЈСТВА НА НЕКОИ ДОМАШНИ И СТРАНСКИ СОРТИ (ЛИНИИ) ТУТУН ОД ТИПОТ БЕРЛЕЈ ОДГЛЕДУВАНИ ВО АГРОЕКОЛОШКИТЕ УСЛОВИ ВО РЕОНОТ НА ПРИЛЕП

Истражувањата направени во 2008 и 2009 во агроеколошките услови на Прилеп со 6 домашни и странски сорти (линии) тутун од типот берлеј беа поттикнати од потребите на македонската фабрикација за суровина од овој тип, која во моментов целосно се увезува. Овие истражувања покажаа дека и во реонот на Прилеп, кој не важи за реон погоден за производство на типот берлеј, домашните сорти Пелагонец ЦМС F_1 и Б-98/N ЦМС F_8, можат да се одгледуваат со успех. Резултатите од бројот и димензиите на анализираните листови (5 ^{от}, 10 ^{от} и 15 ^{от}) упатуваат на тоа дека од овие сорти можат да се очекуваат добри приноси и квалитет карактеристични за овој тип тутун.

Клучни зборови: тутун берлеј, производство на берлеј

INTRODUCTION

Morphological properties are important category for determination of tobacco types and varieties. To a lesser or greater extent, these properties vary from type to type and they are genetically controlled. Unsuitable agroecological conditions and cultural practices, however, can lead to big variations of both bio-morphological and chemical composition of the obtained raw. Such variations appear despite the existence of genetic control and they are indication of tobacco plasticity. Therefore, it is very important to have a knowledge on the basic prerequisites for stable tobacco production, with minimum variations of its morphological and production characteristics.

Up to 2002, Burley tobacco in R. Macedonia was mainly located in the southeast region of the country. It was grown in small, almost symbolic quantities (only 156 tons in 1993) and after 2002 Macedonian fabrication became completely dependent on Burley tobacco imports. In the period that followed, several Burley varieties were created in Tobacco Institute - Prilep, with yields and quality which guarantee a stable and profitable production. Creation of new varieties is a long-term determination of the Institute and it makes continuous efforts in this field. Two-year field experiments have to be conducted before applying to the National Commission for approval of the new varieties.

MATERIAL AND METHODS

Investigation included six varieties (lines) of domestic and foreign origin: check variety B-21 CMS F_8 (US), was compared to fertile varieties BB-16 (France), B-1317 (Bulgaria), B-136/07 and varieties Pelagonec CMS F1 and B-98/N CMS F8 (Tobacco Institute-Prilep, Macedonia). Two-years investigation (2008 - 2009) was conducted on colluvial soil in the field of the Institute. First autumn plowing was made at 40 cm depth and fertilization was made in spring, with 300 kg/ha NPK 8 : 22 : 20 and two additional plowings. Herbicide was applied prior to transplanting. Healthy seedlings were planted manually, in 4 replications, with random distribution of varieties at 90 x 50 cm spacing. Due to the poorer nitrogen content, 26% KAN (5 g/stalk) was added at the second hoeing of the soil. The number and amount of additional irrigations (3-5, with 30 - 40 l/m^2) depended on weather conditions. Average stalk height and leaf number were obtained from 5 stalks of each variety, on the lower and middle belt leaves (5th, 10th and 15th). Tobacco was regularly treated with products for protection throughout the whole period of growth Meteorological data were obtained from the Internal Meteorological Station of the Institute.

RESULTS AND DISCUSSION

Soil and weather conditions

Depending on conditions and countries in which they worked, some authors (Gornik 1953; Haslam, Skott 1963, Georgievski 1971, Benkovic 1981, Pamukov 1992, Apostolova 1996 and Pelivanoska 1999) recommend growing of Burley tobacco in soils rich in humus (2 - 5%) and microelements, with low pH, well aerated, and water-permeable.

CLIMATE CONDITIONS

Temperature, precipitations and relative air humidity have a strong impact both on tobacco plant growth and development and on its morphological, technological and chemical properties. Data on the climate conditions during the growing season (May-September) in the two years of investigation are presented in Table 1.

Table 1 Meteorological data in the period May-September 2008/2009, in the field of Tobacco Institute-Prilep

Meteorological data	Year	Months					X /∑
		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
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

Air temperature - is a very important factor influencing a region's climate. It varies according to the season. Tobacco plant originates from regions with tropic climate and therefore, from the sowing to the end of seed ripening, it requires higher temperatures compared to other crops.

Hawks and Collins (1994), Rubin (1971), Filiposki (2002) and Drazic (1980) reported that optimum temperature range in all stages of tobacco development is 20 ^{0} - 30 ^{0}C. According to the data presented in Table 1, mean monthly air temperatures were lowest in May (16 ^{0}C in 2008 and 15.8 ^{0}C in 2009). Despite deviation from the optimum, above temperatures had no negative effect on growth, because in that period tobacco was in the stage of rooting. Mean monthly temperatures in June were closer to the optimum (19.9^{0}C in 2008 and 18.5^{0}C in 2009), while in July and August they achieved the optimum (21.4 ^{0}C in August 2009 and 23.6 ^{0}C in August 2008). Air temperatures in September, although lower than the optimum (15.8 ^{0}C in 2008 and 17.1 ^{0}C in 2009), had no significant impact on the final results of investigation, because in that period over 80% of leaf mass was formed and harvested. According to the analysis, lower average air temperature from May to September was recorded in 2009 (18.9 ^{0}C) and higher in 2008 (19.7 ^{0}C).

Relative air humidity - is a variable category closely related to precipitations, number and quantity of additional irrigations, air temperature, etc. For good growth of Burley tobacco, higher relative humidity is required.

In the two years of investigations, the highest values of this parameter were recorded in May 2009 (58%) and September 2008 (71%). In June, July and August, when leaves are formed on the stalk, the relative air humidity varies from 42% in July 2009 to 57% in June 2009. The annual values for the period May-September averaged 52% in 2009 to 57% in 2008. However, in the environment where these stalks were growing, the achieved values were higher, in accordance with the requirements for optimum growth of Burly tobacco.

Precipitations - beside temperature, Burley tobacco requires suitable humidity, which in combination with mineral fertilization and photosynthesis would enable maximum size, yield and quality of leaf. Uzunoski (1985) suggests this type to be grown in regions where precipitation amounts between 300 and 650 mm. Donev et al. (1973) reports that additional and heavy irrigations are necessary in all areas where precipitation during the growing season exceeds 260 mm. Pelivanoska (1999) reported that additional irrigations of Burley tobacco in the region of Prilep were necessary because of the low precipitation levels in the vegetation period (112.8 mm in 1997 and 191.1 mm in 1966). According to the presented data, 2009 was characterized by higher sum of precipitations (196.0 mm) compared to 2008 crop and by better distribution of rainfalls (55 mm in May, 75 mm in June, 43 mm in August). Therefore, tobacco was well supplied with water during the warmest days. In July and early September, however, it was necessary to apply additional irrigation of tobacco. In 2008 crop, the sum of precipitations was 183.3 mm, but rains mainly fell when tobacco requirements for water were smaller (55.0 mm in May and 110.0 mm in September). In June, July and August precipitations dropped to only 10.0 mm and in that period tobacco was irrigated more abundantly.

MORPHOLOGICAL PROPERTIES

Morphological properties of tobacco are genetically determined, but they are also affected by agroecological conditions and applied agrotechniques. Our investigations included different varieties of Burley tobacco. Measurements were made on leaf size (5th, 10th and 15th leaf), stalk height and leaf number.

Characteristics of the 5th leaf

This leaf is positioned in the lower leaf belt, but according to its size it can have significant impact on tobacco yield.

Table 2 Characteristics of the 5th leaf

Variety	Crop	length, cm	2008/2009 Average	Difference from the average		5th leaf width, cm	2008/2009 Average	Difference from the average	
				Absolute	Relative			Absolute	Relative
B-21 CMS F_8	2008 / 2009	40,4 / 41,6	42,0	-	100,00	25,6 / 28,7	27,1	-	100,00
Pelagonec CMS F_1	2008 / 2009	46,8 / 52,1	49,4	+7,4	117,62	31,0 / 36,6	33,8	+6,7	124,72
B-98/N CMS F_8	2008 / 2009	49,1 / 50,4	49,7	+7,7	118,33	34,6 / 35,8	35,2	+8,1	129,89
B-136/07	2008 / 2009	47,5 / 48,9	48,2	+6,2	114,76	33,6 / 32,6	33,1	+6,0	122,14
B-1317	2008 / 2009	39,6 / 38,2	38,9	-3,1	92,62	24,6 / 25,2	24,9	-2,2	91,88
BB-16	*2008* / 2009	*39,3* / 39,4	*39,3*	*-2,7*	*93,57*	*24,4* / 24,7	*24,4*	*-2,7*	*90,04*

According to the presented data, the largest length of the 5th leaf was obtained in B-98/N CMS F8 - 49.7 cm, which is 7.7 cm i.e. 18.33% more compared to the standard variety (42.0). The lowest leaf length of 38.9 cm was recorded in B-1317, which is 3.1 cm less than the standard. 5th leaf length in other varieties of the trial ranged from 39.3 cm in BB-16 to 49.4 cm in Pelagonec CMS F1.

The highest values for 5th leaf width were measured again in B-98/N CMS F8 -35,2 cm, which is 8.1 cm (29.89%) more than the standard (27.1 cm). The lowest width of 24.4 cm was measured in BB-16, which is 2.7 cm

(9.96%) less than the standard. In other varieties included in the trial, the width of the 5th leaf ranged from 24.9 cm in B-1317 to 33.8 cm in Pelagonec CMS F1.

Characteristics of the 10th leaf

10th leaf is positioned in the middle belt area, distinguished by the largest leaf size.

Table 3 Characteristics of the 10th leaf

Variety	Crop	length cm	Average 2008/2009	Difference from the average		5th leaf width cm	Average 2008/2009	Difference from the average	
				Absolute	Relative			Absolute	Relative
B-21 CMS F_8 Ø	2008 2009	51,5 51,9	51,7	-	100,00	35,8 26,9	31,3	-	100,00
Pelagonec CMS F_1	2008 2009	68,1 64,4	66,2	+14,5	128,04	38,9 37,4	38,1	+6,8	121,70
B-98/N CMS F_8	2008 2009	62,2 61,6	61,9	+10,2	119,72	41,2 33,6	37,4	+6,1	119,49
B-136/07	2008 2009	57,2 58,2	57,7	+6,0	111,60	35,7 33,7	34,7	+3,4	110,86
B-1317	2008 2009	50,0 51,8	50,9	-0,8	98,45	32,5 32,0	32,2	+0,9	102,87
BB-16	2008 2009	41,3 44,4	42,8	-8,9	82,78	23,8 23,0	23,4	-7,9	74,76

According to the Table, the longest 10th leaf was measured in the variety Pelagonec CMS F1 - 66.2 cm, which is 14.5 cm (28.04%) more than the standard variety (51.7 cm).

In variety VV-16 this leaf was shortest and achieved only 42.8 cm, which is 8.9 cm (17.22%) less compared to the standard variety. In other varieties in the trial, length of the 10th leaf ranged from 50.9 cm in B-1317 to 61.9 cm in B-98/N CMS F8.

The highest values for width of the 10th leaf were measured in Pelagonec CMS F1-38,1%, which is 6.8 cm (21.70%) more than the standard (31.3 cm). The lowest width of 23.4 cm was measured in BB-16, which is 7.9 cm (25.24%) less than the standard. In other varieties, width of the 10th leaf ranged from 32.2 cm in B-1317 to 37.4 cm in B-98/N CMS F8.

Characteristics of the 15th leaf

!5th leaf is also positioned in the middle belt area of the stalk and it is highly estimated in fabrication.

Table 4 Characteristics of the 15th leaf

Variety	Crop	length cm	2008/2009 Average	Difference from the average		5th leaf width cm	2008/2009 Average	Difference from the average	
				Absolute	Relative			Absolute	Relative
B-21 CMS F_8 Ø	2008 / 2009	54,7 / 48,0	51,3	-	100,00	30,1 / 22,8	26,4	-	100,00
Pelagonec CMS F_1	2008 / 2009	65,0 / 60,2	62,6	+11,3	122,02	31,6 / 29,6	30,6	+4,2	115,91
B-98/N CMS F_8	2008 / 2009	57,8 / 57,4	57,6	+6,3	112,29	32,0 / 31,0	31,0	+4,6	117,42
B-136/07	2008 / 2009	55,2 / 55,4	55,3	+4,0	107,80	32,3 / 28,8	30,5	+4,1	115,53
B-1317	2008 / 2009	53,1 / 52,9	53,0	+1,7	103,31	27,9 / 27,8	27,8	+1,4	105,3
BB-16	2008 / 2009	33,0 / 33,1	33,0	-18,3	64,33	16,0 / 18,5	17,2	-9,2	65,15

According to the data presented, the largest length of the 15th leaf was obtained in Pelagonec CMS F1- 62.6 cm. It is 11.3 cm i.e. 22.02% longer compared to the standard variety (51.3cm). The lowest leaf length of 33.0 cm was recorded in BB-16, which is 18.3 cm (35.67%) less than the standard. In other varieties of the trial, 15th leaf length ranged from 53.0 cm in B-1317 to 57.6 cm in B-98/N CMS F8. .

The largest width of 15th leaf was measured in B-98/N CMS F8 - 31.0 cm, which is 4.6 cm (17.42%) more than the standard (26.4 cm). The smallest width of 17.2 cm was measured in BB-16, which is 9.2 cm (34.84%) less than the standard. In other varieties of the trial, the width ranged from 27.8 cm in B-1317 to 30.6 cm in Pelagonec CMS F1.

Stalk height and leaf number

Stalk height is distinction of the type, but some other factors, like agroecological conditions and applied cultural practices, also influence this character. According to Dulgerski (2009), the optimum stalk height in large-leaf tobaccos, to which Burley tobacco also belongs, ranges from 145 to 180 cm, and leaf number from 26 to 32. Uzunoski, (1985) reports that leaf number and size has a direct influence on tobacco yield.

According to the data (Table 5), highest values for stalk height were recorded in the variety B-98/N CMS F8 - 186.3 cm, which is 39.1 cm (26.56%) more compared to the average stalk height of the standard variety (147.2 cm). In other varieties included in the trial, the average stalk height was higher and ranged between 148.0 cm in BB-16 to 185.5 cm in B 136/07. The highest leaf number was obtained in Pelagonec CMS F1, where 33.3 leaves per stalk were recorded, and it is for 4.7 leaves (16.43 %) higher than the standard variety. The lowest leaf number - 27.2 was recorded in B-136/2, which is for 1.4 leaves (4.90%) lower than the standard variety. In other varieties of the trial, the average leaf number ranged between 28.5 in B-1317 and 31.2 in B-98/N CMS F8.

Table 5 Stalk height and leaf number

Variety	Crop	Height of the stalk with inflorescence	2008/2009 Average	Difference from the average: Absolute	Difference from the average: Relative	Leaf number	2008/2009 Average	Difference from the average: Absolute	Difference from the average: Relative
B-21 CMS F_8 Ø	2008 2009	152,5 142,0	147,2	-	100,00	29,8 27,4	28,6	-	100,00
Pelagonec CMS F_1	2008 2009	177,6 173,0	175,3	+28,1	119,08	33,6 33,0	33,3	+4,7	116,43
B-98/N CMS F_8	2008 2009	191,6 181,0	186,3	+39,1	126,56	31,4 31,0	31,2	+2,6	109,09
B-136/07	2008 2009	190,0 181,0	185,5	+38,3	126,02	27,0 27,4	27,2	-1,4	95,10
B-1317	2008 2009	160,4 168,0	164,2	+17,0	111,55	28,6 28,4	28,5	+0,1	99,65
BB-16	2008 2009	145,0 151,0	148,0	+0,8	100,54	29,4 29,0	29,2	+0,6	102,09

CONCLUSIONS

The results of two-year investigations lead to the following conclusions:

- Soil and climate conditions during investigations were not very suitable for production of Burley tobacco and therefore additional irrigations and fertilization were required.

- The largest leaf size (length and width of the 5th, 10th and 15th leaf) was recorded in the varieties Pelagonec CMS F8 and B-98/N CMS F1.

- The highest stalk with inflorescence was recorded in B-98/N CMS F8 (186.3 cm) and the smallest in the standard variety B-21 CMS F8 (147.2 cm).

- The highest leaf number was recorded in Pelagonec CMS F1 (33.3) and the lowest in B-136/07 (27.2).

- Results of the investigations revealed dominance of Pelagonec CMS F8 and B-98/N CMS F1 over the other varieties included in the trial.

- Characteristics of the investigated varieties are typical for Burley tobacco and in agro-ecological conditions of the Prilep’s region they can be preserved only by application of suitable cultural practices.

REFERENCES

1. Апостолова Е., 1996. Технологии за производство на едролисни тютнюи в Северна България Български тютнюн, година XII брой 3/1996, София.
2. Бенковић Ф., 1981. Производња „Burgya" у Босанској Посавини - Дувански Гласник бр. 2 Београд.
3. Ѓеоргиевски К., 1971. Производство и обработка на крупнолисни тутуни - „Југотутун" - Скопје
4. Gornik R., 1953. Proizvodnja duhana tipa burlej – Zagreb
5. Донев Н., Фетфаджиев В.,1973. Наръчник по тютнюнопроизводство - Пловдив.
6. Дюлгерски Ň., 2009. Сортов идеал при тютнюн тип бърлей - Български тютнюн - бр 6/2009
7. Ѓуниќ М., 1983. Производња дувана сорте берлеј - Дувански гласник број 9 - Берлеј
8. Памуков И., 1992. Сортов идеал при тютнюн бърлей означова получуване на високи доходи - Български тютнюн N⁰ – 6 Пловдив
9. Пеливаноска В., 1999. Влијание на наводнувањето и минералната исхрана врз приносот и квалитетот на тутунот од типот берлеј - Докторска дисертација - Прилеп
10. Ристески И., 1999. Корелација помеѓу хранливата површина и некои производно-технолошки својства на тутунот од сортата вирџинија МВ -1 Магистерски труд - Земјоделски факултет - Скопје
11. Rubin B.A., 1971. Физиология сеълскохозяйственнъін растений – Москвского универзитета - 1971
12. Узуноски М., 1985. Производство на тутун Стопански весник - Скопје
13. Филипоски К. (2002). Влијание на некои агротехнички мерки врз приносот и квалитетот на тутунот тип берлеј _ Тутун / Tobacco, Vol. 52 N⁰ 5-6, Институт за тутун - Прилеп
14. Haslam R. J. , Scott W. A. (1965). Производство, берба и сушење на тутунот Берлеј (превод) Тутун бр. 1-12 - Прилеп
15. Hawks s.n. Collins W. K.,1994. Načela proizvodnje virginiskog duhana – Zagreb (Превод)

UDC 633.71 ISSN 0494-3244
Тутун/Tobacco, Vol.60, N^{o} 7-12, 79-87, 2010
University "St. Kliment Ohridski" - Bitola UDC: 633.71-152.4
Scientific Tobacco Institute – Prilep, R. Macedonia Original scientific paper

INHERITANCE AND GENETIC ANALYSIS OF YIELD IN TOBACCO VARIETIES AND THEIR DIALLEL F1, F2 AND BC1 PROGENIES

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ABSTRACT

Investigations were made on the mode of inheritance of green and dry mass yields per stalk and genetic analysis was of six parental tobacco genotypes (P-23, MB-3, SM-1, YV 125/3, FL-5 and O-87), with fifteen diallel crosses of each generation: F1, F2, BC1(P1) and BC1(P2). The trial was set up in 2008-2009 on the Experimental field of Scientific Tobacco Institute - Prilep in randomized block system with four replications, using standard agrotechnical practices during the vegetation period.

The aim of investigations was to study the genetics of inheritance of green and dry mass, using genetic components of variance to determine the types of genes as carriers of the characters and to assess the impact of the environment on their manifestation.

The yield is the most important agronomic character inherited mostly by partial dominance. Positive heterosis was observed in the hybrid MB-3 x YV 125/3 and negative heterosis in FL-5 x O-87. Genetic analysis of the four investigated generations revealed that additive genes were dominant in creation of yield. Results presented in this paper will give good directions in tobacco selection.

Key words: Tobacco (*Nicotiana tabacum L.*), mode of inheritance, heterosis, genetic analysis, components of genetic variance

НАСЛЕДУВАЊЕ И ГЕНЕТСКА АНАЛИЗА НА ПРИНОСОТ КАЈ ТУТУНСКИ СОРТИ И НИВНОТО ДИЈАЛЕЛНО F1, F2 И BC1 ПОТОМСТВО

Трудот содржи проучувања за начинот на наследување на приносот на зелена маса и приносот на сува маса по страк, како и нивната генетска анализа, кај шест родителски генотипови тутун (P-23, MB-3, SM-1, YV 125/3, FL-5 и O-87) и нивните 15 F1, и по исто толку F2, BC1(P1) и BC1(P2) дијалелни крстоски. Опитот беше поставен во 2008 и 2009 година на опитното поле од Научниот институт за тутун - Прилеп по случаен блок - систем во четири повторувања, а за време на вегетативниот период беа применети стандардни агротехнички мерки.

Целта на истражувањата беше да се проучи генетиката на наследување на приносот на зелена и сува маса, преку одредување на генетските компоненти на варијансата да се определи видот на гените - носители на својствата и да се оцени влијанието на надворешната средина врз нивното манифестирање.

Приносот како највам агрономско својство се наследува првенствено парцијално доминантно. Кај крстоската MB-3 x YV 125/3 има појава на позитивен хетерозис, а кај FL-5 x O-87 негативен. Генетската анализа на четирите проучувани генерации покажува дека предност имаат адитивни гени во неговото креирање. Резултатите прикажани во трудот ќе дадат сигурни насоки во селекцијата на тутунот.

Клучни зборови: тутун (*Nicotiana tabacum L.*), начин на наследување, хетерозис, генетска анализа, компоненти на генетска варијанса.

INTRODUCTION

Properties of the living organisms are created by genomes. In their genetic system they are inheritable and have a limited changeability. Each individual is phenotypic manifestation of properties, influenced by its own genotype and environmental factors. In agronomy, selection is based on phenotypic and genotypic investigations, in order to increase the yield and to improve the quality. Tobacco is agricultural crop which gives pleasure to the consumers and they can hardly resist it, in spite of its bad influence on humans health. For this reason, the aim of breeding, accompanied by various chemical, medicinal and technological investigations, is to reduce the risk of disease and death.

The aim of this paper is to study the mode of inheritance of the characters green and dry mass yields per stalk and to make their genetic analysis using biometric methods, which will improve the knowledge on successive generations and give further directions in selection of tobacco.

MATERIAL AND METHODS

Investigations included six tobacco genotypes, of which four oriental (Prilep P-23, Basma MB-3, Samsun SM-1, Yaka YV 125/3) and two semi-oriental (Floria FL-5, Otlia O-87).

15 diallel crosses for F1 were made, from which the following year seed material for F2 generation was obtained. Backcrossings were made with one of the parents for BC1 (P1) and with the second parent for BC1 (P2), after which a diallel for F1 was crossed again. The trial was carried out in 2008 and 2009 in the field of Scientific Tobacco Institute - Prilep in randomized block design with four replications. During the vegetation period, adequate cultural practices were applied on tobacco.

Green mass yield was weighed after each harvest and after the last harvest, for estimation of green mass yield per stalk, the total tobacco weights obtained from each plot were added and then divided with the number of tobacco stalks. The same method was used to estimate the dry mass leaf per stalk, by weighing tobacco after its manipulation and applying the formulae for corrected yield.

Mode of inheritance was determined by the test-significance of mean values of F1, F2, BC1(P1) and BC1(P2) progenies and parental average, according to Borojevic (1).

Components of genetic variance were determined by the methods of Jinks (4), Hayman (2,3) and Mather & Jinks (5, 6).

The following parameters were analysed:

D - Component of variance resulting from additive gene action

H1 - Component of variance resulting from dominant gene action

H2 - Component of variance resulting from dominant gene action, corrected in relation to their distribution,

$H1 = H2$ when $u = v$

(u - dominant alleles, v - recessive alleles)

F - Interaction between additive and dominant effects,

$F = 0$ when $u = v$

F = positive value, with higher number of dominant alleles

F = negative value, with higher number of recessive alleles

E - Noninherited environmental variance, obtained by the analysis of variance with randomized block design

$\sqrt{\frac{H1}{D}}$ - Average degree of dominance

- The value lower than 1 ($\sqrt{\frac{H1}{D}} < 1$) denotes partial dominance

- The value equal to 1 ($\sqrt{\frac{H1}{D}} = 1$) denotes complete dominance

- The value higher than 1 ($\sqrt{\frac{H1}{D}} > 1$) denotes overdominance

$\frac{H2}{4H1} = u \cdot v$ - frequency of dominant - u and recessive - v genes.

During tobacco vegetation in field (May-Sept 2008), mean monthly air temperature was 19.91^{0}C and the total amount of precipitations (in 39 rainy days) was 235.44 mm. In the same period in 2009, mean monthly air temperature was 19.89^{0}C and the total amount of precipitations (in 42 rainy days) was 240.6 mm.

RESULTS AND DISCUSSIONS

Selected parental genotypes significantly differ in relation to green and dry mass yields per stalk. Of all 15 combinations in 2008 and 2009, only between MB-3 and SM-1 did not show significant difference for the two characters. According to the statistical data analysis, there are no significant differences among replications, which indicates that the trial was properly set up and accurately processed.

The highest yield was recorded in the semi-oriental variety O-87, and the lowest in the oriental aromatic varieties MB-3 and SM-1 (Table 1).

Table 1. Green and dry mass yields in parental genotypes in 2008 and 2009

Parental genotypes	Green mass yield per stalk(g)		Dry mass yield per stalk (g)	
	2008	2009	2008	2009
	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
1. P1 (Prilep P-23)	156.00	158.00	24.00	25.22
2. P2 (Basma MB-3)	78.00	79.35	12.00	12.18
3. P3 (Samsun SM-1)	81.25	85.46	12.50	13.39
4. P4 (Yaka YV 125/3)	139.75	141.82	21.50	22.15
5. P5 (Floria FL - 5)	279.50	280.82	43.00	44.30
6. P6 (Otlia O-87)	318.50	320.94	49.00	49.55
LSD 0,05	11.36	11.69	1.09	1.33
0,01	17.81	18.32	1.70	2.09

In F1 progeny of 2008, green mass yield was inherited by partial dominance, intermediate dominance and positive or negative dominance. Positive heterosis was observed in MB-3 x YV 125/3, and negative in FL-5 x O-87. The mode of inheritance of this character in 2009 was intermediate and partially dominant, and heterosis appearance was observed in the same crosses as in the previous year.

All modes were present in inheritance of the character dry mass yield, with advantage of the partial dominance. Positive heterosis was observed in MB-3 x YV 125/3 and negative heterotic effect in FL-5 x O-87 both in 2008 and 2009 (Table 2, Fig. 1).

Table 2. The mode of inheritance of green and dry mass yields in diallel F1 progeny in 2008 and 2009

F1 generation	Green mass yield per stalk (g)				Dry mass yield per stalk (g)			
	2008		2009		2008		2009	
	$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$	
1. P1 x P2	120.05	i	120.95	i	18.35	i	19.05	i
2. P1 x P3	113.98	i	116.55	i	17.55	i	18.23	i
3. P1 x P4	140.15	-d	140.58	pd	21.32	-d	21.84	-d
4. P1 x P5	199.57	pd	182.80	i	30.55	pd	30.15	pd
5. P1 x P6	169.10	-d	172.05	pd	25.75	-d	26.57	-d
6. P2 x P3	81.55	+d	83.44	i	12.50	+d	12.28	-d
7. P2 x P4	160.23	+h	161.92	+h	23.45	+h	24.31	+h
8. P2 x P5	122.30	pd	122.48	pd	18.60	pd	18.99	pd
9. P2 x P6	123.86	pd	128.15	pd	19.15	pd	19.89	pd
10. P3 x P4	102.05	pd	106.50	i	15.55	pd	16.05	pd
11. P3 x P5	156.29	i	145.45	i	23.9	pd	24.27	pd
12. P3 x P6	149.39	pd	149.33	i	22.75	pd	22.85	pd
13. P4 x P5	220.95	i	222.82	i	34.00	i	33.78	i
14. P4 x P6	155.62	-d	153.78	pd	23.65	-d	24.16	-d
15. P5 x P6	173.75	-h	174.29	-h	26.6	-h	27.18	-h

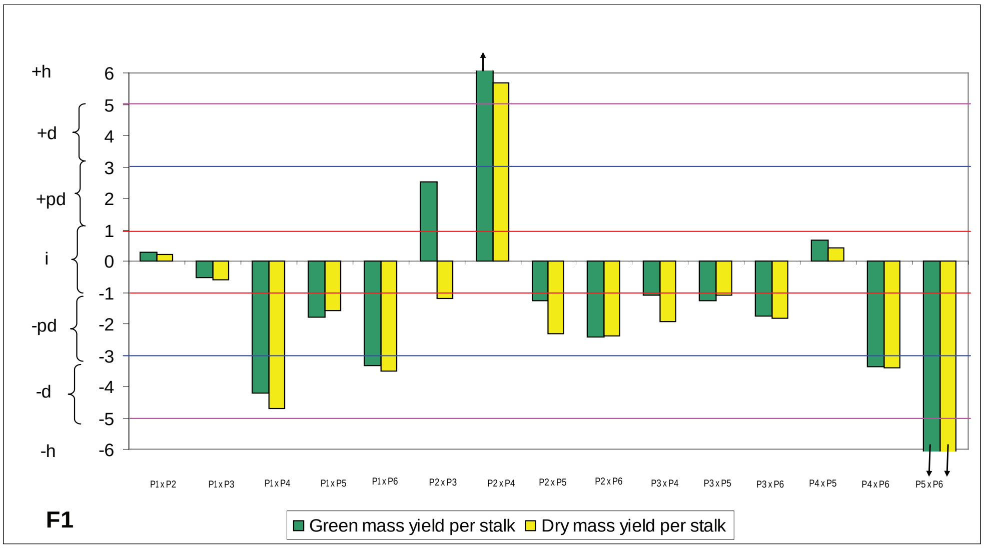

Figure 1 . Graphic presentation of the mode of inheritance of green and dry mass yield per stalk in the diallel F1 progeny

The inheritance of green mass yield in F2 progeny in 2008 and 2009 was intermediate and partially dominant. Negative heterosis was observed in FL-5 x O-87.

Dry mass yield in F2 generation is inherited in the same way as the green mass yield. Intermediate inheritance and partial dominance denote shortening of the selection process and fast fixation and stabilization of the investigated characters (Table 3, Fig. 2).

Table 3. The mode of inheritance of green and dry mass yields in diallel F2 progeny in 2008 and 2009

F2 generation	Green mass yield per stalk (g)				Dry mass yield per stalk (g)			
	2008		2009		2008		2009	
	$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$	
1. P1 x P2	118.15	i	120.06	pd	18.175	i	18.55	i
2. P1 x P3	116.35	i	119.23	i	17.9	i	18.50	i
3. P1 x P4	143.22	pd	145.08	pd	22.035	pd	22.50	-d
4. P1 x P5	208.16	i	206.57	i	32.025	i	32.72	i
5. P1 x P6	202.31	pd	205.90	pd	31.125	pd	31.63	pd
6. P2 x P3	80.45	pd	82.78	i	12.375	pd	12.65	i
7. P2 x P4	130.65	pd	136.21	+d	20.1	pd	20.30	pd
8. P2 x P5	149.82	pd	151.38	pd	23.05	pd	23.42	pd
9. P2 x P6	161.36	pd	163.98	pd	24.825	pd	25.15	pd
10. P3 x P4	105.88	i	109.79	i	16.275	i	16.80	i
11. P3 x P5	167.82	i	164.50	i	25.825	i	26.37	i
12. P3 x P6	173.87	i	176.47	i	26.75	i	27.00	i
13. P4 x P5	215.31	i	216.87	i	33.125	i	33.84	i
14. P4 x P6	191.42	pd	192.46	pd	29.45	pd	29.95	pd
15. P5 x P6	235.95	-h	237.90	-h	36.3	-h	37.27	-h

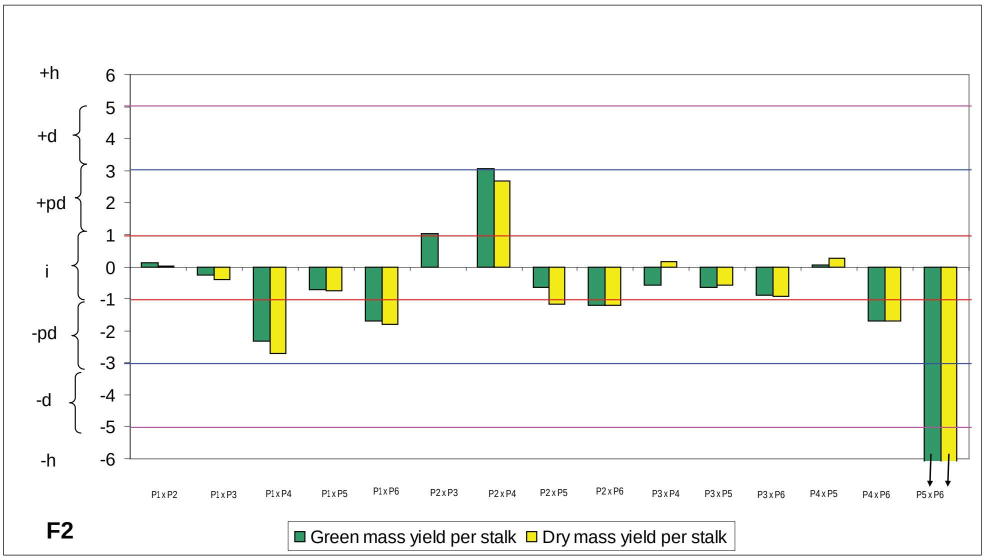

Figure 2 . Graphic presentation of the mode of inheritance of green and dry mass yield per stalk in the diallel F2 progeny

The most frequent mode of inheritance of green mass yield per stalk in the backcross BC1 (P1) generation in 2008 and 2009 was partial dominance. In several combinations both intermediate and negatively dominant inheritance were present. In FL-5 x O-87 the appearance of negative heterosis was observed.

Dry mass yield in this generation is inherited identically as the green mass yield (Table 4, Fig. 3).

Table 4. Mode of inheritance of green and dry mass yields in diallel BC1(P1) progeny in 2008 and 2009

BC1(P1) generation	Green mass yield per stalk (g)				Dry mass yield per stalk (g)			
	2008		2009		2008		2009	
	$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$	
1. P1 x P2	137.64	pd	139.72	pd	21.22	pd	21.78	pd
2. P1 x P3	135.10	pd	137.37	pd	20.84	pd	21.38	pd
3. P1 x P4	147.29	i	149.13	i	22.45	i	23.35	i
4. P1 x P5	177.29	pd	175.86	pd	27.22	pd	27.90	pd
5. P1 x P6	161.70	-d	165.15	-d	24.81	-d	25.50	-d
6. P2 x P3	79.62	i	81.25	pd	12.15	pd	12.51	pd
7. P2 x P4	115.25	i	120.59	pd	17.88	i	17.82	i
8. P2 x P5	99.45	-d	101.01	-d	15.41	-d	15.39	-d
9. P2 x P6	101.24	-d	103.58	-d	15.95	-d	15.70	-d
10. P3 x P4	91.16	pd	95.70	pd	14.05	pd	14.97	pd
11. P3 x P5	118.30	pd	115.65	pd	17.87	pd	18.05	pd
12. P3 x P6	114.56	pd	117.60	pd	17.92	pd	18.19	pd
13. P4 x P5	180.37	pd	182.12	pd	27.94	pd	28.24	pd
14. P4 x P6	146.75	-d	147.68	-d	22.92	-d	23.05	-d
15. P5 x P6	226.20	-h	227.87	-h	34.70	-h	35.55	-h

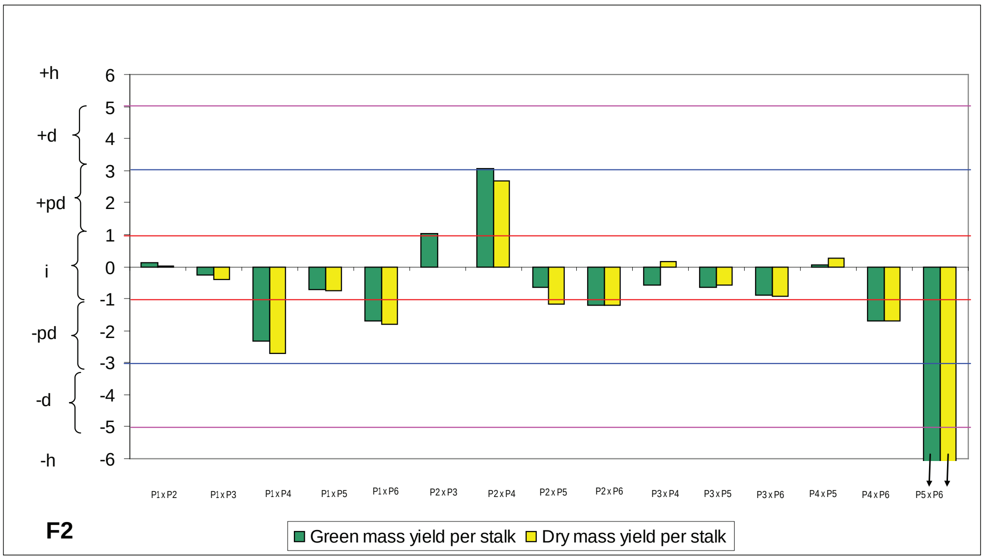

Figure 3 . Graphic presentation of the mode of inheritance of green and dry mass yield per stalk in the diallel BC1(P1) progeny

In BC1 (P2) generation, green mass yield in both investigating yeras was inherited mostly with partial dominance. In a number of combinations intermediary and dominant inheritance was present. In MB-3 x YV 125/3 in 2008 the character was inherited from the higher-yielding parent (YV 125/3), and in 2009 this combination had positive heterotic effect. The hybrid FL-5 x O-87 showed negative heterosis.

Dry mass yield in this generation was inherited mostly with partial dominance. Results from both investigating years were identical, except for the hybrid MB-3 x SM-1, which had positive heterotic effect in 2008 and in 2009 intermediate inheritance was present. This is a result of the insignificant difference for this character between the two parental genotypes, so that small differences appear as a consequence of the poor effect of non- genetic factors (Table 5, Fig. 4).

Table 5. Mode of inheritance of green and dry mass yields in diallel BC1(P2) progeny in 2008 and 2009

BC1(P2) generation	Green mass yield per stalk (g)				Dry mass yield per stalk (g)			
	2008		2009		2008		2009	
	$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$	
1. P1 x P2	98.65	pd	100.40	pd	15.41	pd	15.30	pd
2. P1 x P3	97.66	pd	101.17	pd	15.25	pd	15.50	pd
3. P1 x P4	139.16	-d	141.04	-d	21.28	-d	21.99	-d
4. P1 x P5	239.07	pd	237.27	pd	36.81	pd	37.50	pd
5. P1 x P6	242.94	i	246.65	i	37.59	i	37.85	i
6. P2 x P3	81.25	+d	84.30	pd	12.69	+h	12.91	i
7. P2 x P4	146.14	+d	151.83	+h	22.53	+d	22.70	+d
8. P2 x P5	200.20	i	201.75	i	31.78	pd	31.28	i
9. P2 x P6	221.50	i	224.35	i	34.00	i	34.17	i
10. P3 x P4	120.41	pd	123.88	pd	18.50	pd	19.05	pd
11. P3 x P5	217.42	pd	213.35	pd	33.15	pd	34.30	pd
12. P3 x P6	233.22	pd	235.34	pd	36.57	pd	36.22	pd
13. P4 x P5	250.25	pd	251.62	pd	38.70	pd	39.46	pd
14. P4 x P6	236.11	i	237.24	i	36.33	i	36.59	i
15. P5 x P6	245.70	-h	247.95	-h	37.50	-h	38.20	-h

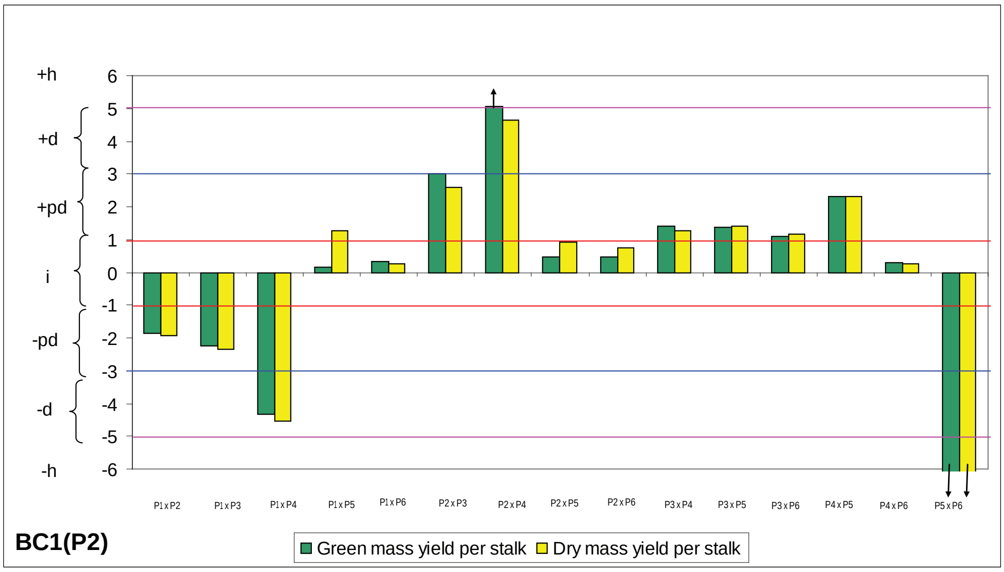

Figure 4 . Graphic presentation of the mode of inheritance of green and dry mass yield per stalk in the diallel BC1(P2) progeny

According to the presented data, mean values of the characters investigated in 2008 were similar to those in 2009, and the laws of inheritance were also identical. High similarities were also observed in meteorological data about mean monthly air temperatures and total amount of precipitations during the vegetation period (May-September).

Genetic analysis of green mass yield per stalk in F1, F2, BC1(P1) and BC1(P2) progenies showed high values of the additive component of genetic variance and low values of the dominant component (D > H1 and H2), which is an indication that inheritance of the character is governed both by dominant and recessive genes, with prevalence of the recessive ones. The positive value of the interaction F reveals dominance of higher-yielding parent in inheritance of this character. The low value of the environmental variability (E) indicates a low impact of the environment in inheritance of the character.

The values for H2/4H1 (genes frequency) in all generations were below 0.25, which denotes absence of symmetrical distribution of dominant and recessive alleles. The average level of dominance is lower than one, which indicates the presence of partially dominant inheritance. The results on genetic analysis of green mass yield per stalk are presented in Table 6.

Table 6. Components of genetic variance for green mass yield per stalk in diallel F1, F2, BC1(P1) and BC1(P2) generations

Components	F1	F2	BC1(P1)	BC1(P2)
D	1685.25	1673.05	1644.31	1672.28
H1	51.54	62.72	283.00	115.15
H2	39.28	55.05	191.05	92.18
F	97.66	204.38	809.55	85.37
E	8.22	11.98	22.36	12.29
H2 / 4H1	0.19	0.21	0.16	0.20
$\sqrt{H1/D}$	0.173	0.173	0.412	0.245

From genetic analysis of the character dry mass yield in F1, F2, BC1(P1) and BC1(P2) progeny it can be concluded that the additive components of genetic variance are much higher compared to those of dominant component (D > H1 and H2), according to which the inheritance is governed mostly by recessive genes. The interaction F for F1 and BC1(P2) has a negative value, indicating that the lower-yielding parent has a dominant role in inheritance of the character, whereas for F2 and BC1(P1) the interaction has a positive value, indicating the dominance of the parent with higher dry mass yield. For this character again, the environmental variance has a low value, which points out to the similar meteorological conditions in both investigation years and to identical application of suitable cultural practices during the vegetation period. The values of H2/H41 are lower than 0.25 in all generations, which indicates an absence of symmetrical distribution of dominant and recessive alleles. The average level of dominance $\sqrt{H1/D}$ is lower than one, which indicates the presence of partially dominant inheritance of this character.. The results of genetic analysis on dry mass yield per stalk are presented in Table 7.

Table7. Components of genetic variance for dry mass yield per stalk in diallel F1, F2, BC1(P1) and BC1(P2) generations

Components	F1	F2	BC1(P1)	BC1(P2)
D	39.55	39.56	39.58	39.47
H1	1.39	1.97	5.04	3.98
H2	1.31	1.82	2.15	3.46
F	-4.72	3.54	13.18	-10.62
E	0.14	0.11	0.10	0.20
H2 / 4H1	0.23	0.23	0.10	0.21
$\sqrt{H1/D}$	0.173	0.200	0.346	0.100

CONCLUSIONS

The following statements can be drawn from the results of investigation:

- Parental genotypes are homozygous, uniform and show significant differences between them.
- The inheritance of green and dry mass yields from the six parents to their diallel F1, F2, BC1(P1) and BC1(P2) progenies is mostly partially dominant and intermediate. Positive heterosis in the first hybrid generation was present in MB-3 x YV 125/3, and negative heterosis in FL-5 x O-87 for both characters and in both years of investigation.
- Genetic analysis reveals that both additive and dominant genes have an impact on creation of the investigated characters, with high dominance of additive genes (the value of the additive component of genetic variance - D is dramatically higher compared to the dominant one - H1 and H2). From the values of the estimated parameters, F denotes a presence of asymmetrical distribution of dominant and recessive alleles, H2/4H1 indicates that inheritance of the green mass yield in four generations and dry mass yield in F2 and BC1(P1) is prevailed by the higher-yielding parent and inheritance of the dry mass yield in F1 and BC1(P2) is dominated by the lower-yielding parent and $\sqrt{H1/D}$ indicates the presence of partially dominant mode of inheritance of the investigated characters.
- Investigations on heritability and genetic analysis have enormous impact on tobacco breeding, because they determine the inheritance of yield as the most important agronomic character and give useful directions for creation of new, higher-yielding varieties. The applied methods of work are also applicable for determination of quantitative characters in many other crops.

REFERENCES

1. Borojević S.,1981. Principi i metode oplemenivanja bilja. Ćirpanov, Novi Sad
2. Hayman B.I., 1954. The analysis of variance of diallel tables. Biometrics, 10-11, p. 235-244.
3. Hayman B.I., 1954. The theory and analysis of diallel crosses (I). Genetics, 39, p. 789-809.
4. Jinks J.L., 1954. The analysis of continuous variation in diallel cross of Nicotiana rustica varieties. Genetics, 39, p. 857-877
5. Mather K., J.L. Jinks, 1971. Biometrical genetics. Champan and Hall, London.
6. Mather K., J.L. Jinks, 1974. Biometrical genetics. Champan and Hall, London.

UDC 633.71
Тутун/Tobacco, Vol.60, N^o 7-12, 88-93, 2010
University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute – Prilep, R. Macedonia

ISSN 0494-3244

UDC: 633.71-184(497.7)
Original scientific paper

THE INTENSITY OF UPTAKE AND UTILIZATION OF NITROGEN AND CHEMICAL CHARACTERISTICS OF ORIENTAL TOBACCO DEPENDING ON THE RATE OF NITROGEN FERTILIZER

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ABSTRACT

The uptake and utilization of nitrogen during the growing season and changes in chemical composition of dry tobacco depending on the rate of nitrogen fertilizer were investigated in stationary field trial with tobacco as a long-term monoculture. The investigation included four rates of N fertilization: 0, 25, 50 and 100 kg N/ha. The increasing rates of nitrogen fertilizer increased the uptake and utilization of nitrogen during the growing season and its concentration in the leaves from the lower, middle and upper stalk position. In oriental tobacco, nitrogen uptake for creation of 1 g dry above-ground biomass over a 24-hour period is the highest in the period from transplanting to the stage of rapid growth. In the check and in the variant treated with 25 kg N/ha, daily nitrogen requirements were the highest from the stage of rapid growth to maturation of leaves from the lower stalk position. With fertilization rates of 50 kg and 100 kg N/ha, the nitrogen requirements were the highest from the stage of maturation of leaves from the lower stalk position to butonization.

In soil and climate conditions in which the trial was set up, the rate of 50 kg N/ha can be considered as optimal for obtaining tobacco with chemical composition that will meet the requirements of cigarette industry.

Key words: Oriental tobacco, nitrogen fertilizer, uptake of nitrogen, utilization of nitrogen

ИНТЕНЗИТЕТОТ НА УСВОЈУВАЊЕ И ИСКОРИСТУВАЊЕ НА АЗОТОТ И ХЕМИСКИТЕ КАРАКТЕРИСТИКИ НА ОРИЕНТАЛСКИОТ ТУТУН ВО ЗАВИСНОСТ ОД ДОЗИТЕ НА АЗОТНИТЕ ЃУБРИЊА

Во ова истражување е проучувана спецификата на усвојувањето и искористувањето на азотот во текот на вегетацијата и промените во хемискиот состав на тутунот во зависност од дозите на азотно ѓуриво, во услови на стациониран, долгогодишен опит на тутунот во монокултура и континуирано ѓубрење со одредени дози на ѓубрења.

За таа цел се поставени черири варијанти, ѓубрени само со азотно ѓубре, со дози од: 0, 25, 50 и 100 kg N/ha.

Испитувањата покажаа дека со зголемување на дозите на азотно ѓубре се зголемува интензитетот на усвојувањео и искористување на азотот во текот на вегетацијата, и неговата концентрација во листовите од долниот, средниот и горниот појас.

Интензитетот на усвојување на азотот, изразен во mg, за создавање на 1 g сува надземна биомаса во едно денонoќие е најголемо во периодот од расадување до фазата на буен пораст на ориенталскиот тутун. Кај контролата и варијантата ѓубрена со 25 kg N/ha, најголемата дневна потреба за азот за создавање на надземната маса се јавува од периодот на буен пораст до созревањето на листовите од долниот бербен појас. При внесување на 50 и 100 kg N/ha, пикот за потребите од азот е во периодот од созревањето на листовите од долниот бербен појас до фазата на бутонизација.

При почвено – климатските услови во кои се извршени испитувањата, може да се констатира дека варијантата ѓубрена со 50 kg N/h е оптимална за добивање на тутун чиј хемиски состав одговара за потребите на цигарната индустрија.
Клучни зборови: ориенталски тутун, ѓубрење со азот, усвојување на азот, искористувње на азот

INTRODUCTION

Nitrogen is essential for tobacco growth, development, yield and quality. The existing information about the needs of oriental tobacco for nitrogen is mainly based on the data for chemical composition of plants (Yancheva, 2002, 2009; Sekin et al., 2002). Other important parameters describing the nitrogen nutrition of plants are the intensity of uptake and utilization of nitrogen. The former characterizes the amount of N needed to build 1 g of dry matter per day and the latter - the absolute quantity of the element required to construct the mass of plants per unit area per day (Mitreva and Iliev, 1984). The intensity of nitrogen uptake depends on the phase of plant growth and the rate of nitrogen fertilization (Apostolova, 1983; Nankova and Stoyanova, 1995). The daily export of nitrogen in plant biomass per unit area varies with the development phase and the level and type of fertilization (Mitreva and Iliev, 1984; Panayotova, 1999; Sifola and Postiglione, 2003).

The aim of the study was to determine the specificity of the intensity of uptake and utilization of nitrogen during the plant growth and changes in chemical composition of cured tobacco depending on the rate of nitrogen fertilization.

MATERIAL AND METHODS

A field experiment with long-term fertilization was conducted with the oriental tobacco variety Plovdiv 7 in 2006. The rendzina soil of the site was heavy sandy loam. For the purpose of the study, four nitrogen rates were tested: 0; 25; 50 and 100 kg ha^{-1}. Nitrogen was applied as urea before transplanting. The fertilizer was uniformly broadcast over the soil surface of each plot before being incorporated into the soil. Tobacco seedlings were transplanted at a 0.5 x 0.12 m distance (166 000 plants/ha). All cultural practices were in accordance with those used by the growers for oriental tobacco production.

For the determination of N in the above-ground biomass, twenty plants were sampled in seedling stage and ten plants per variant - during the following phenological stages: rosette (35 days after transplanting - DAT), ripeness of basal leaves (56 DAT), ripeness of middle leaves (77 DAT) and ripeness of upper leaves (98 DAT). Plants were separated into leaves, stalks and inflorescences. Individual parts were washed to remove all traces of soil. The samples were dried to a constant weight in an oven at 65 ^{o}C and dry weight was recorded. Total nitrogen was determined using the Kjeldahl method. The intensities of nitrogen uptake and utilization were calculated after determining the dry weight of plant parts at the different phenological stages, the nitrogen content and the number of days between periods of observation (Mitreva and Iliev, 1984).

The random samples of sun-cured leaves were analyzed for nicotine (ISO 15152), proteins (BDS 9142-88), reducing sugars (ISO 15154) and ashes (ISO 2817).

RESULTS AND DISCUSSIONS

The intensity of N uptake by tobacco above-ground biomass for formation of 1 g dry matter per 24 hours changes depending on the period of the growing season and the rate of nitrogen fertilization (Fig. 1). It varies from 8.9 to 33.3 mg N/1 g dry matter per day. The highest consumption of nitrogen for formation of 1 g dry matter is during the period from seedling establishment to the beginning of active vegetative growth (1-35 DAT). The uptake of nitrogen during this period gets ahead of accumulation of biomass, which leads to its enrichment with nitrogen. In the later stages of the growing season, the intensity of N uptake decreases. Such a reduction in the amount of nitrogen to build a unit of dry matter during the vegetation was established by Mitreva and Iliev (1984) and Tomov and Manolov (2004). In unfertilized plots, the intensity of N uptake gradually decreases during the growing season. The intensity of nitrogen uptake is influenced by the amount of N applied. Consumption of nitrogen increases from seedling establishment to bud formation (from 1 to 77 DAT) as the rate of applied nitrogen increases. During the bud formation until flowering (78-98 DAT) the intensity of nitrogen uptake does not depend on nitrogen treatments.

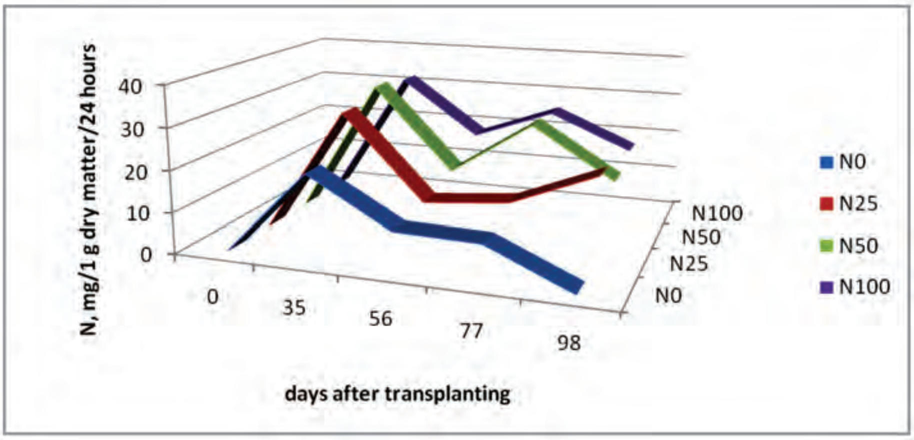

Fig. 1. Intensity of nitrogen uptake by tobacco above-ground biomass

Sifola and Postiglione (2003) reported that the daily uptake of nitrogen for the whole plant formation depends on soil processes, fertilization practices and physiology (uptake, translocation, assimilation and partitioning). During the period of rapid dry matter accumulation (DMA) and nutrient uptake in flue-cured tobacco (between 41 and 75 DAT) the soil must have sufficient amount of available nutrients to satisfy the needs of the plant (Moustakas and Nizanis, 2005). The intensity of nitrogen utilization ranges from 144.5 to 1602 g N/ha/day depending on the observation period and fertilization rate (Fig. 2). In the period between seedling establishment and beginning of active growth (1-35 DAT), the absolute daily amount of nitrogen absorbed by plants per unit area is relatively low (144.5-348.5 g/ha/day). The high total N content of plants and high intensity of nitrogen uptake during this period are indicative of relatively high demand of the plant for this element in the initial phase of development. The utilization of nitrogen during vegetation follows sigmoidal curve. The maximum daily utilization for unfertilized control and plots fertilized with low nitrogen rate – 25 kg N/ha coincides with the period of maximum accumulation of dry matter (36-56 DAT). When plants are fertilized with 100 kg N/ha, the maximum daily consumption of nitrogen is observed between 57 and 77 DAT and once more coincides with the maximum daily DMA. The maximum utilization of N does not coincide with the peak period in the accumulation of dry matter when plants are fertilized with 50 kg N/ha. The intensity of nitrogen utilization strongly depends on the amount of N applied. The effect of increasing N rates on daily consumption of nitrogen in formation of the above-ground biomass is positive in the period beween beginning of active growth and bud formation (36-77 DAT) but insignificant at later interval. The increasing of nitrogen fertilization rates is associated with a linear increase in absolute amount of nitrogen needed for formation of the above-ground biomass from 36 to 77 DAT.

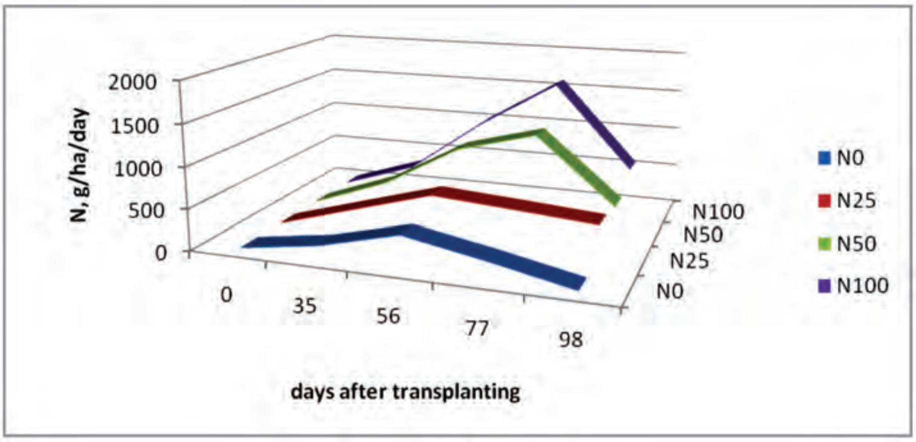

Fig. 2. Intensity of utilization of nitrogen

The specificity of the consumption of nitrogen from the soil influences the leaf nitrogen concentration during the growing season and chemical characteristics of cured tobacco. The concentration of nitrogen in the mature leaves ranged from 1.3 to 3.8% (Fig. 3). The nitrogen content in the leaves depends on the priming and the rate of N fertilizer. It rises from the lower to the upper priming and differences between them are increased in response to the increasing levels of applied N. The rates of nitrogen fertilizer have a strong effect on N in the mature leaves. The nitrogen content in the leaves increases in response to the increasing rates of nitrogen fertilizer.

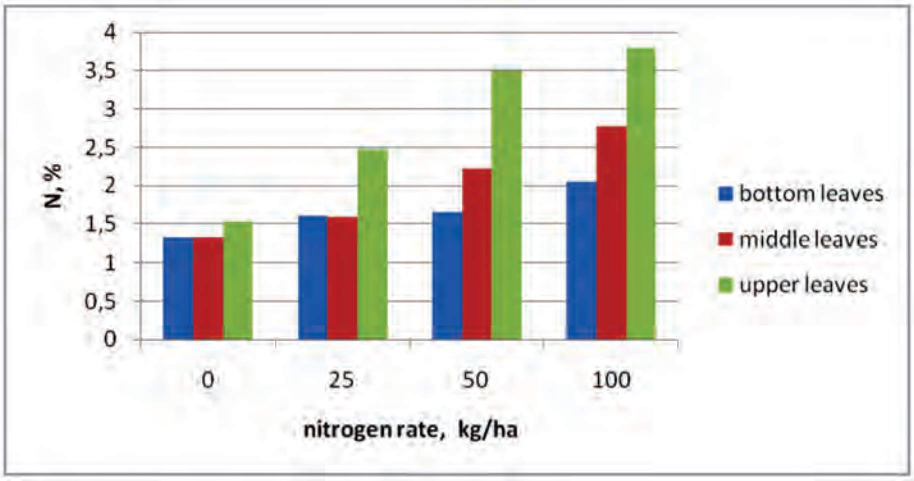

Fig. 3. Concentration of nitrogen in tobacco mature leaves from bottom, middle and upper stalk position during the growing season

Most of the chemical constituents of tobacco, although genetically controlled, have been found to change with the growth stage and a number of cultural practices (Lolas et al., 1979). Chemical characteristics of cured tobacco change depending on nitrogen fertilizer rate (Table 1). The percentage of nicotine and proteins in cured leaves increases when the rate of nitrogen increases from 0 to 100 kg/ha. There is a negative relationship between increasing N levels and sugars content. Ash content increases with increasing nitrogen rate up to 50 kg/ha, while further raise of nitrogen level has an insignificant effect.

These values of chemical characteristics of cured tobacco correspond to the specific content of total nitrogen in the leaves during the growing season. The relationships among leaf total N and the content of nicotine, proteins, reducing sugars and ash are explained by the following regression equations:

$У_{nicotine}$= 0.45+0.48x; R=0.958; R^2=0.918
$У_{proteins}$= 1.73+1.32x; R=0.995; R^2=0.989
$У_{reducing\ sugars}$= 24.72-3.54x; R=0.989; R^2=0.979
$У_{ash}$= 8.06+1.01x; R=0.939; R^2=0.882

Table 1. Chemical characteristics of the variety Plovdiv 7 (% of dry weight)

Nitrogen rate (kg/ha)	Nicotine	Proteins	Reducing sugars	Ash
0	1.05	3.49	20.0	9.40
25	1.50	4.35	17.9	9.92
50	1.59	4.93	15.6	10.88
100	1.83	5.50	14.9	10.73

Smoke delivery and smoke composition depend on the characteristics of the leaf tobacco. The nitrogenous constituents are considered the most important in determining flavor and smoking quality of tobacco. According to Ghiuselev (1983), nicotine content from 1.0 to 1.6% is related to fullness of flavor but higher content leads to strong and pungent smoke. When sugar content increases over 16%, and this is accompanied by low content of nitrogen substances, the resulting taste is of insufficient completeness. The daily consumption of plants receiving 50 kg N/dka ranges from 339 to 1148.8 g N/ha/day, due to which the concentration of nitrogen in mature leaves from the bottom, middle and upper stalk position during the growing season varies from 1.7 to 3.5%. Given the above limits, we can point out that in terms of experience, the chemical composition of tobacco fertilized with 50 kg N/ha would have the beneficial effect on the smoking properties.

CONCLUSIONS

1. The increasing N rates intensify the uptake and utilization of nitrogen as well as its concentration in the mature leaves from the bottom, middle and upper stalk position during the growing season.

2. The intensity of the N uptake for formation of 1 g dry matter per 24 hours is highest during the period from seedling establishment to the beginning of active growth of oriental tobacco. The maximum daily consumption of N for the formation of the above-ground biomass per unit area for unfertilized plants and for plants fertilized with low nitrogen rate (25 kg N/ha) is during the period beween the beginning of active growth and ripeness of basal leaves. Nitrogen utilization rate of plants, fertilized with 50 and 100 kg N/ha is high between ripeness of basal leaves and bud formation.

3. Under the experimental climatic and soil conditions, a nitrogen fertilizer rate of 50 kg ha^{-1} should be considered as optimal to produce tobacco with desirable chemical composition for cigarette manufacturing.

REFERENCES

1. Apostolova E., 1983. Investigation of the root system nutrition and cationic-anionic balance of Virginia tobacco, PhD thesis (Blg.), Plovdiv.
2. Sekin S., A. Peksuslu, R. Küçüközden, 2002. Macro and micro element contents of Izmir tobaccos related with quality, The Second Balkan Scientific Conference "Quality and efficiency of the tobacco production, treatment and processing", Plovdiv, 47-55.
3. Sifola I. M., L. Postiglione, 2003. The effect of nitrogen fertilization on nitrogen use efficiency of irrigated and non-irrigated tobacco (Nicotiana tabacum L.), Plant and Soil, 252, 313-323.
4. Moustakas N. K., H. Ntzanis, 2005. Dry matter accumulation and nutrient uptake in flue-cured tobacco (Nicotiana tabacum L.), Field Crops Research, 94, 1-13.
5. Lolas C. P., W. K. Collins, S. M. Hawks, H. Seltmann, W. Weeks, 1979. Effects of phosphorus rates on the chemical composition of flue-cured tobacco grown in soils with varying phosphorus availability, Tob. Sci., 23, 31-34.
6. Ghiuselev L., 1983. Stokoznanie na tutuna, Hristo G. Danov, Plovdiv.
7. Mitreva N., V. Iliev, 1984. System in root nutrition of the sunflower plant. I. Uptake, utilization and distribution of nitrogen in the above-ground parts during ontogenesis, Soil Science and Agrochemistry, vol. XIX, №1, 10-18.
8. Nankova M., M. Stoyanova, 1995. Effect of nitrogen fertilization on nitrogen intensity uptake and protein quantity and quality of wheat, Plant Science, vol. XXXII, №3, 11-14.
9. Panayotova G., 1999. Nutrition of durum wheat (Tr. durum Desf.) cultivated with cotton in crop rotation, PhD thesis (Blg.), Sofia.
10. Tomov T., I. Manolov, 2004. Necessary nitrogen, phosphorus and potassium for yield formation of wheat variety Prelom, Field Crops Studies, vol. I-2, 285-291.
11. Yancheva D., 2002. Mineral composition of the oriental tobacco leaves depending on the nitrogen fertilizer rate, The Second Balkan Scientific Conference "Quality and efficiency of the tobacco production, treatment and processing", Plovdiv, 162-166.
12. Yancheva D., 2009. Parameters of sufficiency in nutritive regime of Krumovgrad oriental tobacco Plant Science, 46, 260-264.

UDC 633.71 ISSN 0494-3244
Тутун/Tobacco, Vol.60, N⁰ 7-12, 94-102, 2010
University "St. Kliment Ohridski" - Bitola UDC: 633.71(497)
Scientific Tobacco Institute – Prilep, R. Macedonia Original scientific paper

PRODUCTION OF ORIENTAL TOBACCOS IN THE BALKAN COUNTRIES

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ABSTRACT

Production of oriental tobaccos is mainly located in the Balkan countries, as well as in some countries of Asia and Former Soviet Republics. The share of this type in the world tobacco production is gradually decreasing. The tendency of decreasing has been clearly observed in the Balkan countries. Subsidies given by the state helped to maintain the tobacco production in Macedonia and Bulgaria. The leading producer in the last five years was Turkey with 77.570 t, followed by Bulgaria with 23.430 t, Greece - 20.494 t, Macedonia – 20.173 t, Albania-2.453 t and finally Serbia with 308 t. The total share of these countries in the world production of oriental tobacco in the last few years dropped from 60,41% to 52,70%. The main reasons for this decline is the abolishment of subsidies in Greece and the shift from tobacco toward olive production and development of tourism in Turkey. To maintain the production of oriental tobacco in the Republic of Macedonia, the government must continue with subsidization.

Key words: oriental tobacco, production, Basma, Prilep, Krumovgrad, Izmir, Samsun, Katerini

ПРОИЗВОДСТВО НА ОРИЕНТАЛСКИТЕ ТУТУНИ ВО БАЛКАНСКИТЕ ЗЕМЈИ

Производството на ориенталски тутун во светот претежно се одвива во балканските земји, како и во некои азиски земји и поранешни советски републики. Во светски рамки, ова производство полека се намалува, но тенденцијата на намалување осетно се забележува и кај производството во балканските зенји. Субвенциите од страна на државата го задржаа производството во Македонија и Бугарија. Најголем производител во последниве пет години е Турција со околу 77.570 тони, потоа Бугарија со 23.430 тони, Грција со 20.494 тони, Македонија со 20.173 тони, Албанија со 2.453тони и најмало прозводство има Србија со 308 тони. Вкупното производство на овие земји во светското производство на ориенталски тутуни во последниве години е намалено од 60,41% на 52,70%. Најголема причина за намалувањето е укинвањето на субвенциите во Грција и преминувањето кон производство на маслиники и развој на туризмот во Турција. За да се задржи производството во Р Македнија неопходно е потребно да продолжи субвенционирањето на ова произвоство од страна на државата.

Клучни зборови: ориенталски тутун, производство, басма, прилеп, крумовград, измир, самсун, катерини

INTRODUCTION

Oriental tobacco accounts for only about 4 % of the world tobacco production. In world frames, this tobacco is grown mainly in the Balkan countries, Turkey, Greece, Bulgaria, Macedonia, and also in Italy, Egypt, South Africa, Syria, etc. It is named by the area in which it is grown – Eastern Mediterranean, and its varieties are named by the towns or regions of growing (Samsun, Izmir, Katerini, Krumovgrad, Prilep, Djebel, etc.). It is also known as Turkish tobacco, because it had been grown in the Ottoman Empire. Oriental tobaccos are characterized by their small leaves. They are grown on poor soils, in areas with large number of sunny and warm days, which contributes to the formation of their strong aromatic and "sweet and sour" flavor while smoking. Cigarette blends consist of three basic tobacco types: Virginia, Burley and Oriental. The oriental tobaccos are applied in small amounts (up to 20%), as a spice for aromatization of cigarettes. The leading importers of oriental tobaccos in the world are the USA, Japan and Germany. According to our knowledge, multinational tobacco companies are making efforts to substitute the oriental tobaccos with aromatized large-leaf tobaccos and artificial aroma, but so far without some significant success. Still, this information deserves serious attention, having in mind that tobacco growing is the only source of existence to a great number of households. It must also be mentioned that during the growing season, additional labor force composed of seasonal workers is engaged in tobacco production.

MATERIAL AND METHODS

Data from FAOSTAT, Universal Leaf Tobacco Company, SOCOTAB, Alliance One Macedonia, Analyses of the Ministry of Agriculture, Forestry and Water Economy of R. Serbia, Analyses of the Ministry of Agriculture, Forestry and Water Economy of R. Macedonia, Yugotutun, Tobacco Institute-Prilep and our previous research in this field were used as a source for investigation on the oriental tobacco production in the Balkan countries (Turkey, Greece, Bulgaria, Macedonia, Serbia and Macedonia). For higher reliability, the investigations were carried out in a longer period (1995 – 2010), except for Albania where the investigation was carried out between 2005-2010. For data processing and making final conclusions, the comparative-analytical method was applied.

RESULTS AND DISCUSSIONS

The total share of oriental tobaccos is about 4% (2010) of the total tobacco production in the world. Until 2000 this production ranged 500.000 to 600.000 tons, but after 2000 there was a tendency of decreasing and in the last few years the production has been stabilized at about 250.000 tons. The drop in the world production of oriental tobaccos is consecutively followed by a drop in the Balkan tobacco producing countries.

In this paper, analysis was also made on the share of certain tobacco types by country of production.

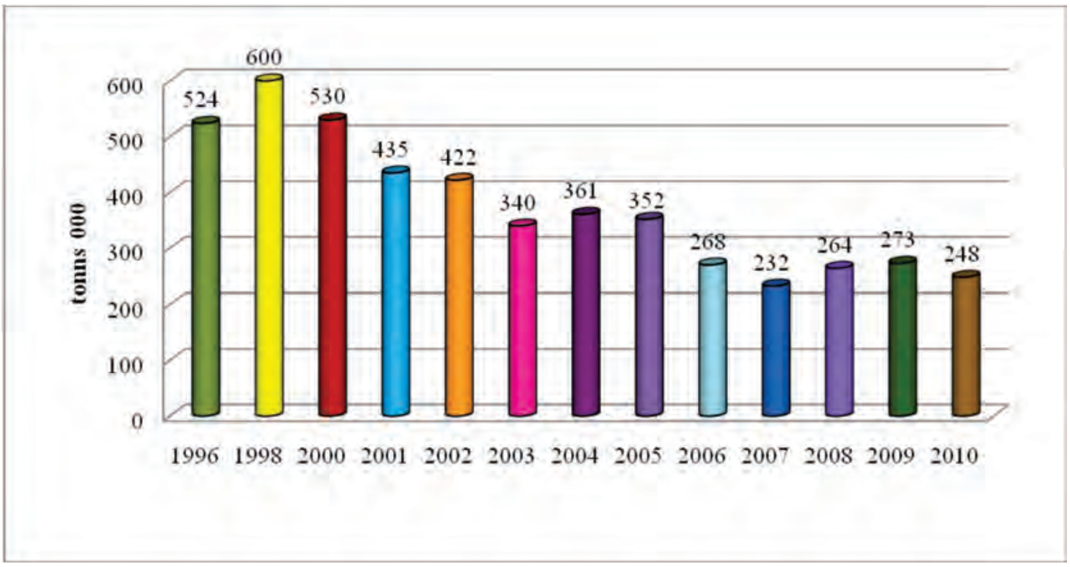

Figure 1. World production of oriental tobacco (1996-2010)

Until 2000, Turkey was the largest producer of oriental tobaccos with over 200.000 tons annual production.

Data on tobacco production in Turkey (Table 1) clearly show a decline from 2000 to 2005 and even more rapid decline after 2005, so that for 2010 it is predicted to fall on 52.742 tons.

Table 1. Classical and non-classical oriental tobacco production in Turkey (in tons)

Years	Variety							Total	Index
	Izmir	Samsun	Basma	Other Bleck Sea	Marmara	East and South East	Katerini		
1996[1)]	127.198	18.203	7.503	5.600	4.101	65.099	0	224.602	100,00
2000	114.171	17.216	9.561	5.746	3.969	46.984	0	197.647	88,00
2001	91.031	12.648	6.209	3.998	3.209	24.304	0	141.399	62,96
2002	106.360	15.870	5.950	3.240	2.320	22.384	0	156.124	69,51
2003	66.287	10.093	3.565	1.740	1.282	25.996	0	108.963	48,51
2004	81.642	9.562	4.500	1.169	1.741	25.403	0	124.017	55,22
2005	83.441	11.221	5.462	969	1.596	26.386	0	129.075	57,47
2006	53.850	9.904	4.404	966	861	23.240	0	93.225	41,51
2007	38.557	8.938	3.542	606	794	18.431	0	70.868	31,55
2008	59.736	7.908	3.641	664	149	19.259	40	91.397	40,69
2009	63.413	9.533	4.513	0	10	1.690	458	79.617	35,45
2010[2)]	41.260	2.800	4.800	0	40	3.080	762	52.742	23,48
$\bar{x}$ (2006-2010)	51.363	7.817	4.180	447	371	13.140	252	77.570	34,54
%	66,22	10,08	5,38	0,58	0,48	16,94	0,32	100,00	-

[2)] Semiofficial data

According to the data for the last 5 years, it can be stated that Izmir, with an average share of 66,22%, has the most important place in the production of oriental tobacco in Turkey. It can be observed that production of non-classical oriental tobaccos is slowly but surely decreasing. In 2010 the production dropped to only 23,48%, which is more than four times lower compared to that in 1996. Decline in the oriental tobacco production in Turkey is a result of the tendency to substitute tobacco with perennial crops (olives), development of tourism in the Aegean part of Turkey - the region where these tobaccos were grown mostly, as well as the lack of labor force due to the outflow of young people to Western countries

From data presented in Table 2, it can be seen that oriental tobacco production in Greece has been significantly reduced since 2000, as a result of the abolishment of tobacco subsidies from the European Union, payment of subsidies to the producers several years after they stopped growing tobacco and providing subsidies for alternative crops (these subsidies are known as “lazy money”). Presently, non-typical oriental (semi-oriental) tobaccos in Greece, like Sochoumi, Kaba-koulak & Elassona, Myrodata Agrinion, Tzebelia and Mavra are not produced anymore. Only the typical and best quality tobaccos Katerini and Basma are still grown, and they account for 99% of tobacco production in Greece. The production of tobacco in this country is almost four times lower compared to that in 2000. The same tendency is observed in Greece and Turkey, with over four times lower production (23,77%) compared to 1995.

Table 2, Classical and non-classical oriental tobacco production in Greece (in tons)

Years	Variety							Total	Index
	Basma	Katerini	Sochoumi	Kaba-koulak & Elassona	Myrodata Agrinion	Tzebelia	Mavra		
1995	23.748	18.166	4.782	5.810	4.592	10.457	3.885	78.670	100,00
2000	27.342	18.059	5.957	12.162	4.610	7.224	3.359	78.713	100,05
2001	26.803	16.732	6.570	9.920	3.294	3.584	2.977	69.880	88,83
2002	27.489	17.143	6.198	6.878	2.889	1.597	1.587	63.781	81,07
2003	26.270	16.421	5.708	5.577	2.223	793	800	57.792	73,46
2004	27.887	16.803	4.844	4.014	1.857	588	603	56.596	71,94
2005	27.376	18.640	4.273	1.165	622	491	303	52.870	67,20
2006	11.585	10.050	85	16	17	2	1	21.756	27,65
2007	10.994	10.448	0	0	0	0	0	21.442	27,26
2008	9.639	10.447	0	0	0	0	0	20.086	25,53
2009	9.083	11.407	0	0	0	0	0	20.490	26,05
2010[1)]	9.000	9.700	0	0	0	0	0	18.700	23,77
$\bar{x}$ (2006/10)	10.060	10.410	17	3.2	3.4	0.4	0.2	20.494	26,05
%	49,09	50,79	0,08	0,01	0,02			100,00	-

[1)] Semiofficial data

Table 3, Classical and non-classical oriental tobacco production in Bulgaria (in tons)

Years	Variety							Total	Index
	Krumov-grad	Krumov-grad K	Nevrokop	East Balkan	Basma	Other Oriental	North Bulgarian		
1995	5.000	0	3.500	500	1.000	3.400	1.500	14.900	-
2000	6.700	0	6.400	1.200	900	4.400	3.000	22.600	100,00
2001	11.000	0	6.000	1.200	400	5.000	4.000	27.600	122,12
2002	14.000	0	5.000	1.800	400	5.900	4.000	31.100	137,61
2003	14.300	0	6.050	2.046	825	3.454	1.870	28.545	126,31
2004	14.000	0	5.600	1.800	1.100	6.000	5.000	33.500	148,23
2005	18.000	0	1.500	1.300	3.850	5.470	5.500	35.620	157,61
2006	12.800	0	250	600	800	1.100	1.800	17.350	76,77
2007	15.950	50	300	733	214	0	1.000	18.247	80,74
2008	16.000	800	400	951	250	0	3.500	21.901	96,91
2009	19.500	1.400	130	1.341	120	50	6.300	28.841	127,62
2010[2)]	21.000	1.900	100	1.021	250	40	6.500	30.811	136,33
$\bar{x}$ (2006/10)	17.050	830	236	929	327	238	3.820	23.430	103,67
%	72,77	3,54	1,02	3,97	1,39	1,01	16,30	100,00	-

[2)] Semiofficial data

Tobacco production in Bulgaria is characterized with big oscillations, but it still maintains certain level, which is due to the state policy and payment of subsidies. According to data presented in Table 3, the average production in the last five years approximated 23.430 tons, with high oscillations. In Bulgaria, the situation is very similar to that in Turkey and Greece: there is a tendency of restructuring of tobacco types and varieties, i.e. reduction of semi-oriental tobaccos, which are characterized by larger and less aromatic leaves. There is insignificant production of the types Krumovgrad K, Nevrokop, Istocen Balkan (East Balkan) and other oriental tobaccos. More stable is the production of the type Severna Bugarija (North Bulgaria)– 3.820 tons, which presents 16% of the production. The most frequently produced oriental tobacco in Bulgaria is Krumovgrad, with a share of 72,77% or almost four thirds of the total production.

Republic of Macedonia is distinguished by tobacco production which consists only of oriental types, unlike other Balkan countries where large-leaf tobacco of the types Virginia and Burley is also present. In Macedonia there were also big oscillations in tobacco production, depending on the state policy. In fact, this production was going to collapse, but with the subsiding policy of the state it has been reestablished, with good opportunities even to increase. This is especially important because there is no alternative crop that would absorb so much labor force and would have such an economic impact as tobacco.

Table 4, Classical and non-classical oriental tobacco production in Macedonia (in tons)

Years	Variety					Total	Index
	Prilep	Yaka	Djebel	Basmak	Otlja		
1995	6.952	5.527	904	0	1.163	14.546	-
2000	12.394	6.169	410	0	546	19.519	100,00
2001	12.698	7.476	336	0	41	20.551	105,29
2002	14.029	9.202	165	0	35	23.431	120,04
2003	10.031	5.225	109	0	0	15.365	78,72
2004	13.104	6.447	215	0	0	19.766	101,26
2005	14.764	7.275	0	1.166	0	23.205	118,88
2006	11.884	6.145	0	1.651	0	19.680	100,82
2007	10.115	4.166	86	1.920	0	16.287	83,44
2008	9.065	4.465	46	2.549	0	16.125	82,61
2009	13.393	6.294	212	3.321	56	23.276	119,25
2010[1)]	19.000	5.000	400	1.000	100	25.500	130,64
$\bar{x}$ (2006/10)	12.691	5.214	149	2.088	31	20.173	103,35
%	62,91	25,85	0,74	10,35	0,15	100,00	-

[1)] Semiofficial data

In relation to the restructuring of tobacco types and varieties in Macedonia, the same tendencies are observed as in other Balkan countries. The highest percentage in the last five years falls on the type Prilep, with average production of 62,91%. It is followed by the type Yaka, which average production is 25,85%. Since 2005, the type Basmak has been added to the list of tobaccos grown in R. Macedonia, with a share of 10,35%, but with a tendency of decreasing. Participation of the types Djebel and Otlia is only symbolic, achieving 0,74 and 0,15%, respectively.

Table 5. Classical and non-classical oriental tobacco production in Albania (in tons)

Years	Variety							Total	Index
	Roskovec -2	Katerini	Llvinë	MS-81	Sheldia	Koplik 23/1	Kabaku-llak 2A		
2005	435	780	360	240	192	216	200	2.423	100,00
2006	448	852	330	210	156	210	300	2.506	103,43
2007	405	900	252	169	225	240	275	2.466	101,77
2008	330	930	206	233	218	218	294	2.429	100,25
2009	270	1.072	168	162	200	188	285	2.345	96,78
2010	262	1.116	192	158	248	190	354	2.520	104.00
$\bar{x}\bar{x}$	358	942	251	195	207	210	285	2.448	101,03
%	14,62	38,48	10,25	7,97	8,46	8,58	11,64	100,00	-

Table 6. Classical and non-classical oriental tobacco production in Turkey, Greece, Bulgaria, Macedonia, Serbia and Albania (in tons)

Years	Balkan countries						Balkan countries, Total	Index
	Turkey	Greece	Bulgaria	Macedonia	Serbia	Albania		
1995/6	224.602	78.670	14.900	14.546	6.797	-	339.515	100,00
2000	197.647	78.713	22.600	19.519	4.949	-	323.428	95,26
2001	141.399	69.880	27.600	20.551	5.309	-	264.739	77,98
2002	156.124	63.781	31.100	23.431	4.679	-	279.115	82,21
2003	108.963	57.792	28.545	15.365	391	-	211.056	62,16
2004	124.017	56.596	33.500	19.766	384	-	234.263	69,00
2005	129.075	52.870	35.620	23.205	254	2.423	243.447	71,70
2006	93.225	21.756	17.350	19.680	220	2.506	154.737	45,58
2007	70.868	21.442	18.247	16.287	277	2.466	129.587	38,17
2008	91.397	20.086	21.901	16.125	300	2.429	152.238	44,84
2009	79.617	20.490	28.841	23.276	322	2.345	154.591	45,53
2010[1)]	52.742	18.700	30.811	25.500	420	2.520	130.693	38,49
$\bar{x}$ 1995/2010	122.473	46.731	25.918	19.771	2.025	-	216.918	63,89
$\bar{x}$ 2006/2010	77.570	20.494	23.430	20.173	308	2.453	144.428	42,54
% 1995/2010	56,46	21.54	11,95	9,12	0.93	-	100,00	-
% 2006/2010	53,71	14,19	16,22	13,97	0,21	1,70	100,00	-
Trend 2011	44.739	10.962	29.030	22.241	417	2.445	107.389	31,07
Trend 2012	33.363	5.819	26.837	21.628	435	2.444	88.082	28,53
Trend 2013	21.990	1	29.940	22.960	475	2.443	75.366	-

Table 7. Average tobacco production expressed in % od World Total production Oriental tobacco

Years	World Total Oriental tobacco production, tons	% Turkey/ World Total Oriental tobacco	% Grecce/ World Total Oriental tobacco	% Bulgaria/ World Total Oriental tobacco	% Macedonia/ World Total production of Oriental tobacco	% Serbia/ World Total Oriental tobacco	% Albania/ World Total Oriental tobacco	% Total production in Balkan countries/ World Total Oriental tobacco
1995	524.000	42,86	15,01	2,84	2,78	1.30	-	64,80
2000	530.000	37,29	14,85	4,26	3,68	0.93	-	61,02
2001	435.000	32,51	16,06	6,34	4,72	1.22	-	60,86
2002	422.000	37,00	15,11	7,37	5,55	1.11	-	66,14
2003	340.000	32,05	17,00	8,40	4,52	0.12	-	62,08
2004	361.000	34,35	15,68	9,28	5,48	0.11	-	64,90
2005	352.000	36,67	15,02	10,12	6,59	0.07	0,67	69,14
2006	268.000	34,79	8,12	6,47	7,34	0.08	0,93	57,73
2007	232.000	30,55	9,24	7,87	7,02	0.12	1,06	55,85
2008	264.000	34,62	7,61	8,30	6,11	0.11	0,92	57,66
2009	273.000	29,16	7,51	10,56	8,53	0.12	0,86	56,74
2010[1)]	248.000	21,27	7,54	12,42	10,28	0.17	1,02	52,70
$\bar{x}$	354.083	33,59	12,40	7,85	6,05	0.46	0,91	60,80

According to the presented data on the oriental tobacco production in the Balkan countries in relation to the world production of this type, it can be stated that Turkey has the largest share, ranging from 21,27% in 2010 to 42,86% in 1995, which is an average of 33,59%. The following country is Greece, with a production of 7,51 in 2009 to 17,00% in 2003. Interesting situation is observed in Bulgaria and Macedonia, with a share ranging respectively from 2,84 and 2,78% in 1995 to 12,42% and 10,28% in 2010. The share of Serbian oriental tobaccos significantly dropped after 2003, both as a result of the transition period for tobacco industry in this country and of the lack of interest among the new owners (Philip Morris, British American Tobacco –BAT, etc.). The average production in Albania is 2453 tons or about 1,7% of the total production of oriental tobaccon in the Balkan.

Among all Balkan countries, Serbia had the smallest share in the total world production of oriental tobacco in the investigated period, ranging from 0,07% in 2005 to 1,30% in 1995/1996. In the total production of oriental tobaccos in the Balkan, the share of Serbia is only 0,22 - 0,93%. After the period of transition in Serbia, there is only one company to purchase and process oriental tobacco, mainly of the variety Prilep P-156.

Actually, even the share of Bulgarian and Macedonian oriental tobacco is not increased as much as it seems; such impression is obtained because the world production of oriental tobacco is in decline, especially in Turkey and Greece. The average share of oriental tobacco from the six Balkan countries dropped from 64,80% in 1995 to 52,70% in 2010.

Summary values on the production of oriental tobaccos in the six Balkan countries-producers of the best quality oriental tobaccos in the world, are presented in Table 6. The data reveal the average movement of production in these countries in the last six years and throughout the whole period of investigation and the trend for the years 2011, 2012 and 2013.

They also show a significant decline of the oriental tobacco production in Turkey and Greece. Big oscillations in this production were observed in Bulgaria and Macedonia, but subsiding policy helped to maintain it at an acceptable level, although still below the multiple-year averages. Unofficially, the production of oriental tobacco in 2010 in the six Balkan countries is about 130.693 tons, which is 38,49% of that achieved over ten years ago.

CONCLUSIONS

Based on our analysis, it can be stated that production of oriental tobacco in Turkey and Bulgaria was reduced for over four times, while in Bulgaria and Macedonia it is maintained at acceptable level, although still below the multiple-year averages. The reason for this decline in Turkey is due to the shift from tobacco toward olive growing but also to the development of tourism in the Aegean part of Turkey, in the region of Izmir, and to the outflow of young people to Western countries for a better and more secure existence. The main reason for the decline in Greece is the abolishment of the EU tobacco subsidies, which values exceeded the price for purchased oriental tobacco. In Bulgaria and Macedonia, production of oriental tobacco has been maintained due to the subsidies provided by the governments of these two countries. In Serbia, the main reason for decrease is the lack of interest of the new owners to produce oriental tobacco.

Another phenomenon to be emphasized is the restructuring of the type assortment of oriental tobaccos and elimination of the semi-oriental and the lower quality tobaccos.

Macedonia and Bulgaria, supported by the governments subsidy policy, should increase their production of oriental tobaccos because the production in Greece and Turkey will continue to decrease.

The trend in production of oriental tobaccos in the investigated Balkan countries shows further linear decline.

We must also mention the global changes and shifting of tobacco production to developing countries, because they will have a great impact on production of oriental tobacco in the Balkan countries.

REFERENCES

1. Analyses of the Ministry of Agriculture, Forestry and Water economy of the Republic of Macedonia
2. Analyses of the Ministry of Agriculture, Forestry and Water economy of Serbia
3. Analyses of AD Yugotutun. Skopje
4. Analyses of Scientific Tobacco Institute. Prilep.
5. Data. www.Socotab
6. Data. www. Universal Leaf Tobacco Company
7. FAO, 2011. FAOSTAT Statistical databases. Rome
8. FAO 2003. Projections of tobacco production, consumption and trade to the year 2010. Food and Agricultural Organization of the Uniteted Nations, Rim,2003.
9. Филипоски К.,2000. Распространетост и динамика на производството на тутун во светот и во република Македонија. Тутун/Tobacco, Прилеп, Год.50, бр. 4-6, p. 94-108.
10. Филипоски К., Митрески М., Христоски Ж., 2000. Производство на тутун во прилепскиот тутунопроизводен реон. Тутун/Tobacco, Прилеп, Год.50,бр.9-10, p.221-234.
11. Филипоски К., Пешевски М., Митрески М., 2003. Можности за надминување на состојбата и проблемите во тутунското стопанство. Тутун/Tobacco, Прилеп, Год.53, бр. 3-4, p.117-123.
12. Филипоски К., Пешевски М.,Стојаноска С. 2004. Реетаблирање и прилагодување на производството на тутун во Р. Македонија кон Европската Унија. Советување на агроекономисти “Одржлив развој на агрокоплексот – придонес кон Европската интеграција“ Јуни. Скопје.
13. Филипоски К., 2011. Статистички методи во земјоделските истражувања. Одбрани поглавја. Прилеп.
14. Marić N., Ralević N., Filipović L.,2002. Poslovna statistika. Beograd.
15. Peševski, M., Filiposki K., 2002., Sexual and age structure of the participants in the production of oriental type of tobacco on family farms in the Republic of Macedonia. Macedonian association of agricultural economists and Ministry of Agriculture, forestry and water economy. Paperworks from First scientific meeting of Balkan’s Agricultural Economists: Cooperation between developing Balkans countries of Agriculture and food production, 306-314, Skopje.

UDC 633.71
Тутун/Tobacco, Vol.60, Nº 7-12, 103-111, 2010
University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute – Prilep, R. Macedonia

ISSN 0494-3244

UDC: 632.488.43
633.71--248.843
Original scientific paper

MORPHOLOGICAL CHARACTERISTICS OF THE CAUSAL AGENT OF BROWN SPOT DISEASE IN NATURAL CONDITIONS

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ABSTRACT

Alternaria alternata is a variable species which morphological characteristics highly depend on the effect of environmental factors. Measurements are usually made on pure culture of the fungus, but the aim of these investigations was to give data on morphological characteristic of the species in naturally infected tobacco leaves.

Microscopic measurements revealed that in this case the hyphae of *A. alternata* are thinner, while conidiophores are wider and longer compared to those in pure culture. It was also determined that the conidial size is larger and the number of transverse and longitudinal septa is higher. Oblique septa are also present. The chains are longer and consist of 3-7 conidia.

Key words: *Alternaria alternata***,** tobacco

МОРФОЛОШКИ КАРАКТЕРИСТИКИ НА ПРЕДИЗВИКУВАЧОТ НА БОЛЕСТА КАФЕНА ДАМКАВОСТ ВО ПРИРОДНИ УСЛОВИ

Alternaria alternata е доста варијабилен вид, чии морфолошки карактеристики можат да зависат од влијанието на еколошките фактори. Најчесто, мерењата се вршат во чиста култура од габата. Но, целта на овие истражувања беше да се дадат податоци за нејзините морфолошки карактеристики во природни услови, односно во инфицирани тутунски листови.

При микрскопските мерења беше утврдено дека во овој случај таа има потенки хифи, а конидиофорите се пошироки и подолги отколку во чиста култура. Исто така и конидиите се со поголеми димензии, со поголем број напречни и надолжни септи, а имаат и коси септи. Формираат подолги низи, со 3-7 конидии.

Клучни зборови: *Alternaria alternata*, тутун

INTRODUCTION

Alternaria alternata is the most frequently mentioned and studied among all *Alternaria* species. It is a saprophyte feeding on dead plants, stored products, soil, skin and textile, an epiphyte on leaves and a parasite on living plants. *A. alternata* is mostly a pathogen of weakness which in suitable moments attacks the susceptible and stressed plants. In some cases, however, it can attack the healthy and vigorous plants too (Rotem, 1994).

Almost all Alternaria infected cultures can be also attacked by *A. alternata*. Thus, plants of the Solanaceae family, beside with *A. solani*, can be infected with *A. alternata* on tomato (Grogan et al., 1975), peppers (Halfon-Meiri and Rylski, 1983) and potato (Cakarevic and Boskovic, 1994).

A. alternata has been recorded on at least 115 host-plants in 43 families. 74% of the publications refer to 14 host-plants, of which tobacco is the most frequently mentioned (in 13% of all publications) (Rotem, 1994).

A. alternata is the causal agent of brown spot disease and it attacks all types of tobacco. The intensity of attack depends on climate conditions, susceptibility of the varieties and many other factors during tobacco production (Gveroska and Taskoski, 2006, 2007).

Typical for this disease is the occurrence of brown spots on leaf surface. As the infection spreads, their centers become brown and concentric rings appear around them. With maturation of leaves a yellowish chlorotic zone is formed around the spots. In periods with high humidity the sporulation is abundant and organs of the pathogen appear in the middle of the spots, forming black powdery cover.

The increased chlorotic zone and total infected area results in reduced humidity, alkaloids and reducing sugars in the leaf (Main, 1971). The increase of disease intensity results in higher nicotine (21 - 31%), lower reducing sugars (1%) and higher filling capacity (51%) compared to the healthy tobacco (Karunakara, 1998).

According to Spurr and Main (1974), the necrosis includes gradual complexication among the oxidized phenols, amino acids and proteins. Beside its direct impact on leaf quality and yield, the pathogen also affects the smoking properties of leaf, reflected in less pronounced aroma and "sweet" taste, and strongly expressed "empty" taste.

Tobacco plant is a suitable substrate for development of this pathogen during the vegetation period and later, in the period of curing and processing. The fungus was isolated from molds typical for storehouses, but it was also found in cigarettes (Kantor et al, 1979).

A. alternata consists of species which release specific toxins with high pathogenocity to certain host-plants. For this reason, Otani and Khmoto (1992) and Rotem (1994) suggested that they should be marked as specialized forms of *A. alternata*. For example, the species marked *A. alternata f. sp tabaci* releases the AT toxin, which is pathogenic to tobacco.

According to Kodama et al. (1990), the purified AT toxin of 0.2 μg/ml inhibits the root development in tobacco seedlings and that of 1-2 μg/ml provokes necrosis on each leaf. All isolates to which this toxin was added, including the spores of the non-pathogenic ones, cause brown spot disease on the inoculated plants. The results show that AT toxin is a distinguishing factor in the pathosystem of the genus *Nicotiana* – *A. alternata*.

The aim of investigations was to give data about morphological characteristics of *A. alternata* on infected tobacco leaves in natural conditions.

MATERIAL AND METHODS

Infested leaves were sampled from 3 tobacco varieties, with severe attack of brown spot disease, i.e. the sporulation was abundant and spots were already covered with black powder.

The fungus morphology was investigated by microscopic measurement of hyphae width and the size (l/w) of conidiophores and conidia.

The material was collected from leaf spots using a sterile needle and 5 microscopic preparations were made for each variety.

Measurements were made on 100 randomly selected samples using ocular micrometer, previously calibrated, according to the method of Ziberoski (1998). Transverse, longitudinal and oblique septa were also counted.

RESULTS AND DISCUSSION

Hyphae of the fungus are septate and they are branching laterally, under different angles. In preparations they are less prominent compared to conidia and conidiophores. They are transparent to light brown, 2.35 - 4.80 µm wide, or 3.87 µm in average (Table 1).

Table 1. Hyphal width of *A. alternata* (µm)

Variety	Width	
	range	average
MV 1	2,35 - 4,00	3,93
O 110	3,20 - 4,00	3,64
P 23	3,20 - 4,80	4,06
Average	2,35 - 4,80	3,87

The width of conidiophores ranges from 2.00 µm to 6.00 µm, or 3.80 - 4.51 µm in average (Table 2). Their length ranges from 16.00 µm to 56 µm, or 32.80 - 45.33 µm in average.

The lowest size of conidiophores from the spots of infected leaves was recorded in MV1variety.

Thus, it can be stated that the size of conidiophores in natural conditions is (2,00 x 6,00)µm x (16.00 - 56.00) µm, or in average 4.19 x 37.95 (Table 2).

Table 2. Dimensions of conidiophores of *A. alternata* (µm)

Variety	width		length		width x lenght
	from - to	average	from - to	average	
MV 1	2,00-6,00	3,80	16,00-56,00	32,80	3,80 x 32,80
O 110	3,20 -6,00	4,27	32,00-47,00	45,33	4,27 x 45,33
P 23	3,20 -6,00	4,51	16,00-52,00	35,71	4,51 x 35,71
Average	2,00- 6,00	4,19	16,00-56,00	37,95	4,19 x 37,95

Conidia are usually single or they can form a chain consisting of higher number of conidia (3-8) (Photo 1).

Photo 1. Conidia of *Alternaria alternata* in chain

Data presented in Table 3 reveal that conidia width ranged from 10.00 µm to 24.00 µm, and the average range was 16.00 - 19.01 µm.

The conidia length ranged from 44.00 µm to 96 µm, and the average values are similar - 61.27 to 65.66 µm.

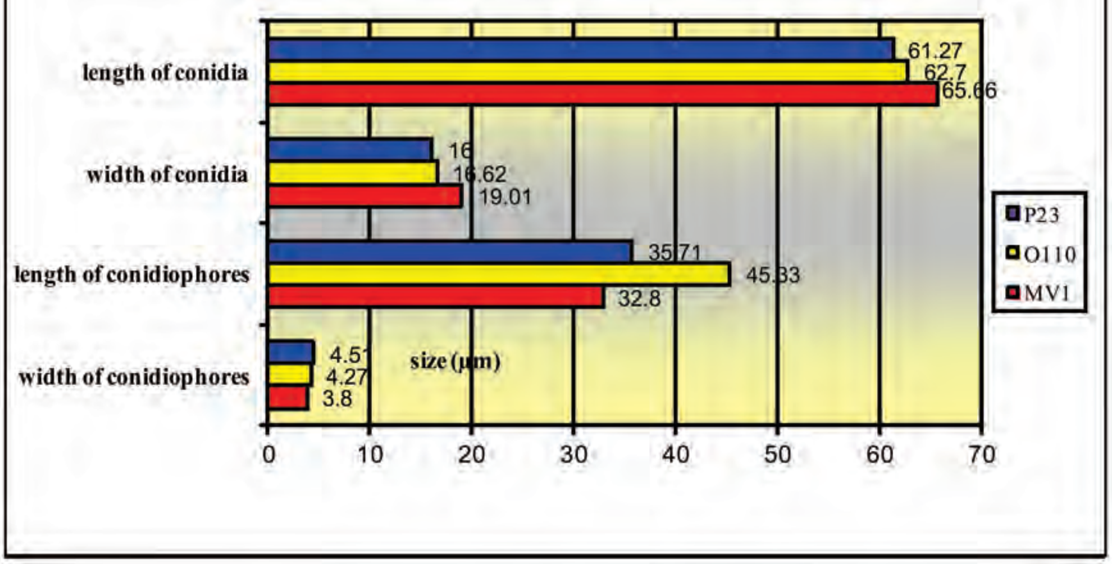
Table 3. The size of *A. alternata* conidia (µm)

Variety	width		length		width x length
	from - to	average	from - to	average	
MV 1	15,20- 24,00	19,01	50,00-92,00	65,66	19,01 x 65,66
O 110	10,00 -24,00	16,62	44,00 -96,00	62,70	16,62 x 62,70
P 23	12,00 -20,00	16,00	52,00 -74,00	61,27	16,00 x 61,27
Average	10,00 -24,00	17,21	44,00 -96,00	63,21	17,21 x 63,21

According to the data presented, conidial size varies in a wide range from 10.00 - 24.00 x 44.00 - 96.00 µm, with average values of 17.21 x 63.21 µm.

The largest conidia were observed on the infected leaves of MV1 variety. (Table 3, Figure 1).

Graph 1. Dimensions of conidiophores and conidia of *A. alternata*



Conidiphores and conidia of *Alternaria alternatra* in natural conditions are presented in Photo 2, 3 and 4.

Conidia have 3-8 transverse septa and 1-6 longitudinal septa. They also have up to four oblique septa (Table 4).

Major percentage of conidia have 5-6 transverse and 2-3 longitudinal septa, and 1-2 oblique septa (Table 5, Fig.2).

Table 4. Number of septa in conidia

Variety	Transverse septa	Longitudinal septa	Oblique septa
MV 1	3 - 8	1 - 6	0 - 4
O 110	5 - 8	1 - 6	0 - 2
P 23	1 - 7	1 - 5	1 - 3
Average	3 - 8	1 - 6	0 - 4

Table 5. Pecentage of conidia with different number of septa

a) Transverse septa

Variety	Number of septa					
	3	4	5	6	7	8
	conidia %					
MV 1	20,00	0,00	46,66	13,33	6,66	26,66
O 110	0,00	0,00	21,42	64,28	0,00	14,28
P 23	8,33	8,33	25,00	33,33	25,00	0,00
Average	9,44	9,44	31,03	36,98	10,55	13,65

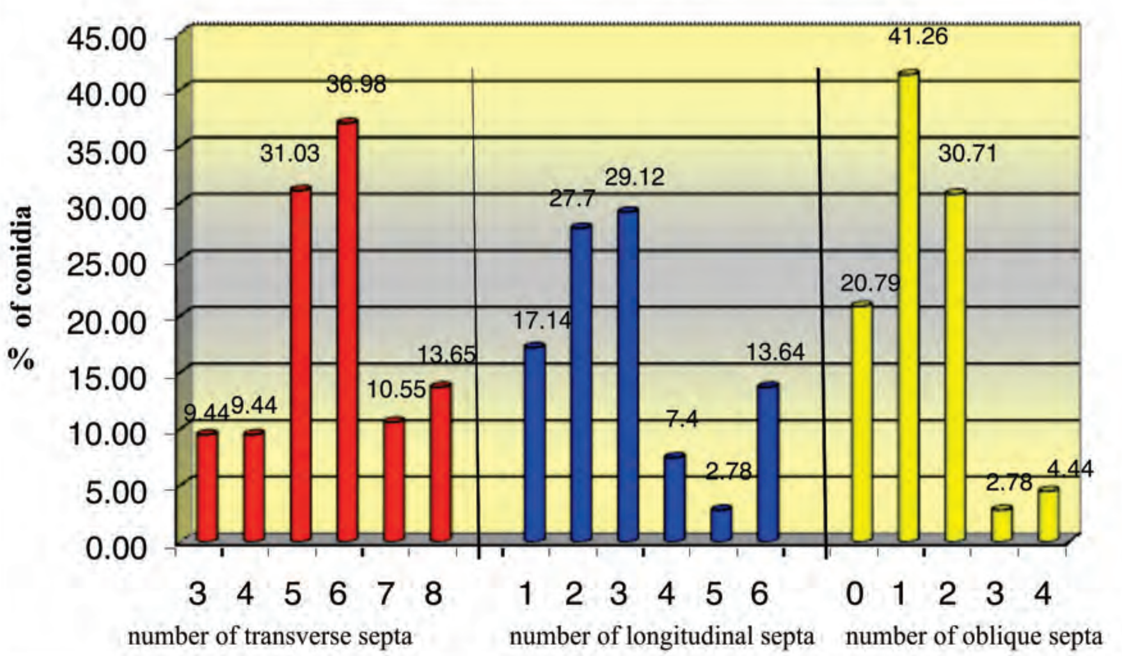
b) Longitudinal septa

Variety	Number of septa					
	1	2	3	4	5	6
	conidia %					
MV 1	13,33	20,00	26,66	6,66	0,00	26,66
O 110	21,43	21,43	35,71	7,14	0,00	14,28
P 23	16,66	41,66	25,00	8,33	8,33	0,00
Average	17,14	27,70	29,12	7,40	2,78	13,64

c) Oblique septa

Variety	Number of septa				
	0	1	2	3	4
	conidia %				
MV 1	26,66	33,33	26,66	0,00	13,33
O 110	35,71	57,14	7,14	0,00	0,00
P 23	0,00	33,33	58,33	8,33	0,00
Average	20,79	41,26	30,71	2,78	4,44

Graph 2. Percentage of conidia with various number of septae



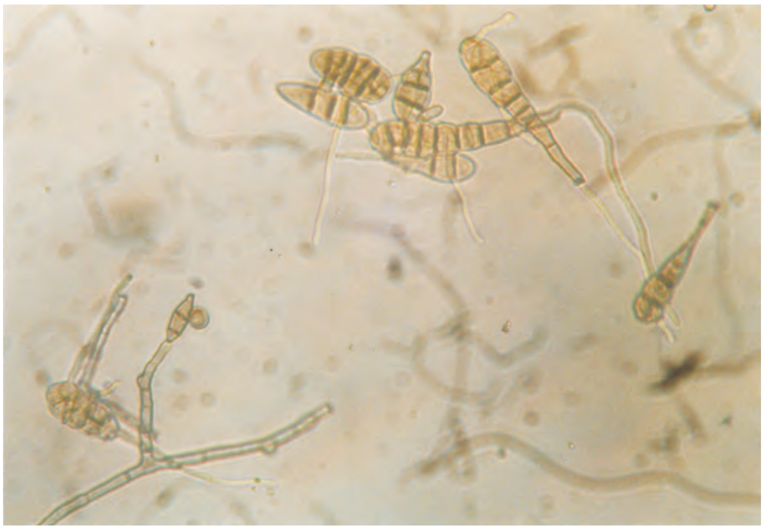


Photo 2. Conidiophores and conidia of *A. alternata* on infected leaves

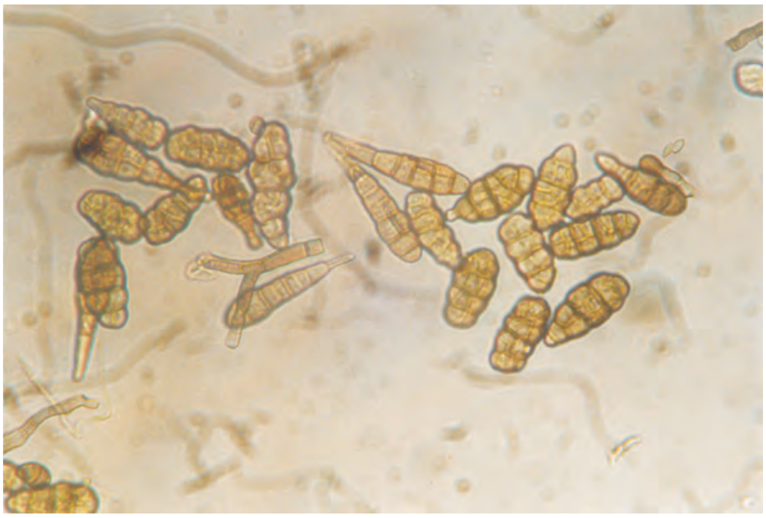


Photo 3. Conidia of *A. alternata* on infected leaves

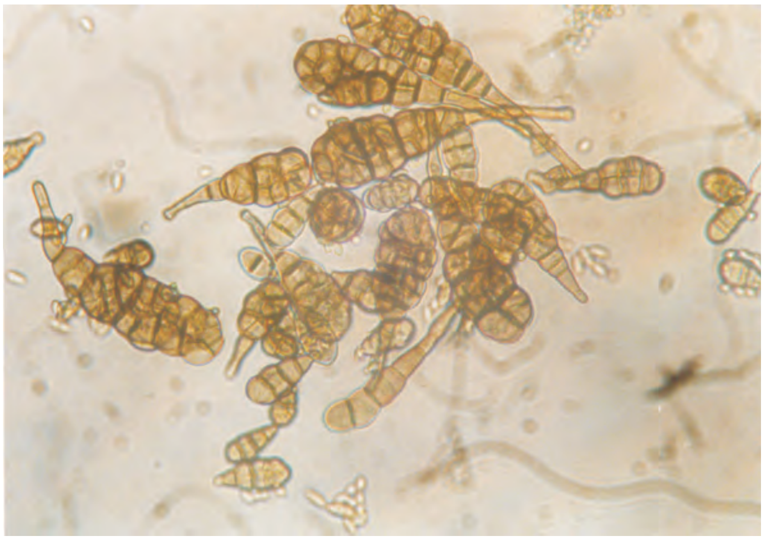


Photo 4. Conidia of *A. alternata* on infected leaves

Morphological data for *A. alternata* on naturally infected plant material are in compliance with the results of Rotem (1994) and Misaghi (1978).

Compared to hyphae of the pure culture (Gveroska and Taskoski, 2009), it can be noted that hyphae of the fungus are thinner and conidiophores are wider and longer. The conidia are single, but they can also form chains containing of higher number of conidia with transverse, longitudinal or oblique septa. According to Misaghi et al. (1978), environmental factors have a significant effect on conidial size.

Su and Sun (1981) determined polymorph conidia in infected tobacco leaves. They had golden brown bodies with light brown to transparent beaks, long chains (10 conidia or more) which are sometimes branched, with transverse, longitudinal and oblique septa. They are also larger compared to the ones from the culture grown on media.

CONCLUSIONS

- In naturally infected tobacco leaves, *Alternaria alternata* created hyphae which were 2,35 - 4,80 μm wide (3,87 μm in average).
- Conidiophore width ranged 2,00 - 6,00 μm and length 16,00 - 56,00 μm. Their average size was 4,19 μm x 37,95 μm.
- Conidial width ranged between 10,00 and 24,00 μm and length between 44,00 and 96,00 μm. Their average size was 17,21 x 63,21 μm.
- Infected tobacco leaves of MV1 variety had the lowest size of conidiophores and the largest size of conidia.
- Number of septa per conidium ranged as follows: 3-8 transversal, 1-6 longitudinal and 0-8 oblique. The highest number of conidia have 5-6 transversal, 2-3 longitudinal and 1-2 oblique septa.
- *Alternaria alternata* in naturally infected tobacco leaves has thinner hyphae and conidiophores are wider and longer compared to those in pure culture. Also, the conidial size is larger and they have higher number of transverse, longitudinal and oblique septa. They form longer chains, consisting of 3-7 conidia.

REFERENCES

1. Cakarevic V., Boškovic T., 1993. Alternaria alternata (Fries) Keissle (sin.A.tenuis Ness., sin.A.longipens (Ell.and Ev.)Meison), potatoes parasite in Yugoslavia. http://www.actahort.org/books/462/462-153.htm
2. Гвероска Б.,Ташкоски П., 2006. Реакција на тутунските сорти кон болеста кафена дамкавост. Тутун / Tobacco, Vol.56, No 7-8, 138-146.
3. Гвероска Б.,Ташкоски П., 2007. Фактори кои влијаат врз појавата и ширењето на болеста кафена дамкавост кај тутунот. Тутун / Tobacco, Vol.57, No 9-10, 209-216.
4. Гвероска Б.,Ташкоски П., 2009. Морфолошки карактеристики на предизвикувачот на болеста кафена дамкавост во чиста култура
5. Grogan R.G., Kimble K.A., Misaghi I., 1975. A Stem Canker Disease of Tomato Caused by Alternaria alternata f.sp. lycopersici. Phytopathology 65:880-886.
6. Halfon –Meiri A., Rylski I., 1983. Internal mold caused in sweet peper by Alternaria alternata: Fungal ingress. Phytopathology 73:67-70.
7. Kantor D., Toth-Aranuos I., Perercsenyi E., 1979. Characteristics of the microbiological state of tobacco products. Acta Aliment., 1979, 8-1, p.13-24.
8. Karunakara K. M., Shenoi M.M., Sreenivas S.S., 1998. Assessment of crop loss due to brown spot disease in FCV tobacco. IPS Symposium. Dec., UAS Bangalore, India.
9. Kodama M., Suzuki T. Otani H.,&al., 1990.

Purification and bioassay of host-selective AT-toxin from Alternaria alternata causing brown spot of tobacco. Nippon Shokubutshu Byori Gakkaiho = Ann. Phytopathol. Soc. Jpn, 56-5, p.628-36., ISSN.0031-9473.
10. Main C.E., 1971. Pathogenesis and Halo Formation of the Tobacco Brown Spot Lesion. Phytopathology 65:1347-1443.
11. Misaghi I.J., Grogan R.G., Duniway J.M., Kimble K.A., 1978. Influence of Environmental and Culture Media on Spore Morphology of, Pathogenicity and Cultural Characteristics of Alternaria alternata. Phytopathology 68: 29-34.
12. Otani H., Kohmoto K. / Chelkowski J. and Wisconti A., 1992). Alternaria Biology, Plant Diseases and Metabolities. Elsevier, Amsterdam-London-New York-Tokyo.
13. Rotem J., 1994. The genus Alternaria. APS PRESS. St. Paul, Minnesota.
14. Spurr H.W., Main C.E., 1974. Brown pigment formation in tobacco leaves infected with Alternaria. Phytopathology 64-5, p.738-45.
15. Зибероски Ј., 1998. Практикум по микробиологија. Универзитет Св. Климент Охридски, Земјоделски факултет, Скопје

UDC 633.71
Тутун/Tobacco, Vol.60, N^{o} 7-12, 112-120, 2010
University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute – Prilep, R. Macedonia

ISSN 0494-3244

UDC: 633.71.248.114.6
632.481.146:633.71
Original scientific paper

TOBACCO REACTION TO TOXIC METABOLITES OF THE PATHOGEN *PHYTOFTHORA PARASITICA VAR. NICOTIANAE*

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ABSTRACT

In phytopathological laboratory of Tobacco Institute-Prilep *in vitro* investigations were made on the presence of toxic matters in inoculum of the pathogen *Phytophthora parasitica var. nicotianae* and their impact on tobacco plant, to determine whether these toxic metabolites cause necrotic reactions and other damages on the host-plant. Comparison was made between symptoms resulting from the effect of toxic metabolites and symptoms caused by the pathogen itself. Inoculum was prepared from suspension of fungus mycelium and filtrate as selective agent obtained from the fungus grown on liquid medium and after that filtrated through filter paper and bacteriological filter of 0.22 μ porosity. Leaves of *Nicotiana nesophila* species and leaves of two oriental and one Burley variety of *Nicotiana tabacum* were used in the investigation. Inoculation was made by two methods: adding a drop of the inoculum on leaf surface and submerging the base of the leaf in the inoculum. In leaves inoculated by both methods, differences in necrosis development on leaf surface could be noticed. The necrosis developed more rapidly in leaves inoculated with a suspension of pathogen's mycelium, where percentage of damaged leaf tissue was also higher compared to the leaves inoculated with selective agent. There were no differences, however, between leaf symptoms caused by toxic metabolites and those caused by the pathogen itself. The aim of the investigation was to determine the infectivity of toxic compounds released by the pathogen and the effects of two methods of inoculation on the percentage of damaged leaf tissue.

Key words: tobacco, pathogen, toxins, inoculum, suspension

РЕАКЦИЈА НА ТУТУНОТ СПРЕМА ТОКСИЧНИТЕ МЕТАБОЛИТИ ОД ПАТОГЕНОТ *PHYTOFTHORA PARASITICA VAR. NICOTIANAE*

Во фитопатолошката лабораторија како и во *in vitro* лабораторијата при Научниот институт за тутун од Прилеп, вршени се испитувања за присуството на токсични материи во инокулумот од патогенот *Phytophthora parasitica var. nicotianae* како и на нивното влијание врз тутунското растение, односно дали овие токсични метаболити причинуваат одредени некротични реакции или други оштетувања кај растението-домаќин. Направена е споредба на симптомите коишто се појавија како резултат на влијанието на токсичните метаболити и симптомите причинети од самиот патоген. Како инокулум беа користени суспензија подготвена од мицелија на габата и филтрат како селективен агенс добиен од габата одгледувана на течна хранлива подлога, кој потоа е филтриран низ филтер-хартија и бактериолошки филтер со порозност од 0,22 микрона. За испитување беа користени листови од видот *N. nesophila* и листови од две ориенталски и една берлејска сорта од *N. tabacum*. Инокулирањето е извршено со поставување на капка од инокулумот на површината од листот и со потопување на основата од листот во користениот инокулум. Кај инокулираните листови по двете методи (со капка и со потопување) и со двата инокулума, можеше да се забележи разлика во развојот на некрозата која се појавуваше по површината на листовите. Некрозата се ширеше многу побрзо кај листовите кои беа инокулирани со суспензија подготвена од мицелија на патогенот, каде и процентот на оштетено лисно ткиво беше повисок, а побавно кај листовите инокулирани со селективниот агенс. Симптомите појавени по површината на листовите причинети од токсичните метаболити на патогенот не се разликуваа од оние кои беа причинети од самиот патоген. Целта во ова истражување беше да се провери инфективноста на токсичните материи што ги ослободува патогенот и разликата во оштетената лисна површина причинета од двата користени инокулума.

Клучни зборови: тутун, патоген, токсини, инокулум, суспензија

INTRODUCTION

Black shank is one of the most devastating diseases in tobacco and a serious threat to tobacco production all over the world. It can be found in all tobacco producing countries and, depending on climate conditions and host resistance, it can cause severe losses of tobacco yield. The causing agent of the disease is phytopathogenic fungus *P. parasitica var. nicotianae*. It is a soilborn pathogen which infects the plant through its root, and symptoms occur along the stem. In the beginning, infected plants suddenly lose their turgor and wilt, and later necrosis appears at basal stem region, spreading along its length. The root system becomes dark, leaves turn to yellow, wilt and shrivel along the stem which becomes brown-black in color, with only few green leaves remaining on the top of the plant (Taskoski, 2003). Losses of tobacco can be more severe in rainy years and in irrigating conditions.

According to Wolf (1954, cit. Lucas 1975), symptoms of wilting are caused by systemic effect of toxins created by the pathogen itself. According to Powers (1954, cit. Lucas 1975), wilting is a result of prevention of water and nutrients movement through plant vessels, as well as of the presence of rubber matters and tylosis, created during decomposition of cells attacked by the pathogen. Subject of our investigation was to find which of these factors have a stronger impact on the process of tobacco wilting .

During development of pathogens, smaller or greater pathological changes occur in leaf tissue. They are usually manifested through necroses or wilting of certain parts of the plant or even of the whole plant. According to Cutova (1983), toxins themselves can cause disease symptoms similar to those of the natural infections and reaction of some varieties to the toxins does not differ from the reaction to the pathogen.

Toxins are metabolitic products of the pathogenic microorganisms which can cause death of cell protoplasts. The process of cell intoxication itself has a harmful effect upon living parts of the cell, having a greater influence on its biochemical and physiological activities than on its structure. Phytopathology literature presents data on the existence of high number of toxins. Some of them are specific, but the importance of non-specific toxins is of equal importance (Sutic, 1986).

The main task of our investigation was to find out whether the pathogenic fungi *P. parasitica var. nicotianae* produce metabolites which would be capable of infecting plant tissue just as the pathogen itself, to study the mode of their activity and to identify eventual differences in symptoms obtained by both inoculation methods.

MATERIAL AND METHODS

Leaves of *N. nesophila* species, which is highly resistant to the pathogen, and leaves of two oriental (P 12-2/1 and P 23) and one Burley (B2) varieties were used in the trial, while non-inoculated leaves of P 12-2/1 were used as a check. Suspension made of fungal mycelium and selective agent obtained from the fungus by filtration was used as inoculum. Inoculation was performed in two variants: by adding a drop of the inoculum on leaf surface and by submerging the base of the leaf in the inoculum. Four replications were made for each variant. Two of the variants were inoculated with suspension and the other two with selective agent. Five healthy middle belt leaves from each variety were prepared for each variant. Prior to inoculation, each leaf area was measured by the formula P=a.b.K, where the middle value (0.63) was used as correction factor (K) (Saric et co., 1990).

Pure *P. parasitica var. nicotianae* culture was used for inoculum preparation. The fungus was previously isolated from infected tobacco plants on oat agar medium, by standard laboratory method. The culture was grown 15-20 days at 25^0C.

Suspension of the fungal mycelium was prepared from the culture on Petri dish and blended in 100 ml distilled water. The suspension prepared in this way was used for inoculation of tobacco leaves.

In the first variant, leaf surface was injured with sterile needle prior to inoculation and a drop of the suspension was added in the injured area. Inoculated leaves were stored 15 days in a wet chamber. Leaves which served as a check were inoculated in the injured area byadding a drop of sterile distilled water.

In the second variant, the base of tobacco leaves was submerged in 20 ml mycelial suspension, and check leaves were submerged in 20 ml sterile distilled water.

For investigation of the selective agent infectivity, Ppn was grown on liquid nutrient medium by the method of Slavov (2002). The liquid medium was passed through filter paper and bacteriological filter of 0.22 μ porosity, followed by filtrate concentration and another filtration through bacteriological filter. The concentrated filtrate of the culture presented a selective agent.

The mode of work with selective agent was the same as that with mycelial suspension.

In the first variant with drop inoculation the selective agent was used undiluted, and in the second variant the selective agent was diluted with sterile distilled water in 1:10 ratio, where the leaves were placed.

Estimation and measurement of necrosis development on leaf surface was made on the 2nd, 4th, 9th, 11th and 14th day of inoculation. In leaves inoculated with drop, the necrosis diameter was measured, and in leaves submerged in the inoculum the length of necrosis was measured from the leaf base, i.e from the starting point of necrosis to its end. Based on measurements of necrotized leaf tissue, the percentage of damaged leaf tissue was estimated. Results of the investigations are presented as a mean value of all replications.

RESULTS AND DISCUSSION

Necrosis was observed on leaf surface in both types of inoculation, with certain differences in its growth and size, depending on the type of inoculum.

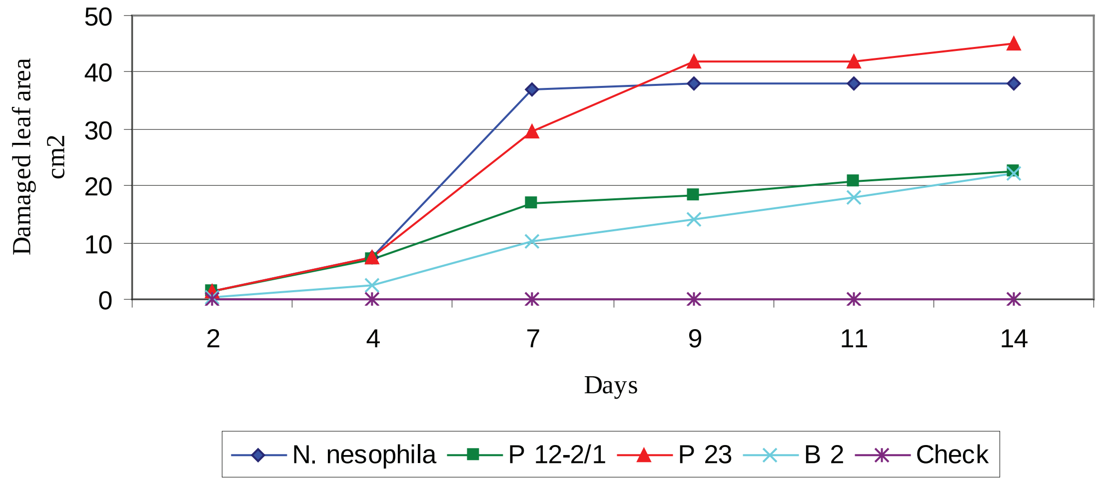
Results on occurrence and growth of necrosis in leaves inoculated with drop of suspension on leaf surface are presented in Table 1 and Figure 1. As soon as the second day of inoculation, necrosis of about 1.50 cm in size was observed in all inoculated leaves, which developed with different intensity during the period of observation. It should be emphasized that necrosis developed quite rapidly in inoculated leaves of *N. nesophila*, a species which is otherwise known as resistant to this pathogen (Taskoski, 2005).

Table 1 - Leaves inoculated with a drop of mycelium suspension

Species-Tob. variety	Leaf size, l/w	Leaf area cm^2	Spread of necrosis by days, in cm^2					
			2	4	7	9	11	14
N. nesophila	9.5/6.5	38.90	1.50	7.30	36.85	37.95	37.95	38.11
P 12-2/1	9.0/6.0	34.02	1.50	7.05	17.01	18.27	20.79	22.52
P 23	13.2/7.2	59.87	1.50	7.56	29.69	41.89	41.89	45.21
B 2	12.9/6.6	53.64	0.51	2.31	10.29	13.91	17.91	22.28
Check P 12-2/1	15.1/7.5	71.34	-	-	-	-	-	-

On the 14th day of observation, the following level of necrosis was observed: 45.21cm^2 of leaf tissue was damaged in variety P 23 , 38.11 cm^2 in *N. nesophila* species, 22.52cm^2 in P12-2/1 and 22.28 in B2 cm^2. No symptoms of necrosis were observed in leaves that were used as a check.

Fig.1 Necrosis development



On the basis of damaged leaf tissue, the level of infection was estimated, and it ranged fro 41.53 in B2 variety to 97.96% in *N. nesophila*. Varieties P 23 and P 12-2/1 (Table 2) also showed high level of infection of leaf tissue, ranging from 75.51% and 66.19% (Table 2).

Table 2 - Level of infection in leaves inoculated with a drop of mycelium suspension

Species-tob. variety	Leaf size, l/w cm	Leaf area cm^2	Damaged leaf area cm^2	Infected leaf tissue %
N. nesophila	9.5/6.5	38.90	38.11	97.96
P 12-2/1	9.0/6.0	34.02	22.52	66.19
P 23	13.2/7.2	59.87	45.21	75.51
B 2	12.9/6.6	53.64	22.28	41.53
Check P 12-2/1	15.1/7.5	71.34	0.00	0.00

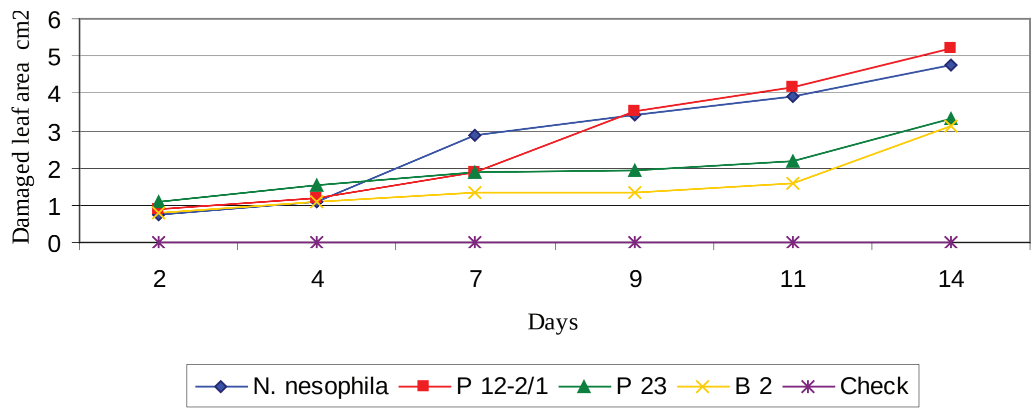
Tissue of the leaves inoculated with a drop of the selective agent was also damaged. Necrosis was observed even on the second day of inoculation, only to somewhat lesser extent (Table 3, Fig. 2). During all days of observation, necrosis was spreading almost identically in all inoculated leaves, and on the 14th day the heaviest damage was measured in P 12-2/1 (5.23 cm^2), N. nesophila (4.762 cm^2) and P 23 and B2 (3.12 cm^2).

In this case, too, the check leaves remained healthy and free of damage.

Table 3 - Leaves inoculated with a drop of selective agent

Species-Tob. variety	Leaf size l/w cm	Leaf area cm^2	Spread of necrosis by days, in cm^2					
			2	4	7	9	11	14
N. nesophila	8.8/5.6	31.05	0.73	1.07	2.88	3.41	3.93	4.76
P 12-2/1	14.3/7.1	63.96	0.89	1.20	1.87	3.50	4.17	5.23
P 23	13.0/7.0	57.33	1.10	1.56	1.86	1.92	2.19	3.30
B 2	9.9/5.3	33.05	0.81	1.08	1.34	1.36	1.60	3.12
Check P 12-2/1	15.5/9.3	90.81	-	-	-	-	-	-

Fig.2 Necrosis development



Considering that in this variant lower damage of leaf surface was observed, the infection percentage was lower, too. The highest percentage of infection was measured in *N. nesophila* species (15.33%), and the lowest in P 23 variety (5.75%).

Table 4 - Level of infection in leaves inoculated with a drop of selective agent

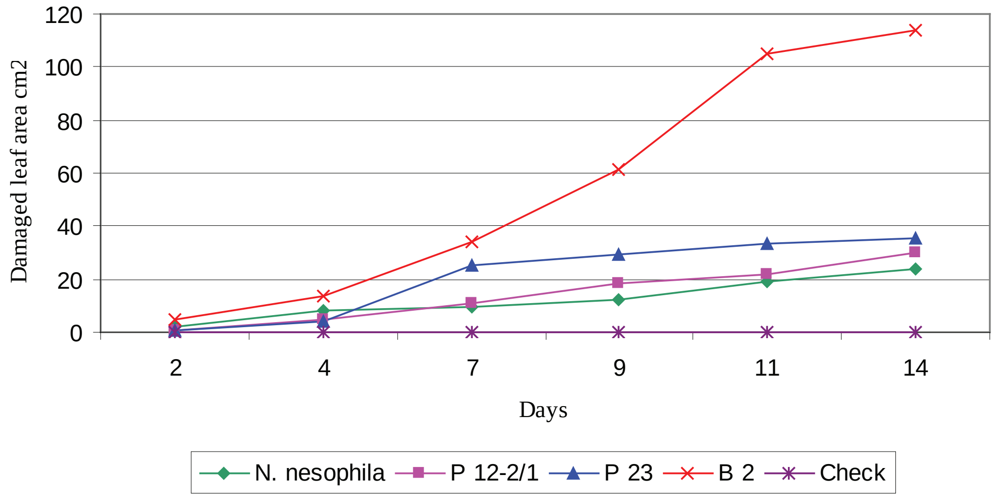
Species-Tob. variety	Leaf size l/w cm	Leaf area cm^2	Damaged leaf area cm^2	Infected leaf tissue %
N. nesophila	8.8/5.6	31.05	4.76	15.33
P 12-2/1	14.3/7.1	63.96	5.23	8.17
P 23	13.0/7.0	57.33	3.30	5.75
B 2	9.9/5.3	33.05	3.12	9.44
Check-P 12-2/1	15.5/9.3	90.81	0.00	0.00

Similar results were obtained in leaf inoculation by the second method, i.e. by submerging the leaf base in the inoculum. Results of investigation on necrosis in leaves inoculated with suspension of fungus mycelium are presented in Table 5, Figure 3. On the second day of observation, the infection intensity was similar to the one observed in inoculation with a drop selective agent and ranged between 1.00 cm^2 in P 12-2/1 and P 23 to 4.75 cm^2 in B2. Higher intensity of necrosis during observation was measured in B2, reaching 113.66 cm^2 on the 14th day of observation. The lowest intensity in this mode of inoculation was measured in the leaves of *N. nesophila* - 23.56 cm^2.

Table 5 - Leaves inoculated by submerging in mycelium suspension

Species-Tob. variety	Leaf size l/w cm	Leaf area cm^2	Spread of necrosis by days, in cm^2					
			2	4	7	9	11	14
N. nesophila	10.3/6.0	38.93	1.83	8.25	9.28	12.46	18.98	23.56
P 12-2/1	15.5/6.1	59.56	1.00	5.10	10.60	18.23	21.73	30.12
P 23	12.6/6.5	51.59	1.00	4.11	25.45	29.40	33.07	35.33
B 2	21.8/9.9	135.96	4.75	13.80	33.93	61.26	104.67	113.66
Check P 12-2/1	17.6/8.4	93.13	-	-	-	-	-	-

Fig.3 Necrosis development



Damages and percentage of infection, estimated on the basis of total and infected leaf surface are presented in Table 6. As could be seen from the Table, there are no major differences in the level of infection of investigated varieties and the wild species. The lowest percentage of infection was assessed in P 12-2/1 (50.57%), and the highest in B2 (83.59%).

Table 6 - Level of infection in leaves submerged in mycelium suspension

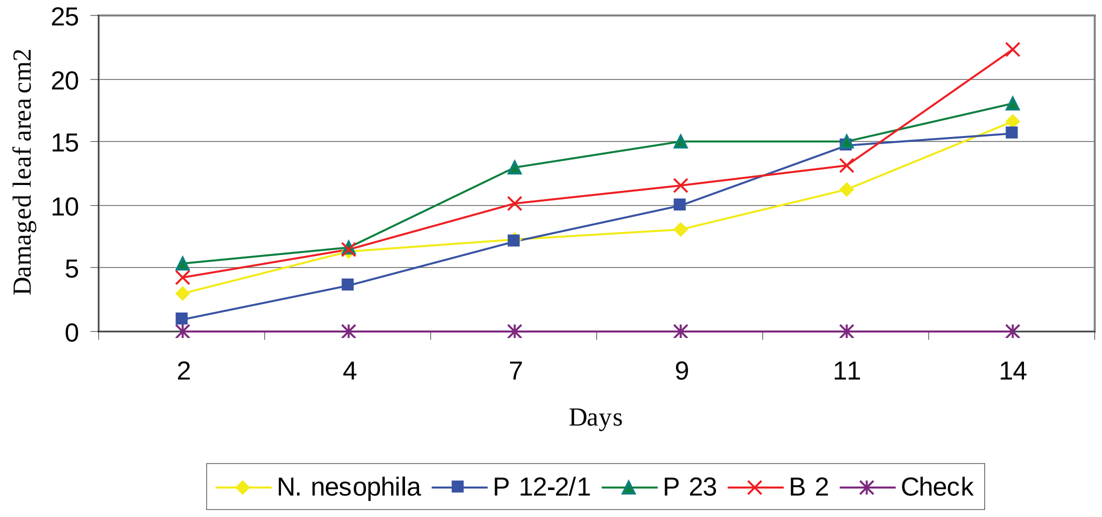
Species-Tob. variety	Leaf size l/w cm	Leaf area cm^2	Damaged leaf area cm^2	Infected leaf tissue %
N. nesophila	10.3/6.0	38.93	23.56	60.51
P 12-2/1	15.5/6.1	59.56	30.12	50.57
P 23	12.6/6.5	51.59	35.33	68.48
B 2	21.8/9.9	135.96	113.66	83.59
Check P 12-2/1	17.6/8.4	93.13	0.00	0.00

In leaves submerged in selective agent of the pathogen, higher damage, compared to other variants, was observed on leaf surface on the second day of inoculation, ranging from 1.00 cm^2 in P 12-2/1 to 5.40 cm^2 in P 23. On the last day, the highest damage was measured in B2 (22.30 cm^2) and the lowest in P12-2/1 (15.68 cm^2). (Table 7, Fig. 4). In this investigation, too, no symptoms of disease were observed in the check leaves.

Table 7 - Leaves inoculated by submerging in a selective agent

Species-Tob. variety	Leaf size l/w cm	Leaf area cm^2	Spread of necrosis by days, in cm^2					
			2	4	7	9	11	14
N. nesophila	9.5/5.0	29.92	3.00	6.40	7.20	8.00	11.20	16.60
P 12-2/1	13.3/6.0	50.27	1.00	3.63	7.10	9.98	14.71	15.68
P 23	10.0/5.5	34.65	5.40	6.60	13.00	15.00	15.00	18.00
B 2	9.8/5.2	32.10	4.20	6.50	10.10	11.50	13.10	22.30
Check P 12-2/1	14.9/6.3	59.13	-	-	-	-	-	-

Fig.4 Necrosis development



Percentage of infected leaf surface in this variant ranged between 31.19 % in P 12-2/1 and 69.47% in B2 (Table 8).

From the four variants investigated it could be seen that in both inoculation methods, daily growth of necrosis was highest in the leaves inoculated with suspension prepared from fungus mycelium. The daily growth is much lower in the leaves inoculated with selective agent.

Percentage of infected area differs significantly. It is much lower in the leaves inoculated with a drop of the selective agent and higher in the leaves inoculated with a drop of the suspension. The difference in percentage of infected leaf tissue was very small when leaves were inoculated by the second method, i.e. by submerging the base of the leaf in the inoculum.

Table 8 - Level of infection in leaves submerged in a selective agent

Species-Tob. variety	Leaf size l/w cm	Leaf area cm^2	Damaged leaf area cm^2	Infected leaf tissue %
N. nesophila	9.5/5	29.92	16.60	55.48
P 12-2/1	13.3/6.0	50.27	15.68	31.19
P 23	10.0/5.5	34.65	18.00	51.94
B 2	9.8/5.2	32.10	22.30	69.47
Check- P 12-2/1	14.9/6.3	59.13	0.00	0.00

The increased necrosis in leaves inoculated with suspension is due to the prolonged active metabolism of the pathogen, which thereby becomes more infective. According to Kutova (1983), cells affected by toxins do not die immediately, but are partially damaged by decrease of their physiological functions. More obvious changes appear in cells that are closer to the damage. Under the influence of toxins, the ability of wilted leaves placed in pure water to regain their turgor is very poor, unlike the mechanically provoked wilting, where turgor recovers very quickly.

As it was also reported, many varieties that are resistant to the pathogen show high susceptibility to the toxins. These statements are in compliance with investigations of Sutic (1986), who described toxins as low-molecular-weight compounds which spread easily from the place of infection, causing necrosis of healthy neighboring cells. This type of toxic effect is characterized by chlorotic zone around necrosis.

CONCLUSIONS

The investigations confirm the negative effect of toxic metabolites of the pathogen on the cells of plant tissue. The results obtained lead to the following statements:

- The fungus of *P. parasitica var. nicotianae* creates metabolites that are toxic to tobacco plant cells.
- 2. As a result of toxic metabolites, necrosis appears on leaf surface or along the vessels of tobacco inoculated with a drop of selective agent or a drop of suspension of the fungus mycelium, i.e. when they are submerged in selective agent or in suspension prepared with mycelium of the pathogen.
- Inoculated leaves from different tobacco varieties had a different reaction to the toxic metabolites of the pathogen.
- Higher necrosis level and higher percentage of damaged leaf tissue in almost all varieties were observed in leaves inoculated with suspension of the fungus mycelium in both investigated variants.
- Significantly lower percentage of infection was observed in leaves inoculated with selective agent in both variants, but it was particularly low in leaves inoculated with a drop of this inoculum.
- Selective agent infects the cells of tobacco leaves, which can be observed as a lesion surrounded by a chlorotic ring.

REFERENCE

1. Кутова И., 1983. Вздејствие на токсичните метаболити на Phytophthora parasitica var. nicotianae врху тютюневите растения. Растениевдни науки, год XX, No 3, София.
2. Lucas G., 1975. Diseases of tobacco. Biological Consulting Associates Box 5726, Raleigh, North Carolina.
3. Sariċ M., Kastori R., Petoviċ M., Stankoviċ Ž., Krstiċ B. and Petroviċ N., 1990. Praktikum iz fiziologije biljaka. Naučna knjiga, Beograd.
4. Славов С., Бачварова Р., Благоева-Николаева В., 2002. In vitro селекциона шема за отпорност на тутунот кон Phytophthora parasitica var. nicotianae. Зборник на резимеа, 20-ти Симпозиум за тутун, Охрид.
5. Ташкоски П., 2003. Phytophthora parasitica (Dastur) var. nicotianae (Breda de Haan) Tucker причинител на болеста црнилка на тутунот. Тутун, Vol. 53, No 11-12, 345-355.
6. Ташкоски П., 2005. Реакција на видовите од родот Nicotiana на расата 0 и 1 од Phytophthora parasitica var. nicotianae. Тутун, Vol. 55, N^{o} 3-4, 78-86.
7. Šutić D., 1986. Anatomija i fiziologija bolesnih biljaka. Nolit, Beograd.

UDC 633.71
Тутун/Tobacco, Vol.60, N° 7-12, 121-126, 2010
University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute – Prilep, R. Macedonia

ISSN 0494-3244

UDC: 633.71-238(497.2)"2008/10"
Original scientific paper

EXAMINING THE RESPONSE OF SOME PERSPECTIVE LINES BURLEY TOBACCO VARIETIES TO ECONOMICALLY IMPORTANT VIRAL PLANT DISEASES

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ABSTRACT

The research was carried out during the period 2008–2010 on the experimental fields of Tobacco and tobacco products Institute – Markovo. Eight perspective Bulgarian Burley tobacco lines – 1354, 1478, 3199, 2131, 1435, 3149, 1409, 1421, with different degrees of resistance to viral diseases were included in the study. The line 1354 was used as a control genotype. All samples were tested on a naturally and an artificially infected background . The percentage of infection by Tomato spotted wilt virus (TSWV), Potato virus Y (PVY) and Tobacco mosaic virus (TMV) was calculated on the basis of symptoms observation.

Immunological assessment of the lines reaction to TMV and PVY was made. The results of the research showed that none of the lines studied are resistant to TMV, PVY and TSWV, and the varieties Burley tobacco are attacked to some extent by the viruses.

Key words: tobacco, Burley tobacco lines, TSWV, TMV, PVY, naturally and artificially infected background, percentage of infection, viral diseases.

ИСТРАЖУВАЊЕ НА РЕАКЦИЈАТА ОД НЕКОИ ПЕРСПЕКТИВНИ ЛИНИИ НА ТУТУНОТ ОД ТИПОТ БЕРЛЕЈ ВРЗ ЕКОНОМСКО ВАЖНИ ВИРУСНИ БОЛЕСТИ

Истражувањето беше извршено во периодот од 2008 до 2010 година на опитното поле од Институтот за тутун и тутунски призводи – Марково. Во опитот беа вклучени осум перспективни линии тутун од типот берлеј (1354, 1478, 3199, 2131, 1435, 3149, 1409, 1421), со различен степен на отпорност кон вирусни заболувања. Сортата 1354 беше користена како контролен генотип. Сите примероци беа тестирани на природна и на вештачки заразена површина.

Процентот на инфекција од вирусот на бронзена некроза на тутунот (TSWV), цртичеста некроза на тутунот или т.н. компиров Y вирус (PVY) и мозаикот на тутунот (TMV) се пресметани врз основа на набљудуваните симптоми.

Од добиените резултати на истражувањето се констатира дека ниту една од истражуваните линии не е отпорна на TMV, PVY и TSWV, а сите линии на тутун беа нападнати од вируси до одреден степен.

Клучни зборови: тутун, линии тутун од типот берлеј, TSWV, TMV, PVY, природна и вештачки заразена површина, процент на инфекција, вирусни заболувања.

INTRODUCTION

Tobacco is subject to attacks by a number of viruses, most of which spread every year and turn into epiphytoties. For example, in Bulgaria in 1956 the losses caused by the Tomato spotted wilt virus (TSWV), reached up to 100% in some areas (Gabrovska, 1984; Kovachevski et al, 1999). More devastation was caused by epiphytoties of PVY and TMV in 1983 and 1986, as well as in 1989 in some places.

Tomato spotted wilt virus is one of the most widespread and economically important plant virus (Golbach and Peters,1994, cit. Dimitrov, 2003). The virus infects at least 900 plant species, and number of recorded natural host species is steadily increasing (Peters, 1998, cit. Kaliciak, 2009) – among them are many vegetables and ornamental plants (Mason et al., 2003, cit. Kaliciak, 2009). Data from a study by Dimitrov (2003) about the survival of TSWV during winter period and its persistent spread during the spring season, shows the exceptional role of the *Thrips tabaci Lind* wintering in the soil and the plants' remains. To this day in the world, from 5000 types of trips described, only these 9 kinds are vectors of TSWV: *Franklinella occidentalis; Franklinella schultzei; Franklinella fusca; T. tabacci; Thrips setosus; Thrips moultoni; Franklinella tenicornis; Lithrips dorsalis; Scirtothrips dorsalis* (Zitter, 1989).

Potato virus Y (PVY) is a typical member of the Potyviridae family. It has a wide host range and is spread worldwide, causing serious losses in Solanaceous crops (Voster,1990; Chatzivessilion et al, 2004). During the pollination period PVY is spread by more than 50 aphid species in a non-persistent manner. The peach aphid (*Myzus persicae* Sulz) has the ability to spread the virus very quickly and is the most efficient vector of the virus (De Box et Hutinga, 1981; Di Fonzo, 1995 cit. Kanavaki, 2006). The PVY is spread by *Acyrthosiphon, Aphis, Myzus, Neomyzus* (Lukas, 1975).

PVY is represented in three major strain groups: ordinary (PVY^0), tobacco veinal necrosis (PVY^n) and aphid non-transmissible stipple streak (PVY^c) (Kaliciak, 2009). In Bulgaria, tobacco strains of PVY belong to two groups: 1) PVY^0 (ordinary form) causing tobacco veinal chlorosis, and 2) PVY^n necrosis strains, causing veinal necrosis (Kovachevski, 1999).

Another worldspread and economically important viral plant disease is the Tobacco mosaic virus (TMV). It is a typical member of the group Tobamovirus. The virus is very unstable in the natural environment, which determines the big number of varieties and strains. Most important of these are: tomato strains, acuba strains, ringspot strains, masked strains (Kovachevski et al, 1999). TMV is economically the most important viral disease for the tobacco plants, because it could reduce the dry tobacco product more then 50 % - the leaves have lower quality and physiological property, reduced fragrance and minimized smoking index (Kovachevski et al, 1999).

Study data of four Bulgarian researchers (Dimitrov and Bosukov, 2004 ; Kovachevski et al, 1999; Stoimenova, 1995) shows that Tobacco-growing in Bulgaria suffers economically most from TSWV, PVY, CMV (Cucumber mosaic virus), TMV and ToMV (Tomato mosaic virus).

The aim of the present research is to investigate the responses of perspective Bulgarian lines of Burley tobacco, naturally and artificially infected, to the economically important viral plant diseases TMV, PVY and TSWV.

MATERIAL AND METHODS

The study was carried out during 2008–2010 on the experimental field of Tobacco and tobacco products Institute – Markovo. The research included eight perspective Bulgarian Burley tobacco lines – 1354, 1478, 3199, 2131, 1435, 3149, 1409 and 1421, with different degrees of resistance to viral diseases. The line 1354 was used as control genotype. All samples were tested on a naturally and an artificially infected background. Immunological assessment of the plants' reaction to TMV and PVY was made. The percentage of infection by economically important plant viral diseases TMV, PVY and TSWV it was detected.

The identification of the viruses accruing in the natural environment was based on observation of the characteristic symptoms through regular monitoring of the fields. As the symptoms of TMV and ToMV do not differ, they are registered together as Tobamoviruses.

The artificial infection was carried in the green houses of Tobacco and tobacco products Institute – Markovo, using the method of mechanical inoculation of Noordam (1973). Serologically tested isolates of TMV and PVY were used, and plants of N. tabacum cv. Samsun NN, Nevrokop 1146, cv. Samsun N`N` were used as indicators, as well as Petunia hybrida, Chenopodium amaranticolor, Solanum nigrum.

The necessary agro-technical measures were taken for the good safekeeping of cultivated tobacco.

The statistic processing of results was made by dispersional analyses. The study looked for detection of significant differences between variants.

RESULTS AND DISCUSSION

The results obtained from the spread of TMV, PVY and TSWV of the Burley tobacco lines on naturally infected background are presented in Table 1 and Figure 1, 2 and 3. They show that under local ecological conditions and the virulence of the strains during the three years of research, all Burley tobacco lines are not affected by the Tobacco mosaic virus.

Infection of PVY was found only on the control genotype line 1354, the rest of the genotypes were not attacked by the virus.

The results of the dispersional analyses show that there is a statistically significant difference in percentage of infection by PVY, between the control and other lines – the difference is significant at $P_{0.1\%.}$(Table 1)

During the years, the research was looking at the differences of TSWV attacks. The average percentage of attacks of TSWV in 2008 was 4.2%. The most attacked of this virus lines were line 1478 - 8.8% and line 1399 - 6.7%, while the most weak infection the virus caused on line 1349 - 0.6%. The average percentage of attack of TSWV in 2009 is considerable lower - 1%. The highest percentage of infection was detected in lines 1354 and 1409 - 1.9%.

Year 2010 was characterized with average percentage of attacks 1.4%, as most susceptible to attacks of TSWV is line 1421 - 2.5%. The results of the dispersional analyses show statistically insignificant differences in the degree of infection of TSWV between the control (line 1353) and the other lines included in the study (Table 1).

The results of the immunological tests during the study period, on the artificially infected perspective Burley tobacco lines, are presented in Table 2. They show that the studied genotypes lines 1478; 1399; 2131; 1435; 1349; 1409; 1421 were resistant, while the control (line 1345) was vulnerable to PVY. Lines 1354; 1478; 3199; 1435; 1349; 1409; 1421 reacted with resistance (on the basis of super sensitivity) to TMV, while line 1231 was sensitive to it. Lines 1478; 1399; 1435; 1349; 1409 and 1421 were resistant to both PVY and TMV. Lines sensitive to both viruses have not been found.

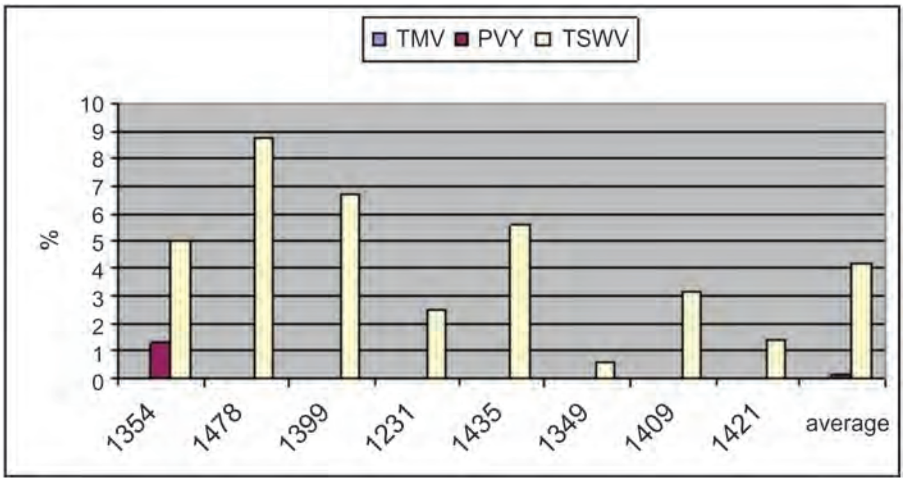

Fig.1 Percent of infection by TSWV, PVY, TMV in 2008

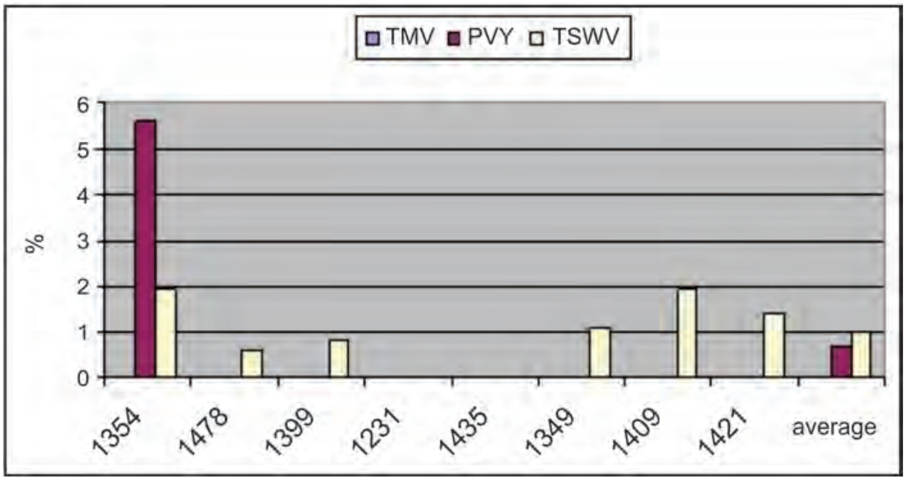

Fig.2 Percent of infection by TSWV, PVY, TMV in 2009

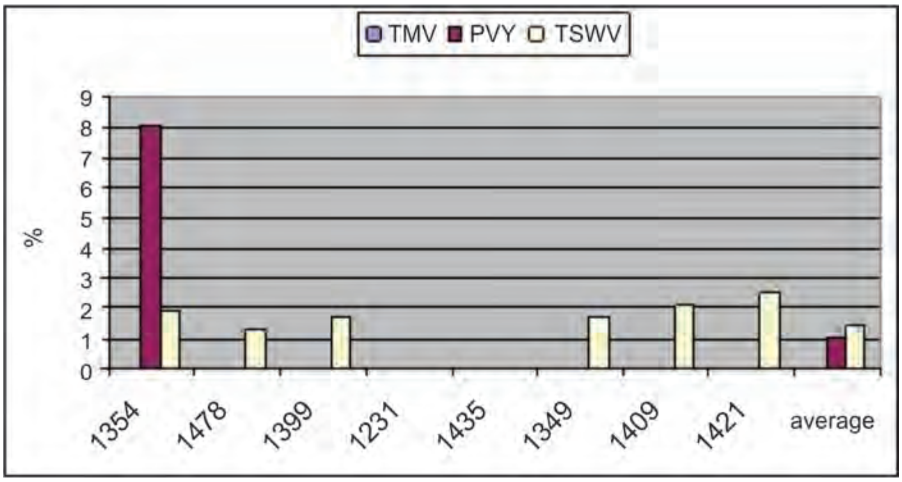

Fig.3 Percent of infection by TSWV, PVY, TMV in 2010

Tab.1 Percent of infection by virus diseases

Genotypes	TMV, %	PVY, %	TSWV, %
lines 1354 Ø	0	5	2.9
lines 1478	$0^{n.s.}$	0^{---}	$3.6^{n.s}$
lines 1399	$0^{n.s.}$	0^{---}	$3.1^{n.s}$
lines 1231	$0^{n.s.}$	0^{---}	$0.8^{n.s}$
lines 1435	$0^{n.s.}$	0^{---}	$1.9^{n.s}$
lines 1349	$0^{n.s.}$	0^{---}	$1.1^{n.s}$
lines 1409	$0^{n.s.}$	0^{---}	$2.4^{n.s}$
lines 1421	$0^{n.s.}$	0^{---}	$1.8^{n.s}$

(n.s.) non – significant differences; (+++) (---) differences are significant at $P_{0,1\%}$

Tab.2 Immunological assessment of the to Burley tobacco lines reaction to economically important viral plant diseases

Genotypes	TMV	PVY
lines 1354 Ø	R	MS
lines 1478	R	R
lines 1399	R	R
lines 1231	MS	R
lines 1435	R	R
lines 1349	R	R
lines 1409	R	R
lines 1421	R	R

CONCLUSIONS

The study found that on both naturally and artificially infected backgrounds, there were distinctive tobacco lines in which no infection to TMV and PVY were found.

During the research period none of the Burley tobacco lines were attacked of Tobacco mosaic virus in the naturally infected background. Line 1231 had reacted to the viruses when artificially infected.

Only the control line was attacked by PVY, while none of the other studied genotypes were affected by the virus in both infection backgrounds.

During the individual years of study, varied differences of TSWV attacks were found. The highest average percentage of infection by the virus was detected in 2008 - 4.2%. During that year the strongest attack was on lines 1478 - 8.8% and 1399 - 6.7%, while the poorest attack was on line 1349 - 0.6%.

REFERENCES

1. Габровска, Т. 1984. Проучвания върху доматената бронзовост (Tomato spotted wilt virus) по тютюна в България. Дисертация, Пловдив
2. Димитров, А. 2003. Наръчник по защита на тютюна от болести неприятели и плевели. Пловдив, 59-63
3. Димитров, А., Бозуков, Х. 2004. Вирусни болести по тютюна в България и борбата с тях. Български тютюн, 5, 11-18
4. Chatzivessilion, E. 2004. A survey of tobacco viruses in tobacco crops and native flora in Greece. Eur.J. of Plant Pathol.110:1011-1023
5. Kaliciak, A. et al, 2009. New hosts of Potato virus Y (PVY) among common wild plants in Europe. Eur. J. of Plant Pathol.124:707-713
6. Kanavaki, M. et al. 2006. Transmission of Potato virus Y in Tobacco Plants by Myzus persicae nicotianae and M. persicae s.str. Plant Dis.90:777-782
7. Ковачевски, И. и др., 1999. Вирусни и вирусноподобни болести на културните растения. София, 189-196
8. Lukas, G. 1975. Diseases of tobacco. Biological Consulting Associates, North Carolina, 447-463
9. Noordam D., 1973. Identification of plant viruses – methods end experiments, Wagenigen.
10. Stoimenova, E. 1995. Investigations on the strain variability of tobamoviruses and cucumovirus isolated from Bulgaria. Journal of culture collections, vol. 1, 46-52
11. Voster, L. 1990. Differentiation of strains of potato virus Y affecting tobacco in South Africa. Phytophylactica 22/1/:129-132
12. Zitter, T., M. Daughtrey, J. Sanderson. 1989. http://vegetablemodonline.ppath.cornell.edu /factsheets/virus_SpottedWilt.htm

UDC 633.71
Тутун/Tobacco, Vol.60, N° 7-12, 127-133, 2010
University “St. Kliment Ohridski” - Bitola
Scientific Tobacco Institute – Prilep, R. Macedonia

ISSN 0494-3244

UDC: 663.977
Original scientific paper

INVESTIGATION ON THE INFLUENCE OF SOME PHYSICAL CHARACTERISTICS OF CIGARETTES UPON SMOKE COMPOSITION

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ABSTRACT

Despite increased restrictions on tobacco use and cigarette smoking ban, a variety of blends and designs is on the rise. Various technical-technological measures have been made to reduce the amount of harmful matters in cigarette smoke. The most recent but insufficiently investigated approach of reduction of smoke yield is filtration of smoke particles through the acetate filters.

Investigations were made on the effect of pressure drop of the filter rod and cigarette and the rate of free combustion on the content of tar, nicotine and carbon monoxide in test cigarettes.

Cigarettes were produced on the industrial cigarette maker, and then tested on the Smoking Machine RM 20/CSR according to ISO 3308. The content of tar was measured according to ISO 4387, and the pressure drop of the filter rod and cigarette was measured according to BDS 11353.

The results have shown that both types of filters used, influence the smoke yield and the amount of harmful matters in smoke. Reducing denier per filament increases smoke removal efficiency. Filter type X (denier 3.0Y35 000) effectively filters the smoke so that the nicotine, tar and carbon monoxide contents are lower than the contents obtained by the filer Y (denier 2.7Y35 000).

Key word: cigarette, filter rod, pressure drop, physical characteristics of cigarettes, nicotine, smoke

ИСПИТУВАЊЕ НА ВЛИЈАНИЕТО НА НЕКОИ ФИЗИЧКИ СВОЈСТВА НА ЦИГАРИТЕ ВРЗ СОСТАВОТ НА ЧАДОТ

И покрај зголемените ограничувања на употребата на тутун и забраната за пушење на цигари, на пазарот се се’ поприсутни цигари со различни тутунски мешавини и дизајн.

Воведени се различни техничко-технолошки мерки за намалување на количината на штетни материи во чадот од цигарите. Еден од најстарите, но недоволно истражен пристап за намалување на приносот на чадот е филтрацијата на честички на чадот преку ацетатноцелулозни филтри. Истражувањата се направени со цел да се се одреди ефектот на отпорот на повлекување на филтер-стапчето, отпорот на повлекување на цигарата и брзината на слободно согорување врз содржината на катран, никотин и јаглероден моноксид во тест-цигарите.

Цигарите се произведени на индустриска машина за цигари и потоа тестирани на машина за пушење RM 20/CSR во согласност со стандардот ISO 3308. Содржината на катран е мерена во согласност со стандардот ISO 4387, а отпорот на повлекување на филтер-стапчето и на цигарата е измерен според стандардот БДС 11353.

Резултатите покажаа дека и двата типа на филтри влијаат врз приносот на чадот и количината на штетни материи во чадот. Намалувањето на денијажата по влакно ја зголемува ефикасноста за отстранување на чадот. Филтерот од типот X (дениер 3.0S35 000) поефикасно го филтрира чадот, така што содржината на никотин, катран и јаглерод моноксид е пониска од содржината добиена со филтерот S (дениер 2.7S35 000).

Клучни зборови: цигари, физички карактеристики на цигарите, никотин, пушење, отпор на повлекување

INTRODUCTION

Nowadays, manufactured cigarettes are the most widespread form of tobacco use. Despite the ban on tobacco use and smoking, there are many cigarette brands on the market made from different tobacco blends and with various designs.

For the tobacco industry, the most important thing is not only the survival of cigarette brands which have already been designed, but also, the production of new ones according to the demand and in accordance with the law. On the other hand, for the science, it is important to discover the relationships between the physical characteristics of cigarettes which form not only the taste, but also the composition of tobacco smoke.

The design of the cigarettes includes; cigarette geometrical characteristics, choice of tobacco blend, paper elements, as well as type and construction of the filter rods.

The pressure drop of a filter rod is a feature that smokers recognize and its varying depends on the mass of tobacco blends, width of the cut, density, and the characteristics of the preproduction materials (filter rod, filter strip, cigarette paper and etc.). That being said, it means that the pressure drop is a result of the complex action of all cigarette technological parameters and its varying has a direct impact upon the formation of tar and nicotine (Nikolic 2004).

The efficiency of the filter rod can be optimized by setting up the intersection, the length and deniers of the fibbers, as well as by varying the length and diameter, the pressure drop, and the ventilation of a filter rod (Nikolic, 2004, Kirkova, 2004).

The pressure drop of the filter rod is one of the most significant parameters as the efficiency of the filter rod for removal of tar and nicotine from the mainstream smoke directly depends on it.

By setting up the degree of fiber separation in the cellulose acetate track and its mass, the pressure drop of the filter can be directly regulated for +-10% around the average of the capacity (Georgiev, 2002).

The tar is a solid residue of tobacco smoke aerosol. Carbon monoxide is a dominant gas, whereas the nicotine, as a semi-volatile compound, is divided into two phases of the smoke (Pillsbury et al., 1969, Ingebrethsen B.J., 1986).

The tar, nicotine and carbon monoxide (TNCO) are generated during the process of distillation and pyrolysis (thermal decomposition) of tobacco and in the process of ash oxidation during the heating of tobacco.

The primary role of the filter rod is to remove the tar particles through the process of mechanical filtration of the smoke aerosol (Keith, 1978, Baker, 1980). Filter fibers have very slight effect on the gas phase of the smoke. Some of the semi-volatile constituents of smoke present in the solid phase remain on the filter rod fibers (Morie and Baggett, 1975).

The remaining of the smoke constituents depends on two mechanisms such as selective filtration and elution. Constituents that have a great affinity for the filter remain on, whereas those of slight affinity elute. The acetate filter is less efficient at retaining the nicotine compared to the tar (Curran and Keifer, 1973, Georgiev, 2002, Kirkova, 2005 and 2007).

At present, many cigarettes have filter rods made of cellulose acetate which reduce the tar by 30-40%, and nicotine by 25-30% compared to the cigarettes without filter rods.

At the last conference of signatory countries of the ratified framework convention for tobacco control of the World Health Organization FCTC/COP4, it has been recommended that attention should be paid to design characteristics and on their influence on smoke composition. The purpose of this examination is to determine the relationship between the pressure drop of filter rod, cigarette pressure drop, cigarette combustion speed and their impact upon the reduction of tar, nicotine and carbon monoxide (TNCO) in the mainstream smoke.

MATERIAL AND METHODS

For the purpose of this examination, manufactured cigarettes of Virginia tobacco blend were used, with the following physical characteristics: king size length - 84 mm, diameter - 7.92 mm, weight - 0,760 g, absolute moisture content - 12,50% and permeability of cigarette paper - 46 CU. The filter rod was made of mono acetate cellulose, with Y shape of the fibers, 25mm long and covered with impermeable paper.

Cigarettes differ only by the deniers of the filter rod and the variants are marked as a brand X/denier 3,0Y35 000 and a brand Y/denier 2,7Y35 000.

Measurements of the pressure drop of filter rod and cigarette were taken according to BDS 11353, whereas the free combustion according to BDS 12975.

The cigarettes are conditioned according to ISO 3402 and tasted by smoking machines Borgwaldt M 20/CSR according to ISO 3308.

The tar content was determined according to ISO 4387 and water content in the capacitor according to ISO 10362-2. The presence of alkaloids expressed as nicotine was determined according to ISO 10315, and the carbon monoxide by ISO 8454.

For both samples of cigarettes, all tested parameters were measured in 8 iterations. In processing the results, arithmetic average value of the arithmetic average X av was used, as well as rithmetic average value of the root-mean-square deviations S av.

RESULTS AND DISCUSSION

The results on pressure drop of filter rod and cigarette and the speed of free combustion of the cigarette for both test- cigarettes are shown in Table 1.

Table 1 Physical characteristics of test-cigarettes

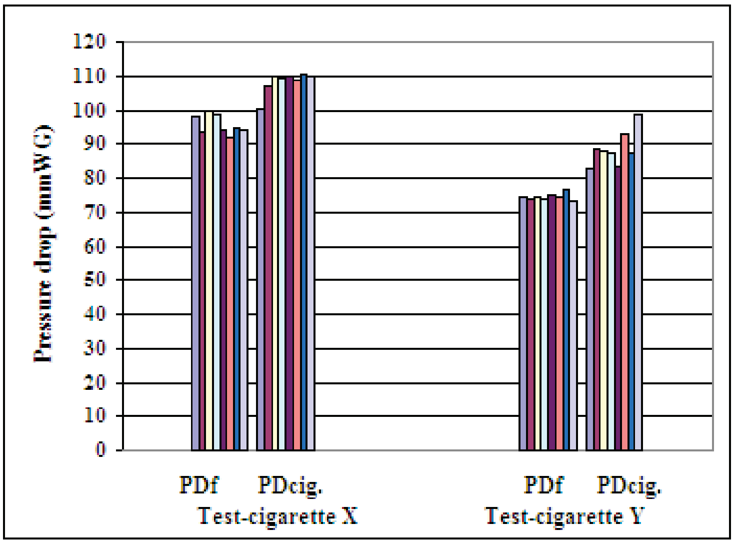
Physical characteristic	X av	*s* av	*v* av	*min*	*max*
Test cigarettes X					
Pressure drop of filter rod (mmWG)	95.56	2.830	2.96	91.90	99.80
Pressure drop of cigarette (mm WG)	108.12	5.126	5.78	100.20	110.30
Free combustion (min)	7.04	0.311	4.41	6.72	7.49
Test-cigarettes Y					
Pressure drop of filter rod (mmWG)	74.32	1.041	1.40	73.05	76.40
Pressure drop of cigarette (mm WG)	88.64	3.387	3.13	82.90	98.74
Free combustion (min)	6.91	0.246	3.56	6.40	7.10

In test-cigarette X, the pressure drop of the filter rod is 95.56 mmWG, whereas in test-cigarette Y, the pressure drop decreased at 74.32 mmWG. Consequently, the test-cigarette X has a greater pressure drop compared to test-cigarette Y.

It means that with the decrease of denier of the filter rod fibers from 3, 0 to 2, 7, the pressure drop of filter rod and cigarette also decreases.

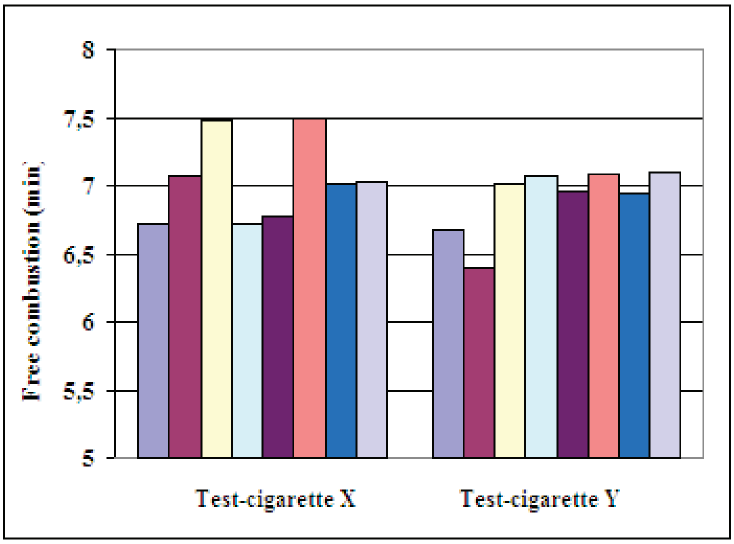
The variation between the values for pressure drop of the filter rod in test-cigarette X is higher compared to the values for this parameter in test-cigarette Y. Thus, it indicates uneven charges in cigarettes.

The change of the values for pressure drop of the filter rod and of the whole cigarette is shown in Graph 1.



Graph 1 - Movement of pressure drop of filter rod and cigarette (mmWG) in both test-cigarettes

PDf(mmWG) pressure drop of filter rod
PDf(mmWG) pressure drop of cigarette



Graph 2 Movement of free combustion (min) in both test-cigarettes

The parameter values of free combustion in both test-cigarettes are very close. There is a slight tendency for slowing the combustion in test-cigarette X. The speed of free combustion depends not only on the pressure drop but also on the type of paper, width of burning tobacco, weight, smoothness of tobacco fill in the cigarette and the mutual influence of all these factors.

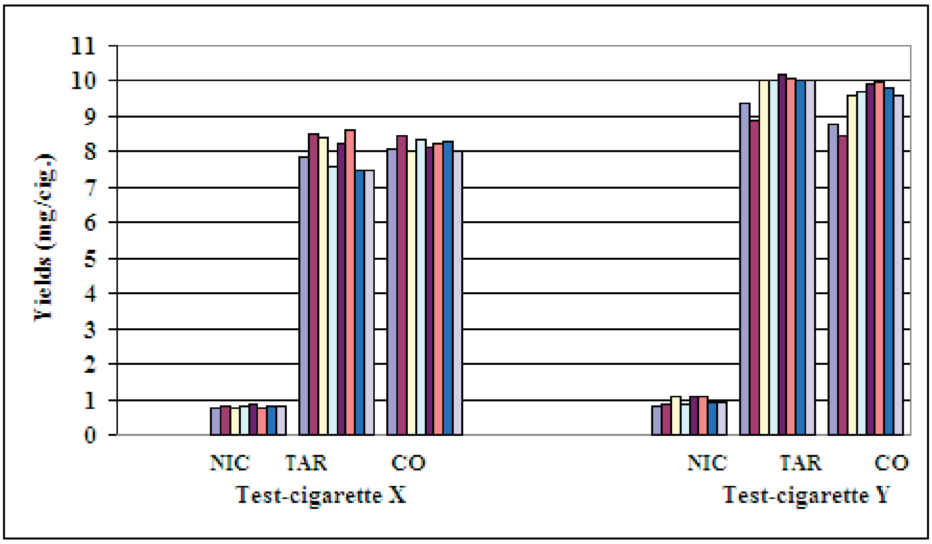
The change of values in free combustion in the examined test-cigarettes is shown in Graph2.

Measurements of the harmful elements tar, nicotine, and carbon monoxide in the mainstream smoke are shown in Table 2.

Table 2. Chemical characteristics of test-cigarettes

Chemical characteristics	X av	*s* av	*v* av	*min*	*max*
Test-cigarette X					
Nicotine (mg/cig)	0.80	0.031	3.92	0.75	0.84
Tar (mg/cig)	8.02	0.478	5.96	7.46	8.64
Carbon monoxide (mg/cig)	8.21	0.166	2.02	8.02	8.48
Test-cigarette Y					
Nicotine (mg/cig)	0.96	0.111	4.96	0.83	1.11
Tar (mg/cig)	9.82	0.449	4.57	8.90	10.20
Carbon monoxide (mg/cig)	9.47	0.555	5.86	8.45	9.90

The presence of tar, nicotine, and carbon monoxide (TNCO) in the mainstream smoke in both test-cigarettes is shown in Graph. 3.



Graph 3 - Presence of nicotine (NIC), tar (TAR) and carbon monoxide (CO) (mg/cig.) in both test-cigarettes.

It is safe to say that the change in pressure drop leads to a change in the process of combustion, and it unarguably influences the quantity and composition of smoke.

The decrease in pressure drop of the filter-rod in the test-cigarette Y increases the content of nicotine in the mainstream smoke by 20% and the tar by 22% compared to the same parameters in cigarette X.

As for the content of carbon monoxide, this parameter increases by 15% in test-cigarette Y compared to cigarette X. If these values are expressed in ratio, in test-cigarette X, the yield of tar compared to the one of nicotine is 10.0, whereas in test-cigarette Y, the yield is slightly higher - 10.2.

In both cigarettes, the tar and carbon monoxide ratio is approximately 1:1. It is 0,97 in test-cigarette X, and 1,04 in test-cigarette Y.

The speed of free combustion of cigarette is proportionally reduced with the increase of tobacco density in cigarette and the diameter of filter-rod.

CONCLUSION

According to the results, there is dependence between the pressure drop of filter-rod, the pressure drop of cigarette and the free combustion of cigarette. It can be seen that the change in the pressure drop of test-cigarettes strongly influences the obtained yields of tar, nicotine and carbon monoxide in the mainstream smoke.

In test-cigarette Y, the decrease in the pressure drop of the filter-rod contributes to the increase of nicotine content in the mainstream smoke by 20% and of the tar by 22% compared to the content of these parameters in cigarette X.

Thus, there is a higher retention of harmful substances if the cigarette is more efficiently filtered as in cigarette X, which has higher density and pressure drop of the filter-rod.

Unlike the pressure drop of cigarettes, the speed of free combustion remains unchanged in both cigarettes.

It still has not been proved how it reflects the smoking characteristics of cigarette and it consequently remains to be the aim of the following examinations.

The results of the investigation show that many factors influence the content of the mainstream smoke, which will be gradually disclosed according to the design change of the cigarette.

REFERENCES

1. Nikolić M., 2004. Tehnologija prerade duvana, Beograd
2. Georgiev S., 2002. Tehnologija na tutunevite izdelija, Plovdiv
3. Pillsbury H.C., Bright C.C., O'Conor K.J., Irish F.W., 1969. Tar and nicotine in tobacco smoke.J.Assoc.off.Anal.Chem.52, 458-462
4. Ingebrethsen B.J., 1986. Aerosol studies of cigrette smoke. Rec. Adv. Tob. Sci. 12, 54-142
5. Keith C.H., 1978. Physical mechanisms of smoke filtration. Rec. Adv. Tob. Sci. 4, 25-45
6. Baker R.R., 1980. Mecanisms of smoke formation and delivery. Rec. Adv. Tob. Sci. 6, 184-224
7. Morie G.P. and Bagett M. S., 1975. Selective removal of semivolatile components from cigarette smioke by various filters. Beitr. Tabakforsh. 8,(3), 150-152
8. Kirkova S., 2004. Influence of the constancy of the main characteristics of auxiliary materials on the quality of cigarettes, Scientific Conference with international participation „60 years ITTP" 357-362
9. Kirkova S., 2004. Studies on the influence of some factors in lowering the levels of nicotine, tar and carbon monoxide in cigarette smoke 15 th National Conference with International Participation & Quality for better life "2004", 113-118

10. Kirkova S., 2005. Studies on the influence of aperture on the mass of cigarettes, Scientific Conference with international participation "Food Science and Technology 2005" УХТ, HT-LII, Iss. 2, 206-210
11. Kirkova S., 2007. Studies on the interdependence of nicotine, tar and carbon monoxide in cigarette smoke and its manageability, Scientific Conference with international participation "Food Science and Technology 2007", УХТ, HT-LIV, Iss. 1, 243-246
12. Kirkova S., 2007. Research on design elements of cigarettes to reduce the content of nicotine, tar and carbon monoxide in smoke, Scientific Conference with international participation "Food Science and Technology 2007", УХТ, HT-LIV, Iss. 1, 253-257
13. Directive 2001/37/EC of the European Parliament and of the Council, Official Journal of European Communities, I 194, 26-34, 2001
14. FCTC/COP4(10) Partial guidelines for implementation of Articles 9 and 10 of the WHO Framework Convention on Tobacco Control (Regulation of the contents of tobacco products and Regulation of tobacco product disclosures)